Mueller Hinton Agar

Intended Use
Mueller Hinton Agar is used for antimicrobial disk diffusion susceptibility testing of common, rapidly growing bacteria (contains low levels of sulfonamide and trimethoprim antagonists, thymine and thymidine and controlled levels of calcium and magnesium ions).

Summary
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.

Principle
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.

Formula*

Ingredients	**g/L**
Casein Acid Hydrolysate	17.5
Beef Extract Powder	2.0
Starch	1.5
Agar	17.0
Final pH (at 25°C)	7.3 ± 0.1

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the 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.

Specimen Collection and Handling
Ensure that all samples are properly labelled. 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.

Directions
1. Suspend 38.00 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. Mix well before pouring.

Quality Control

Dehydrated Appearance: Cream to yellow coloured, homogeneous, free flowing powder
Prepared Appearance: Light yellow to amber coloured, clear to very slightly opalescent gel forms in petridishes.
Cultural Response: Cultural characteristics observed after an incubation of 18-24 hours at 30-35°C.

Organism (ATCC)	**Growth**
Escherichia coli (25922)	Good
Staphylococcus aureus subsp. *aureus* (25923)	Good
Enterococcus faecalis (29212)	Good
Pseudomonas aeruginosa Strain Boston 41501 (27853)	Good

Note: For Good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.

Interpretation of Results

1. 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).
2. *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.
3. The results obtained with specific organisms may be reported as resistant intermediate or susceptible.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions / Limitations

1. Unsupplemented Mueller Hinton Agar, although adequate for susceptibility testing of rapidly growing aerobic pathogens, is not adequate for more fastidious organisms such as *S. pneumoniae.*
2. Numerous factors can affect the result: inoculum size, rate of growth, media formulation, pH, length of incubation, disc content, drug diffusion rates, and measurement of endpoints. Hence, strict adherence to protocol is required to ensure reliable results.
3. Mueller Hinton Agar deeper than 4 mm may cause false resistant results, and agar less than 4 mm deep may be associated with a false-susceptibility report.
4. pH outside the range of 7.3 ± 0.2 may adversely affect susceptibility test results. If the pH is too low, aminoglycosides and macrolides will appear to lose potency; others may appear to have excessive activity. The opposite effects are possible if the pH is too high.
5. The following technical and human errors may occur which compromise on reliability and accuracy and must be avoided: -

i) Improper disc storage.
ii) Inoculum not properly adjusted (too light or too heavy).
iii) Incubation temperature deviating from 30°C-35°C.
iv) Use of an increased CO_2 atmosphere. Reading plates before or after the full 16-18 hours of incubation. Transcribing errors.
v) Reading error while measuring zone diameter.

vi) Deterioration of the McFarland Turbidity Standard.

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.

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.
4. National Committee for Clinical Laboratory Standards. 2000. Approved Standard: M2-A7. Performance Standards for Antimicrobial Disk Susceptibility Tests, 7th edition. NCCLS, Wayne, P.A
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201130650100	Dehydrated Culture Media	100 g
201130650500	Dehydrated Culture Media	500 g
201130652500	Dehydrated Culture Media	2.5 k
203130830250	Bottle Media	6 x 250 mL
203130830100	Bottle Media	100 mL
205130960100	Ready Prepared Plate	(90 mm) 100 Plates
205130960020	Ready Prepared Plate	(90mm) 20 Plates
205130970020	Ready Prepared Plate	(150 mm) 20 Plates