Mueller Hinton Agar

Cat. 1058

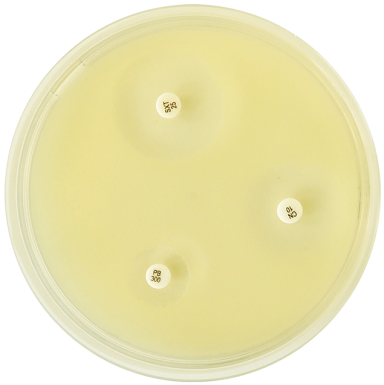
For sensitivity tests on antibiotics and sulfamides, and for the primary isolation of Neisseria and other pathogens from clinical samples

Practical information

Aplications	Categories
Antimicrobial susceptibility tests	General use

Industry: Clinical / Antimicrobial susceptibility testing

CE
IVD



Principles and uses

Mueller Hinton Agar, together with the Mueller Hinton Broth (Cat. 1214), is used to test the antimicrobial susceptibility of rapidly growing aerobic organisms from clinical samples and has become the standard medium for the Bauer Kirby method in accordance to the standards of the Clinical and Laboratory Standards Institute (CLSI) and European Committee and Antimicrobial Susceptibility Testing (EUCAST).

The main objective of in vitro antimicrobial susceptibility testing is to provide a guide for the therapeutic management of infectious diseases through the sensitivity or resistance of facultative aerobic and anaerobic pathogenic bacteria to different antimicrobial compounds.

Because it is impossible to predict the susceptibility of a bacterium responsible for a specific infection to antimicrobials, the antibiotic susceptibility tests carried out in the microbiological laboratory become an essential instrument for the therapeutic management of patients.

In the medium, beef infusion and acid casein peptone (H) provide nitrogen, vitamins, minerals and amino acids essential for growth. The starch absorbs any toxic metabolite produced by microbial growth and the bacteriological agar is the solidifying agent.

Formula in g/L

Acid casein peptone (H)	17,5	Bacteriological agar	17
Beef infusion	2	Starch	1,5

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 38 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121 ºC for 15 minutes. Cool to 45 or 50 ºC and add defibrinated blood if desired. The blood mixture should be chocolated by heating to 80 ºC for 10 minutes if Neisseria development is desired. DO NOT OVERHEAT. To remelt the cold medium, heat as briefly as possible.

Instructions for use

»For clinical diagnosis through antibiotic susceptibility testing, the type of sample is bacteria isolated from any type of clinical sample (urine, blood, rectal, sputum etc.):
- Dispense medium into sterile Petri dishes to give a level depth of 4±0,5 mm (approximately 25 mL in a 90 mm circular plate, 31 mL in a 100 mm circular plate, 71 mL in a 150 mm circular plate, 40 mL in a 100 mm square plate).
- Adjust the density of the organism suspension to McFarland 0,5 by adding saline or more bacteria. A denser inoculum will result in reduced zones of inhibition and a decreased inoculum will have the opposite effect.
- The suspension should optimally be used within 15 min and always within 60 min of preparation.
- Dip a sterile cotton swab into the suspension.

- To avoid over-inoculation of gram negative bacteria, remove excess fluid by pressing and turning the swab against the inside of the tube.
- For gram positive bacteria, do not press or turn the swab against the inside of the tube.
- Apply disks within 15 min of inoculation.
- Incubate at a temperature of 37±1 ºC for 24 hours.
- Zone edges should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye.
- Read MH plates from the back against a dark background illuminated with reflected light.
- Interpret the inhibition zones as susceptible (S), intermediate (I), or resistant (R) according to the categories established by the NCCLS
- In case of distinct colonies within zones, check for purity and repeat the test if necessary.
- For Proteus spp., ignore swarming and read inhibition of growth.
- In case of double zones, read the inner zone.

Cultivation of Neisseria specimens from clinical samples:
- Incubate in plates at a temperature of 37±1 ºC in a CO_2 atmosphere for 18-24 hours.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
Slightly opalescent	Fine powder	Cream	w/o blood: Amber opalescent. with blood: Red	7,4±0,2

Microbiological test

Disk diffusion sensitivity testing. Incubation conditions: (37±1 ºC / 24 h).

Diammeter halo in mm.

Microorganisms	Gentamycin 10 µg	Ampicillin 10 µg	Tetracycline 30 µg	Polymyxin B 300 µg	SXT: Trimethoprim (1,25µg)+Sulfamethoxazole (23,75 µg)
Escherichia coli ATCC 25922 CLSI	19-26	15-22	18-25	13-19	23-29
Escherichia coli ATCC 25922 EUCAST	19-26	15-22		13-19	23-29
Staphylococcus aureus ATCC 25923 CLSI	19-27	27-35	24-30		24-32
Staphylococcus aureus ATCC 25923 EUCAST					
Pseudomonas aeruginosa ATCC 27853 CLSI	17-23			14-18	
Pseudomonas aeruginosa ATCC 27853 EUCAST	17-23				
Enterococcus faecalis ATCC 29212 CLSI					
Enterococcus faecalis ATCC 29212 EUCAST					26-34
Staphylococcus aureus ATCC 29213 CLSI					
Staphylococcus aureus ATCC 29213 EUCAST	19-25		23-31		26-32

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

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