BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) with Ampicillin

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) with Ampicillin is used for the isolation of *Aeromonas* spp.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Streak inoculate 10 µL (0.01 mL) from a 18 – 24 h culture of **Trypticase** Soy Broth diluted to yield 10^3 – 10^4 CFU/mL.
   b. Incubate at 35 ± 2 °C under appropriate atmospheric conditions.
   c. Include plates of a previously tested lot of TSA with 5% Sheep Blood as controls.
2. Examine plates after 18 – 48 h for growth and selectivity.
3. Expected Results

Organisms	ATCC™	Recovery
**Aeromonas hydrophila*	7965	Fair to heavy growth
Pseudomonas aeruginosa	10145	Fair to heavy growth
**Staphylococcus aureus*	25923	Inhibition (partial to complete)
Streptococcus pyogenes	19615	Inhibition (partial to complete)

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Note the firmness of plates during the inoculation procedure.
4. Incubate uninoculated representative plates at 33 – 37 °C and for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Trypticase Soy Agar with 5% Sheep Blood with Ampicillin is used for the isolation of *Aeromonas* spp.

V SUMMARY AND EXPLANATION

Aeromonas spp. are widely distributed in nature, primarily occuring in natural fresh and salt waters, where they infect animals, amphibians, reptiles and fish.[1] They have been recovered from a variety of specimens; gastroenteritis, however, is the most common infection associated with this organism.[2]

They grow on standard media used for the isolation of gram-negative bacilli, e.g., blood agar and MacConkey Agar, but some strains are reported to be inhibited on selective media used to isolate *Salmonella*, *Shigella* and *Campylobacter*.[2] Isolation is facilitated, however, on 5% sheep blood agar containing ampicillin as a selective agent.[1]

VI PRINCIPLES OF THE PROCEDURE

The combination of casein and soy peptones in the **Trypticase** Soy Agar base renders the medium highly nutritious by supplying organic nitrogen. The sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood supplies nutrients and, simultaneously, it allows detection of hemolytic reactions.

Ampicillin is active against gram-negative as well as gram-positive organisms, although *Aeromonas* spp. are resistant to it.

VII REAGENTS

Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) with Ampicillin

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	14.5 g	Growth Factors	1.5 g
Papaic Digest of Soybean Meal	5.0 g	Ampicillin	0.01 g
Sodium Chloride	5.0 g	Sheep Blood, defibrinated	5%
Agar	14.0 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[3-6] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8 °C 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.

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.[2,7-8]

Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Trypticase Soy Agar with 5% Sheep Blood (TSA II) with Ampicillin

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface and streaking the remainder of the plate to obtain isolated colonies. Material not being cultured from swabs should be streaked onto the medium with a sterilized inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

Incubate the plates in an inverted position (agar side up) at 35 ± 2 °C in an aerobic atmosphere for 18 – 48 h.

User Quality Control: See "Quality Contol Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Colonies of *Aeromonas* may be round, raised, with an entire edge, smooth surface and may be surrounded by a zone of beta hemolysis. Gram staining, biochemical tests and other procedures should be performed to confirm findings.

XI LIMITATIONS OF THE PROCEDURE

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Specimens cultured on selective media should, therefore, also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for identification purposes. Consult appropriate texts for detailed information and recommended procedures.[1,2,9]

Ampicillin-susceptible species of *Aeromonas* have been reported.[10]

XII AVAILABILITY

Cat. No.	Description
297346	**BBL™ Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) with Ampicillin, Pkg. of 20 plates

XIII REFERENCES

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Technical Information: In the United States contact BD Technical Services and Support at 800-638-8663 or www.bd.com/ds.

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