OXALIS Diagnostics Pvt. Ltd.

Technical Data

Mueller Hinton Agar. 100 g / 500 g

Mueller Hinton Agar is used for antimicrobial disc diffusion susceptibility testing of common, rapidly growing bacteria.

Product Presentation:

Cat No.	Product description	Pack Size
11130030100	Mueller Hinton Agar	100 Gram
11130030500	Mueller Hinton Agar	500 Gram

Principle and interpritation

Mueller Hinton Agar was originally developed for the cultivation of *Neisseria.* These organisms are now isolated on selective media. Since clinical laboratories were using a wide variety of procedures for determining the susceptibility of bacteria to antibiotic and chemotherapeutic agents, Bauer, Kirby and others developed a standardized procedure in which Mueller Hinton Agar was selected as the test medium. Subsequently, international collaborative study confirmed the value of Mueller Hinton Agar for this purpose due to its relatively good reproducibility, the simplicity of its formula, and the wealth of experimental data that had been accumulated using this medium. Mueller Hinton Agar complies with the requirements of World Health Organization and is specified in the FDA's Bacteriological Analytical Manual for food testing. For additional details refer to The National Committee for Clinical Laboratory Standards (NCCLS) which contains the performance standard for the Bauer-Kirby procedure. This 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* species and *Acinetobacter* species, Enterococci and *Vibrio cholerae*. The procedure is modified for testing fastidious species; i.e. *H. influenza, N. gonorrhoeae, S. pneumoniae* and other *Streptococci*. The NCCLS Document M2, Performance for Antimicrobial Disc Susceptibility Tests, recommends Mueller Hinton Agar supplemented with 5% defibrinated sheep blood for fastidious organisms.

Casein acid hydrolysate and beef extract supply 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 starch during autoclaving provides a small amount of dextrose, which is a source of energy.

Composition

Ingredients	Grams / Litre
Casein Acid Hydrolysate	17.50
Beef Extract Powder	2.00
Starch	1.50
Agar	17.00

Final pH (at 25°C) 7.3±0.1

*Formula adjusted, standardized to suit performance parameters

Type of specimen

Clinical samples

Specimen Collection and Handling

Ensure that all samples are properly labeled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

FACTORY & OFFICE
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Directions

- ✓ Suspend 38.00 g of powder in 1000 mL distilled water.
- ✓ Mix thoroughly.
- ✓ Boil to dissolve the medium completely.
- ✓ Sterilize by autoclaving 121°C for 15 minutes or as per validated cycle.
- ✓ Mix well before pouring.

Storage and Stability

- ✓ Store Dehydrated culture media in cool, dry place at 10°C-30°C away from direct light.
- ✓ Store prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Quality Control

Dehydrated Appearance: Cream to yellow coloured, homogenous, free flowing powder.
Prepared Appearance: Light yellow to amber coloured, clear to very slightly opalescent gel forms in petriplates .
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP and growth is observed after an incubation at 30°C-35°C for 18 to 24 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 18 hours.

Cultural Response :

Organism	Type Culture	Growth	Incubation Temperature	Incubation Period
Staphylococcus aureus	ATCC 25923	Good	30°C -35°C	18 Hours
Escherichia coli	ATCC 25922	Good	30°C -35°C	18 Hours
Pseudomonas aeruginosa	ATCC 27853	Good	30°C -35°C	18 Hours
Enterococcus faecalis	ATCC 29212	Good	30°C -35°C	18 Hours

Interpretation of Results

- ✓ A confluent “lawn” of growth should be obtained. Too light inoculum gives isolated colonies and the test should be repeated. Measure the diameter of the zones of complete inhibition, including the diameter of the disc, to the nearest whole millimeter, using calipers, a ruler, or a template prepared for this purpose. The measuring device is held on the back of the inverted plate over a black, nonreflecting background, and illuminated from above. The endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies, which can be detected with difficulty near the edge of the obvious zone of inhibition. The zone diameters measured around the discs should be compared with those in the NCCLS Document M100 (M2).
- ✓ *S. aureus* when tested with oxacillin discs is an exception, as are Enterococci when tested with vancomycin. In these cases, transmitted light should be used to detect a haze of growth around the disc, which is shown, by “occult resistant” MRSA strains or vancomycin-resistant Enterococci. With *Proteus* species, if the zone of inhibition is distinct enough to measure, disregard any swarming inside the zone. With trimethoprim and sulphonamides, antagonistics in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.
- ✓ The results obtained with specific organisms may be reported as resistant intermediate or susceptible.

Warranty

- ✓ This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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Disposal

Disposal of infectious material and material that comes in to contact with clinical sample must be decontaminated and dispose of by autoclaving or incineration or established laboratory procedures.
User must be ensure safe disposal of used or unusable preperation of the products.

Reference

1. Mueller and Hinton, 1941, Proc. Soc. Exp. Bio. And Med; 48:330.
2. Bauer et al., 1966, Am. J. Clin. Patho., 45:493.
3. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8th Ed; Rev. A, AOAC, International, Gaithersburg, Md.

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