



PRODUCT SPECIFICATION SHEET

Mueller Hinton Agar (BS003)

Intended Use

Mueller Hinton Agar (BS003) is used for cultivation of *Neisseria* and for determination of susceptibility of microorganisms to antimicrobial agents.

Product Summary and Explanation

Mueller Hinton Agar was originally developed for the cultivation of pathogenic *Neisseria*.⁽⁶⁾ However, these organisms are now commonly isolated on selective media. In the early 1960s clinical microbiology laboratories used 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.^(1,2) A subsequent international collaborative study confirmed the value of Mueller Hinton Agar for this purpose because of the relatively good reproducibility of the medium, the simplicity of its formula, and the wealth of experimental data that had been accumulated using this medium.⁽⁷⁾ The CLSI has written a performance standard for the Bauer-Kirby procedure and this document should be consulted for additional details.⁽⁴⁾ The procedure is recommended for testing rapidly growing aerobic or facultatively 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.

Mueller Hinton Agar is manufactured to contain low levels of thymine and thymidine^(8,9) and controlled levels of calcium and magnesium.⁽¹⁰⁻¹²⁾ Thymine and thymidine levels of raw materials are determined using the disc diffusion procedure with trimethoprim-sulfamethoxazole (COT) discs and *Enterococcus faecalis* ATCC 33186 and/or 29212. Calcium and magnesium levels are controlled by testing raw materials and supplementing with sources of calcium and/or magnesium as required to produce correct zone diameters with aminoglycoside antibiotics and *Pseudomonas aeruginosa* ATCC 27853.⁽¹³⁾

Mueller Hinton agar complies with requirements of the World Health Organization⁽¹⁴⁾ and is specified in the FDA *Bacteriological Analytical Manual* for food testing.¹⁵ 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*. The CLSI Document M2, *Performance Standards for Antimicrobial Disk Susceptibility Tests*, recommends Mueller Hinton agar supplemented with 5% defibrinated sheep blood. Details of quality control procedures and interpretive criteria for use with *S. pneumoniae* and other *Streptococcus* spp. are contained in supplemental tables.⁽⁵⁾ These documents should be consulted for additional details.^(4,5)

Principles of the Procedure

Acid hydrolysate of casein and beef extract supply amino acids and other nitrogenous substances, minerals, vitamins, carbon and other nutrients to support the growth of microorganisms. Starch is added to absorb any toxic metabolites produced and acts as a protective colloid against toxic substances. Hydrolysis of the starch during autoclaving provides a small amount of dextrose, which is a source of energy. Agar is the solidifying agent. A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim.

Formula / Liter

Ingredients	Gms / Litre
Beef, infusion	300.00
Casein acid hydrolysate	17.50
Starch	1.50
Agar	17.00
Final pH: 7.3 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	



PRODUCT SPECIFICATION SHEET

Precautions

1. For Laboratory Use only.

Directions

1. Boil the bottle media at 100°C for 10 minutes in boiling Water bath.
2. Heat if necessary, to dissolve the medium completely.
3. Mix well and pour into sterile petri plates.
4. DO NOT OVERHEAT.
5. The surface of the medium should be dry when inoculated.

Quality Control Specifications

Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in the petri plates
Reaction of 3.8% Solution	pH 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.7% Agar gel
Quantity	100ml

Sterility Check: Passes release criteria.

Expected Cultural Response: Cultural response on Mueller Hinton Agar at 35 - 37°C for 18 - 24 hours. of incubation.

Sr No.	Organisms	Inoculum (CFU)	Growth	Recovery
1.	<i>Escherichia coli</i> ATCC 25922	50-100	Luxuriant	≥70%
2.	<i>Haemophilus influenzae</i> ATCC 49247	50-100	Good-luxuriant (on Mueller Hinton Chocolate Agar)	≥70%
3.	<i>Neisseria gonorrhoeae</i> ATCC 49226	50-100	Luxuriant	≥70%
4.	<i>Enterococcus faecalis</i> ATCC 29212	50-100	Luxuriant	≥70%
5.	<i>Streptococcus pneumonia</i> ATCC 6305	50-100	Luxuriant (on Mueller Hinton Blood Agar)	≥70%
6.	<i>Escherichia coli</i> ATCC 35218	50-100	Luxuriant	≥70%
7.	<i>Staphylococcus aureus</i> ATCC 43300	50-100	Luxuriant	≥70%
8.	<i>Pseudomonas Aeruginosa</i> ATCC 77853	50-100	Luxuriant	≥70%
9.	<i>Staphylococcus aureus</i> ATCC 25923	50-100	Luxuriant	≥70%

The organisms listed are the minimum that should be used for quality control testing.



Pseudomonas Aeruginosa ATCC 27853 on Mueller Hinton Agar



Streptococcus pneumoniae ATCC 49619 on
Mueller Hinton Agar
with 5% sheep blood

Test Procedure



PRODUCT SPECIFICATION SHEET

For a complete discussion on antimicrobial susceptibility testing, refer to procedures outlined in appropriate references.^(19,20) Protocols developed by the CLSI and used by manufacturers to evaluate the performance of Mueller Hinton Agar in comparison to a reference medium are published in CLSI document M6-A.⁽²¹⁾

Results

Zone diameters measured around discs should be compared with those in the CLSI Document M100 (M2). Results obtained with specific organisms may then be reported as resistant, intermediate or susceptible.

Storage

Store the sealed bottle containing the medium at 10-25°C. Once opened then not repackable. Place bottle in a low humidity environment. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the pack.

Limitations of the Procedure

1. Numerous factors can affect results: inoculum size, rate of growth, medium formulation and pH. Strict adherence to protocol is required to ensure reliable results.⁽²²⁾
2. When Mueller Hinton agar is supplemented with blood, the zone of inhibition for oxacillin and methicillin may be 2-3 mm smaller than those obtained with unsupplemented agar.⁽²³⁾ Conversely, sheep blood may markedly increase the zone diameters of some cephalosporins when they are tested against enterococci.⁽²⁴⁾ Sheep blood may cause indistinct zones or a film of growth within the zones of inhibition around sulfonamide and trimethoprim discs.⁽²³⁾
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.⁽²³⁾
4. A pH outside the range of 7.3 ± 0.1 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.⁽²³⁾ The opposite effects are possible if the pH is too high.⁽²³⁾

Packaging

Product Name: Mueller Hinton Agar (BS003)

Product Code : BS003

Available Pack sizes : 100ml

References

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PRODUCT SPECIFICATION SHEET

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Further Information

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