



Hi-Sensitivity Test Agar

M485

Hi-Sensitivity Test Agar is used for antimicrobial susceptibility tests.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	11.000
Peptic digest of animal tissue	3.000
Dextrose	2.000
Sodium chloride	3.000
Starch, soluble	1.000
Disodium phosphate	2.000
Sodium acetate	1.000
Magnesium glycerophosphate	0.200
Calcium gluconate	0.100
Cobaltous sulphate	0.001
Cupric sulphate	0.001
Zinc sulphate	0.001
Ferrous sulphate	0.001
Manganous chloride	0.002
Menadione	0.001
Cyanocobalamin	0.001
L-Cysteine hydrochloride	0.020
L-Tryptophan	0.020
Pyridoxine hydrochloride	0.003
Calcium pantothenate	0.003
Nicotinamide	0.003
Biotin	0.0003
Thiamine hydrochloride	0.00004
Adenine	0.010
Guanine	0.010
Xanthine	0.010
Uracil	0.010
Agar	8.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.4 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The goal of an antimicrobial susceptibility test is to predict through an in vitro assessment the likelihood of successfully treating an infection with a particular antimicrobial agent. There are several continual or novel methods for performing antibacterial susceptibility testing. These include the disk diffusion test, broth microdilution, agar gradient and rapid automated instrument methods (1). Hi-Sensitivity Test Agar, which is used for antimicrobial susceptibility tests, is a semi-defined medium in which the mineral contents have been stabilized to give reproducible results. The thiamine and thymidine content is very low thus making it most suitable for testing antimicrobial activity of sulphonamides. However some mutant strains which are totally dependent on thiamine and thymidine for their growth, will not grow on Hi-Sensitivity Test Agar, due to very low levels of these compounds in the media as they are the naturally occurring antagonist of trimethoprim. These strains should be carefully recognized (2,3,4).

Hi-Sensitivity Test Agar has been so designed to overcome the problems occurring in Mueller-Hinton Media that are as follows (5-11).

1. Mueller Hinton Agar and Mueller Hinton Broth give different MIC values.
2. Mueller Hinton Agar shows antagonistic effect towards tetracycline.
3. High levels of sulphonamide and trimethoprim antagonists.
4. Media prepared using peptone of different manufacturers give poor reproducibility.
5. Poor growth supporting ability for Streptococci and variable growth rates with gram-positive organisms.

Some pathogenic organisms are nutritionally dependent due to their intrinsic demands for special growth factors. Supplemental nutrients can be added to Hi-Sensitivity Test Agar to improve the growth of these organisms (12). The following nutrients can be used.

Nutrient	Microorganism(s)
1. Laked blood (5% v/v)	<i>Neisseria</i> and Streptococci
2. Fildes Peptic Digest of Blood (5% v/v)	<i>Haemophilus</i>
3. Menadione (0.5 mcg/ml) and Thiamine hydrochloride (2 mcg/ml)	Dwarf colonies of <i>Staphylococcus aureus</i> and coliform bacteria.
4. Pyridoxine hydrochloride	Symbiotic Streptococci (1mcg/ml)

Supplementation of Hi-Sensitivity Test Agar with 10% horse blood can be used for susceptibility testing of *Helicobacter pylori* (13).

Casein enzymic hydrolysate, peptic digest of animal tissue, dextrose, and vitamins provides nitrogen, carbon compounds and other essential growth nutrients.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 0.8% Agar gel.

Colour and Clarity of prepared medium

Basal medium : Light amber coloured, clear to slightly opalescent gel. After addition of 5% v/v laked blood : Red to chocolate coloured, opaque gel forms in Petri plates.

Reaction

Reaction of 3.14% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

M485: Cultural characteristics observed with added 5% w/v laked blood, after an incubation at 35 - 37°C for 18 - 24 hours .

Organism	Inoculum (CFU)	Growth	Recovery
<i>Bacillus subtilis</i> ATCC 6633	50-100	good-luxuriant	≥70%
<i>Bacteroides vulgatus</i> ATCC 8482	50-100	good-luxuriant	≥70%
<i>Enterococcus faecalis</i> ATCC 29212	50-100	good-luxuriant	≥70%
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good-luxuriant	≥70%
<i>Staphylococcus aureus</i> ATCC 25923	50-100	good-luxuriant	≥70%

