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MUELLER HINTON AGAR TM 339

INTENDED USE

For cultivation of *Neisseria* & for determination of susceptibility of microorganisms to antibiotics.

COMPOSITION

Ingredients	Gm\Ltr.
Casein acid hydrolysate	17.500
Agar	17.000
Beef, infusion from 300 gms	2.000
Starch	1.500

PRODUCT SUMMARY

Mueller Hinton agar is used for cultivation of *Neisseria* & for determination of susceptibility of microorganisms to antibiotics. It is formulated by Mueller and Hinton for the primary isolation of *Neisseria* species. Bauer and Kirby recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration. It has become the standard medium for antimicrobial susceptibility testing and its performance is in accordance to Clinical and Laboratory Standard Institute (CLSI), formerly NCCLS and complies with requirements of the WHO, FDA and EUCAST.

PRINCIPLE

The medium consist of Beef extract and Casein acid hydrolysate which provides nitrogen, vitamins, carbon, and amino acids. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. The thymine/thymidine content of this medium is minimized (determined by disc diffusion procedure with *Enterococcus faecalis* ATCC 29212 and sulfamethoxazole-trimethoprim antibiotic) and levels of calcium and magnesium are adjusted (determined by *Pseudomonas aeruginosa* ATCC 27853 and aminoglycoside antibiotics) to give consistent zones of inhibition as per specified diameters in the CLSI standards. For fastidious organisms, Mueller–Hinton fastidious (MH-F) agar is prepared by supplementing the basal media with 5% (v/v) mechanically defibrinated horse blood and 20 mg/liter β-NAD. The standardized disc diffusion procedure is based on the ability of an antimicrobial agent impregnated on a paper disc to diffuse through agar gel.

INSTRUCTION FOR USE

1. Dissolve 38.0 gms in 1000ml of distilled water.
2. Gently heat to boiling with gentle swirling and dissolve the medium completely.

3. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes. **DO NOT OVERHEAT**.
4. Cool at 45 - 50°C and pour into sterile Petri plates to achieve an even depth of 4.0 ± 0.5 mm.

Note: For fastidious organisms, Mueller–Hinton fastidious (MH-F) agar is prepared by supplementing the basal media with 5% (v/v) mechanically defibrinated horse blood and 20 mg/liter β-NAD. Prepare the media according to instruction and add supplements after cooling to 42–45°C. Mix well and pour into sterile Petri plates to achieve an even depth of 4.0 ± 0.5 mm.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder: Cream to yellow colour, homogeneous mixture, free flowing powder.

Appearance of prepared medium:
Basal medium: Light to medium amber colour, clear to slightly opalescent gel
After addition of blood: Bright red colour, opaque gel

pH (at 25°C): 7.3 ± 0.2

Antibiotic susceptibility testing

Autoclave and cool the medium to 45-50°C. Pour into sterile Petri plates. The agar must be 4 ± 0.5 mm thick. Let solidify on a cold surface. Dry the plates in an incubator with the covers partially removed in order to avoid the formation of water droplets on the surface of the agar, a phenomenon, which can deteriorate the diffusion qualities of the medium. Prepare, inoculate and dispense antibiotic discs following the procedure described by CLSI. Press the disk containing the antimicrobial on the agar surface. Incubate at 35 ± 1°C for 16-20 hours and then measure the inhibition zone with a compass and scale.

Microbiological parameters

- **Growth promotion test**

Cultural characteristics observed after inoculation (103 CFU/ml) and incubation at 35 ± 1°C for 16 – 20 hours.

Test strains	ATCC	Inoculum (CFU/ml)	Growth
Staphylococcus aureus	25923	10^3	Luxuriant
Escherichia coli	25922	10^3	Luxuriant
Pseudomonas aeruginosa	27853	10^3	Luxuriant
Enterococcus faecalis	29212	10^3	Luxuriant

Antibiotic susceptibility test

Cultural characteristics observed after inoculating 0.5 McFarland culture (1-2 x 108 CFU/ml) by lawn technique, dispensing antibiotic discs and incubation at 35 ± 1°C for 16 - 20 hours. After incubation, inhibition zone diameter measured in mm.

	Escherichia coli (ATCC 25922)	*Staphylococcus aureus*(ATCC 25923)	*Pseudomonas aeruginosa* (ATCC 27853)
Antibiotics	**Std.**	**Std.**	**Std.**
Amoxicillin (10 mcg)	19 - 25	28 - 36	Resistant
Ampicillin (10 mcg)	15 - 22	27 - 35	Resistant
Amikacin (30 mcg)	19 - 26	20 - 26	18 - 26
Azithromycin (15 mcg)	Resistant	21 - 26	Resistant
Colistin (Methane Sulphate) (10 mcg)	11 - 17	Resistant	11 - 17
Cefaclor (30 mcg)	23 - 27	27 - 31	Resistant
Cefoperazone (75 mcg)	28 - 34	24 - 33	23 - 29
Cefazolin (30 mcg)	21 - 27	29 - 35	Resistant
Cefuroxime (30 mcg)	20 - 26	27 - 35	Resistant
Cephalothin (30 mcg)	15 - 21	29 - 37	Resistant
Chloramphenicol (30 mcg)	21 - 27	19 - 26	Resistant
Ciprofloxacin (5 mcg)	30 - 40	22 - 30	25 - 33
Erythromycin (15 mcg)	Resistant	22 - 30	Resistant
Gentamicin (10 mcg)	19 - 26	19 - 27	17 - 23
Kanamycin (30 mcg)	14 - 17	19 - 26	Resistant
Norfloxacin (10 mcg)	28 - 35	17 - 28	22 - 29
Lomefloxacin (10 mcg)	27 - 33	23 - 29	22 - 28

Aztreonam (30 mcg)	28 - 36	Resistant	23 - 29
Cefepime (30 mcg)	31 - 37	23 - 29	25 - 31
Linezolid (30 mcg)	Resistant	25 - 32	Resistant

**Incubation at 35 ± 1°C for 16 - 20 hours, in air with 4-6% CO_2 on MH-F agar.*

STORAGE & STABILITY

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Precautions

1. The agar depth should be 4.0 ± 0.5 mm.
2. The inoculum suspension should optimally be used within 15 and always within 60 min of preparation to ensure the correct number of viable cells.
3. During inoculation, prohibit the use of flooding the plates.
4. Antibiotic disks should be applied within 15 min of inoculation and start incubation within another 15 minutes.
5. Do not to exceed the incubation period of 16–20 h, because prolonged incubation often results in indistinct zone edges or colonies within the inhibition zones, which might result in reporting isolates as falsely resistant.
6. A maximum of six disks can be accommodated on a 90-mm circular plate and 12 on a 150-mm circular plate.

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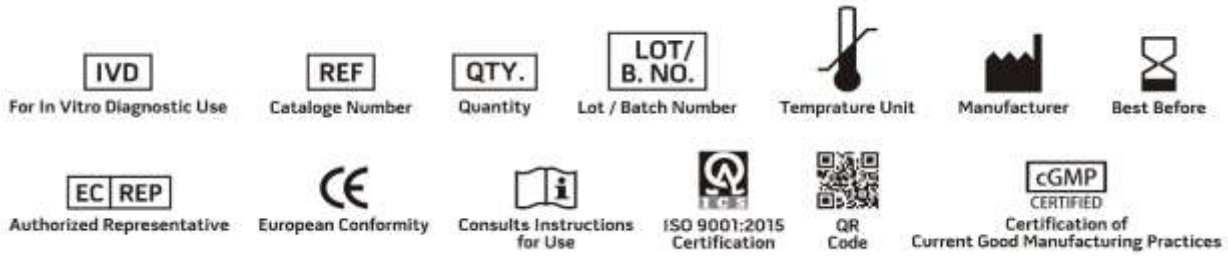

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.