Mueller Hinton Agar II

Cat. 1055

For antibiotic sensitivity tests and for the primary isolation of gonococci, meningococci, and other pathogens from clinical samples

Practical information

Aplications	Categories
Antimicrobial susceptibility tests	General use

Industry: Clinical

CE IVD

Principles and uses

Mueller Hinton Agar II was originally developed for the cultivation and isolation of pathogenic strains of Neisseria. It was found that this medium was useful in identifying sulfonimide-resistant and responsive strains of gonococci. However, Mueller Hinton Agar is now used in antimicrobial disk susceptibility testing.

Bauer and Kirby developed a standardized procedure for determining the susceptibility of bacteria to antibiotic, in which Mueller Hinton Agar was selected as the test medium, because clinical microbiology laboratories in the 60s were using a wide variety of different mediums and procedures. Its performance is specified by CLSI (Clinical and Laboratory Standards Institute), and it is recommended for testing rapidly growing aerobic or facultatively anaerobic bacterial pathogens and fastidious species like H. influenziae, N. gonorrhoeae or S. pneumoniae, when desfibrinated sheep blood is added.

Mueller Hinton Agar II is manufactured to contain low levels of thymine and thymidine, and controlled levels ofcalcium and magnesium. The use of a medium with suitable growth characteristics is essential to test susceptibility of microorganisms to antibiotics. It is also recommended for testing most commonly encountered aerobic and facultative anaerobic bacteria.

Beef infusion and acid Casein peptone (H) provide nitrogen, vitamins, minerals and amino acids essential for growth. The starch absorbs any toxic metabolites produced. 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

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 45-50 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, the type of sample is bacteria isolated from urine:
- Inoculate according to the Bauer-Kirby method.
- Incubate in aerobic conditions at 35±2 ºC for 24-48 hours.
- Reading and interpretation of the results.

For sensitivity tests on antibiotics according to EUCAST:
- 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 35±2 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.
- 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.

For cultivating of Neisseria specimens:
- Incubate in plates at a temperature of 35±2 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)
Ligeramente opalescente	Fine powder	Cream	w/o blood: Amber, opalescent. With blood: red	7,4±0,2

Microbiological test

Disk diffusion sensitivity testing. Incubation conditions: (35±2 ºC / 24 h).

Diammeter halo in mm.

Microrganisms	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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