

TECHNICAL DATA SHEET

MUELLER HINTON AGAR WITH 5% SHEEP BLOOD PLATES

P90/MHA (SB) - 20

INTENDED USE

Mueller Hinton Agar with 5% Sheep Blood is recommended for antimicrobial disc diffusion susceptibility testing of *Streptococcus pneumoniae* with selected agents, i.e., Chloramphenicol, erythromycin, ofloxacin, tetracycline and vancomycin, in addition to oxacillin screening for susceptibility to penicillin, as standardized by the Clinical and Laboratory Standards Institute (CLSI).

TYPES OF SAMPLE

- Clinical

PRINCIPLE

Acid hydrolysate (digest) of casein and beef extract supplies amino acids and other nitrogenous substances, minerals, vitamins, carbon, and other nutrients to support the growth of microorganisms. Starch acts as a protective colloid against toxic substances that may be present in the medium. Hydrolysis of the starch during autoclaving provides a small amount of dextrose, which is a source of energy. Agar is a solidifying agent.

The Bauer-Kirby procedure is based on the diffusion through an agar gel of antimicrobial substances which are impregnated on paper discs. In contrast to earlier methods which used discs of high and low antimicrobial concentrations, and which used the presence or absence of inhibition zones for their interpretation, this method employs discs with a single concentration of antimicrobial agent and zone diameters are correlated with minimal inhibitory concentrations. The CLSI has written a performance standard for the Bauer Kirby procedure and this document should be consulted for additional details. The procedure is recommended for testing rapidly growing aerobic or facultative anaerobic bacterial pathogens, such as staphylococci, members of the Enterobacteriaceae, aerobic gram-negative rods, e.g., *Pseudomonas* spp. and *Acinetobacter* spp., enterococci, and *Vibrio cholerae*. The procedure is modified for testing fastidious species, i.e., *H. influenzae*, *N. gonorrhoeae* and *S. pneumoniae* and other streptococci.

In the test procedure, a standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specified amounts of antibiotics or other antimicrobial agents are then placed on the surface of the medium, the plate is incubated and zones of inhibition around each disc are measured. The determination as to whether the organism is susceptible, intermediate, or resistant to an agent

is made by comparing zone sizes obtained to those in the CLSI Document. Various factors have been identified as influencing disc diffusion susceptibility tests. These include the medium, excess surface moisture on the medium, agar depth, disc potency, Inoculum concentration, pH and β -lactamase production by test organisms.

INGREDIENTS

Approximate Formula Per Liter	
Acid Digest of Casein	17.5 g
Agar	17.0 g
Beef Extract Powder	2.0 g
Starch	1.5 g
Sheep Blood	5 %

- Final pH 7.3 ± 0.2 at 25°C

PHYSICAL PARAMETERS OF PREPARED PLATES

- Appearance: 90 mm petri plates with a smooth surface and absence of any particles, cracks, or bubbles.
- Colour: Cherry Red Color
- Clarity: Slightly Opalescent
- Volume: 20-22 ml

STERILITY CHECK

Sterility of the plates is checked by incubating the plates at $35 \pm 2^{\circ}\text{C}$ for 3 days.

MICROBIAL PERFORMANCE DATA

Culture characteristics observed after inoculating 50-100 CFU and incubate at $35 - 37^{\circ}\text{C}$ for 24-48 hours. Examine plates after 24 to 48 h for amount of growth, colony size and colour.

Test Strains	ATCC No.	Growth
<i>Escherichia coli</i>	ATCC 25922	Good
<i>Enterococcus faecalis</i>	ATCC 33186	Good

<i>Pseudomonas aeruginosa</i>	ATCC 27853	Good
<i>Staphylococcus aureus</i>	ATCC 25923	Good

LIMITATIONS & COMPLEMENTARY TESTS

- It is a non-selective, non-differential medium. This means that almost all organisms plated on here will grow.
- The zone will be smaller if heavy inoculum is tested on the plates.

PRECAUTIONS

- For in-Vitro diagnostic use. Read the label details and storage before opening the pack.
- Wear protective gloves / protective clothing / eye protection / face protection.
- Follow good microbiological lab practices while handling specimens and culture.

PACK SIZE AND PACKAGING

20 plates per kit packed with gamma irradiated packing material.

STORAGE & SHELF LIFE

- Store at 10 -15 °C.
- Use before the expiry date mentioned on the label.
- Product is temperature sensitive; protect from direct sunlight, excessive heat, moisture, and freezing.

DISPOSAL

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Materials that have come in contact with infectious / clinical samples must be decontaminated and disposed of in accordance with current laboratory techniques and regulations.

REFERENCE

- Åhman J, Matuschek E, Kahlmeter G. Evaluation of ten brands of pre-poured Mueller–Hinton agar plates for EUCAST disc diffusion testing. Clin Microbiol Infect. 2022 Nov;28(11):1499.e1-1499.e5.