Iso-Sensitest Agar

Cat. 1001

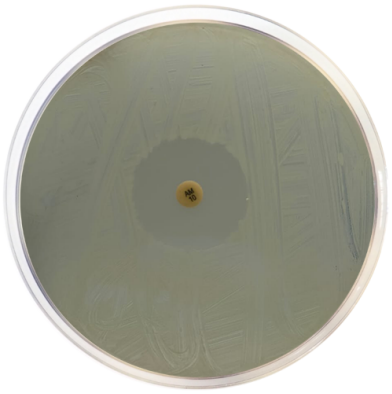
Defined medium designed for antimicrobial susceptibility testing

Practical information

Aplications	Categories
Antibiotic Assay	General use

Industry: Clinical

CE
IVD



Principles and uses

Iso-Sensitest Agar is used as a semi defined medium designed for antimicrobial susceptibility test. This media was developed under a specific formulation to create a controlled formula with stability in mineral content and avoid the undefined components in order to perfectly control and allow reproducible assays. This media allows the growth of the great majority of microorganisms without supplementation.

With this media it is avoided the main objections against Mueller Hinton Formula, both in broth and agar:

- Agar versions showing antagonistic effects towards tetracycline.
- High levels of sulphonamide and trimethoprim antagonists
- Poor growth-supporting ability for streptococci and variable growth rates with gram-positive organisms generally.

Bacteriological agar is the solidifying agent. Dextrose is the fermentable carbohydrate providing carbon and energy. Hydrolyzed peptone and peptone casein provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Magnesium sulphate, calcium gluconate, cobaltous sulphate, cupric sulphate, zinc sulphate, ferrous sulphate, manganous chloride are ions required in a big variation of enzymatic reactions. Menadione is a synthetic chemical compound sometimes used as a nutritional supplement because of its vitamin K. Cyanocobalamin is a source of B12. L-Cysteine hydrochloride is the reducing agent. Biotin, also known as vitamin H or coenzyme R is a water-soluble B-vitamin (vitamin B7). Biotin is necessary for cell growth, the production of fatty acids, and the metabolism of fats and amino acids. Pyridoxine is one of the compounds that can be called vitamin B6. Pantothenate is the source of B5. Nicotamide is the source of B3. Thiamine is the source of vitamin B1. Tryptophan, Adenine, Guanine, xanthine and uracil are the source of aminoacids.

Formula in g/L

Glucose	2	Bacteriological agar	10,7
Biotin	0,0003	Casein peptone	3
Ferrous sulfate	0,001	L-Cysteine hydrochloride	0,02
Manganese (II) chloride	0,002	Sodium chloride	3
Soluble starch	1	Thiamine	0,00004
Zinc sulfate	0,001	L-Tryptophan	0,02
Magnesium glycerophosphate	0,2	Pyridoxine	0,003
Casein hydrolysate	11	Di-sodium hydrogen phosphate	2
Calcium gluconate	0,1	Cyanocobalamin	0,001
Pantothenate	0,003	Nicotinamide	0,003
Adenine	0,01	Guanine	0,01
Xanthine	0,01	Uracil	0,01
Cobaltous Sulphate	0,001	Cupric Sulphate	0,001
Mensadione	0,001		

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

Preparation

Suspend 34,1 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 50 ºC, mix well and dispense into plates.

Instructions for use

For clinical diagnosis, the type of sample is pure cultures isolated from clinical samples.
- 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 the 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.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Amber, slightly opalescent	Beige	7,4±0,2

Microbiological test

Incubation conditions: (35±2 ºC / 18-48 h).

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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