BD™ Tryptic Soy Agar

INTENDED USE

BD Tryptic Soy Agar, provided in bottles, is a partially completed general purpose medium which, after pouring into Petri dishes or tubes, supports the growth of nonfastidious as well as moderately fastidious microorganisms. In clinical microbiology, it is not used for the isolation of pathogens from clinical specimens but may be used for cultivating bacterial strains. After supplementation with blood, it may be used as a primary isolation medium in clinical microbiology.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

The nutritional composition of Tryptic Soy Agar has made it a popular medium for many years. It is the medium specified as Soybean-Casein Digest Agar Medium in the United States Pharmacopeia and in the European Pharmacopeia for the total aerobic microbial count portion of the microbial limit testing procedures.[1,2] The prepared medium is used for a multitude of purposes including maintenance of stock cultures, plate counting, isolation of micro-organisms from a variety of materials.[3-5] It is included in the compendia of methods for the examination of water, waste-water and foods.[6,7]

The use of this medium in clinical microbiology is limited since it does support the growth of a variety of fastidious bacteria. However, since Tryptic Soy Agar does not contain the X and V growth factors, it may be used in determining the requirements for these growth factors by isolates of *Haemophilus* species by the addition of X, V and XV factor strips to inoculated plates.[8] **BD Tryptic Soy Agar** may also be used as a medium for maintaining or subculturing reference strains, e.g., *Enterobacteriaceae* and staphylococci. The unsupplemented medium is not used as a primary isolation medium for clinical applications. After the addition of blood (e.g., 5% sheep blood), it can be used for the isolation of bacteria from clinical specimens.[9,10]

In **BD Tryptic Soy Agar**, the combination of casein and soy peptones renders the medium nutritious by supplying organic nitrogen, particularly amino acids and longer-chained peptides. Sodium chloride maintains the osmotic equilibrium. **BD Tryptic Soy Agar** (Bottled Media) are partially completed (=semi-finished) media supplied in bottles from which the user can prepare a plated or tubed medium. These media are manufactured from **Difco** dehydrated medium.

REAGENTS

BD Tryptic Soy Agar

Formula* Per Liter Purified Water

Bacto™ Tryptone (Pancreatic Digest of Casein)	15.0 g
Bacto Soytone (Papaic Digest of Soybean Meal)	5.0
Sodium Chloride	5.0
Agar	15.0

pH 7.3 ± 0.2

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

PRECAUTIONS

IVD . For professional use only.

Do not use bottles if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration. For completion of this partially completed bottled medium, follow the methods and observe the warnings described under **PROCEDURE** - **Reagent Preparation**. Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store bottles in the dark at 5 to 25° C. Avoid freezing and overheating. The medium may be used up to the expiration date and incubated for the recommended incubation times. Bottles from opened packages can be used up to the expiration date. Opened bottles must be used immediately. To prepare plates or tubes from the bottled medium, it must first be liquefied (see **PROCEDURE** - **Reagent Preparation**). Do not liquefy the medium more than once after solidification.

USER QUALITY CONTROL

Unsupplemented medium: Prepare plates or tubes from the partially completed medium (see **PROCEDURE** - **Reagent Preparation**), without adding supplements such as blood. Inoculate plates or tubes with the test strains indicated in the Table below. For further details, see **GENERAL INSTRUCTIONS FOR USE** document. Incubate the bacteria for 18 to 48 hours aerobically at 35 +/- 2° C. Incubate *Aspergillus niger* for 3 to 4 days aerobically at 25 to 28° C. According to the USP and EP, the medium should be incubated between 30 to 35° C.[1,2]

Strains	Results
Aspergillus niger ATCC™ 16404	Growth
Bacillus subtilis ATCC 6633	Growth
Escherichia coli ATCC 25922	Growth
Staphylococcus aureus ATCC 6538	Growth
Uninoculated	Light amber to amber, slightly opalescent

Medium supplemented with 5% defibrinated sheep blood: Prepare plates from the partially completed medium (see **PROCEDURE** - **Reagent Preparation**) and supplement with 5% sheep blood after cooling to 48 to 50° C. It is recommended to test the supplemented medium according to NCCLS Standard M22-A2.[11] Incubate strains for 20 to 24 hours in an aerobic atmosphere enriched with carbon dioxide.

Strains	Results
Streptococcus pyogenes ATCC 19615	Growth; beta hemolysis
Streptococcus pneumoniae ATCC 6305	Growth; alpha hemolysis
Staphylococcus aureus ATCC 25923	Growth; may or may not be beta-hemolytic
Uninoculated	Red (blood color)

PROCEDURE

Materials Provided

BD Tryptic Soy Agar (partially completed bottled media). See **AVAILABILITY** for fill volumes and package sizes.

STERILE

Materials Not Provided

Autoclave (set at 100 +/- 2° C), steam cooker, or hot plate; waterbath (48-50°C); defibrinated blood (for preparation of blood plates only); sterile glassware and sterile plastic Petri dishes or tubes.
Ancillary reagents and laboratory equipment as required.

Reagent Preparation

Liquefy **BD Tryptic Soy Agar** (Bottled Media) by heating in an autoclave or steam cooker. Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. **Slightly loosen the cap before heating to allow pressure exchange.**

Warning: it is not recommended to use microwave ovens for liquefaction of the medium. Do not place media bottles with metal closures into a microwave oven.

When using an autoclave, set the temperature to not more than 100 +/- 2° C as excessive heating may deteriorate the ingredients, possibly leading to unsatisfactory microbiological

performance. When using a hot plate and/or a waterbath, boil sufficiently long to dissolve the whole medium. The time needed for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device before use, its wattage, size, and the volume and temperature of the medium in the container. It is recommended to test and record the time needed for liquefaction after the first use.
After complete liquefaction, remove the container from the heating device and place into a waterbath set at 48 to 50° C.
Warning: Wear heat-protective gloves! Do not place the hot container into an icebath or in cold water to accelerate cooling as this might cause cracks in the glass. Risk of severe scald!
Leave the container in the waterbath sufficiently long to allow cooling of the complete medium to the set temperature.
If defibrinated blood (e.g., 5% sheep blood) or other heat-sensitive supplements (which must be brought to room temperature before use) are added, the temperature of the medium must not be higher than 50° C. Apply aseptic conditions during addition of the supplement and during pouring of the plates! Use sterile dishes or tubes. Mix the medium gently after addition of the supplement, but avoid formation of foam and bubbles.
Pour the medium into the dishes if surface inoculation is desired. For a normal 90 to 100 mm dish, 19 to 21 ml is an appropriate volume. Allow the completed medium to solidify, invert the plates and allow to dry at room temperature for an adequate time (for complete solidification, store overnight at 18 to 23° C). Wrap in fresh plastic bags and store at 2 to 8° C. Prepared plates of this medium may be used for 5 to 7 days.

If the pour plate method shall be applied, add the material to be tested or its dilution into the empty dish, overlay with the medium, rotate the dish gently to mix, and allow to solidify completely.

For the preparation of slants in tubes, add the appropriate amount of liquefied medium, cooled to 48 to 50° C, to the tubes and allow to solidify in the desired slanted position. Tubes, when closed tightly with screw caps, can be used for 2 to 3 weeks when stored in the dark at room temperature or at 2 to 8° C.
Before use, the medium surfacs must not be excessively wet.

Liquefy the bottled medium only once. Do not allow any leftovers to solidify and liquefy thereafter for a second time as repeated heating will damage the ingredients of the medium, leading to unsatisfactory microbiological performance.

Specimen Types
The unsupplemented medium, poured into Petri dishes is used in a variety of procedures, e.g., for pharmaceutical tests. In clinical microbiology, it must not be used as an isolation medium for pathogens from clinical specimens, unless supplemented with blood (e.g., 5% sheep blood). If supplemented with 5% blood, the plated medium can be universally used for primary isolation of pathogens from all types of specimens. Consult the references for specimen collection and processing.[4,10] The tubed, slanted medium must not be used directly with clinical specimens but only for the growth and maintenance of bacterial cultures.

Test Procedure
Before use, agar surfaces of the completed medium (in Petri dishes or in tubes) should be smooth and moist, but without excessive moisture because this could cause confluent growth. Consult the appropriate references for specific methods.[2,6,7]
Plates supplemented with blood: 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. Incubate the plates or tubes under the conditions chosen.

- If used for clinical specimens, incubate for 18 to 48 hours (or longer if necessary) at 35 +/- 2° C or as appropriate for the organisms.

- If used for hygiene monitoring, incubate at 30 to 35° C, for up to 5 days.

If used for pharmaceutical materials, consult the references.[1,2]

The slanted medium in tubes is used for the cultivation and maintenance of bacterial cultures. Streak the strain directly of after suspension in sterile water or saline onto the whole slanted surface. Incubate as appropriate for the isolate. During incubation, caps may be slightly loosened to allow venting. After incubation and during storage, close completely.

Results

After incubation, plates may show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. In addition, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

- Pour plate method: Consult the appropriate references.[1,2,6,7]

If inoculated with an appropriate inoculum, slants will show growth on the whole surface. Tubes may be stored refrigerated for several weeks without loss of viability of the culture. The survival time depends on the individual strains.

The number and types of organisms growing on the completed media prepared from **BD Tryptic Soy Agar** (Bottled Media) is very large. Therefore, no specific details on their appearance can be given here. Consult the references.[4,9,10]
From the isolates obtained on the media, appropriate subcultures must be set up to allow a further differentiation and identification.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Tryptic Soy Agar is used in a variety of industrial microbiology procedures, e.g., in microbial limit testing and in water and food microbiology.[1-3,6,7]
Unsupplemented Tryptic Soy Agar is used for cultivation of many less fastidious bacteria, e.g., *Enterobacteriaceae,* nonfermenting Gram negative rods (*Pseudomonas* and many others), enterococci, staphylococci, sporeforming bacteria (*Bacillus* and related genera), and other organisms with similar growth requirements. The medium is not suitable for the isolation and cultivation of very fastidious bacteria, such as *Neisseria* or *Haemophilus* species, or other organisms with special nutritional requirements, and it is not an optimal medium for the isolation of fastidious strict anaerobes. Therefore, the use in clinical microbiology is limited to special tests, e.g., the differentiation of *Haemophilus* with X, V, and XV factors strips.[8]

Tryptic Soy Agar supplemented with blood (e.g., 5% sheep blood), is frequently used as a primary isolation medium for aerobic bacteria in clinical microbiology. For details, consult the references.[3-5,8-10]

Unsupplemented Tryptic Soy Agar does not contain compounds that actively neutralize disinfectants or preservatives. If materials containing such compounds or surfaces that have been previously disinfected shall be monitored, it is recommended to use Tryptic Soy Agar with Lecithin and Polysorbate or to supplement the medium appropriately.

REFERENCES

1. U.S. Pharmacopeial Convention, Inc. 1994. The U.S. Pharmacopeia 24/The national formulary 19-1999. U.S. Pharmacopeial Convention, Inc., Rockville, Md.
2. Council of Europe, 2002. European Pharmacopoeia, 4th edition, and Supplement 4.2. European Pharmacopoeia Secretariat. Strasbourg/France.
3. MacFaddin, J.F. 1985. Media for isolation-cultivation- identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore.

4. Baron, E.J., L.R. Peterson, and S.M. Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis.
5. Nash, P., and M.M. Krenz. 1991. Culture media. *In:* A. Balows, W.J. Hausler, Jr., K.L. Herrmann, H.D. Isenberg, and H.J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
6. Eaton, A.D., L.S. Clesceri, and A.E. Greenberg (ed.). 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
7. Downes, F.P., and K. Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
8. Campos, J.M. 1995. *Haemophilus*. *In:* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
9. Chapin, K.C., and T.-L. Lauderdale. 2003. Reagents, stains, and media: bacteriology. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
10. Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
11. NCCLS M2-A7. 1996. Quality assurance for commercially prepared microbiological culture media - 2nd edition; approved standard. National Committee of Clinical Laboratory Standards (NCCLS), Wayne, PA, USA.

PACKAGING/AVAILABILITY

BD Tryptic Soy Agar (partially completed bottled media)

Cat. No. 256665	cpu 10	100 ml fill volume; in 250 ml sirup bottle
Cat. No. 257105	cpu 12	250 ml fill volume; in 500 ml flat bottle
Cat. No. 257106	cpu 10	500 ml fill volume; in 500 ml sirup bottle
Cat. No. 257240	cpu 4	400 ml fill volume; in 500 ml laboratory bottle

FURTHER INFORMATION

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