Mueller-Hinton Agar

Art. No. 01-136

CE IVD

Also known as

M-H Agar

Specification

Recommended medium used to perform Antibiotic and Sulphonamide sensitivity testing with pathogenic microorganisms from clinical specimens, according to the Kirby-Bauer and Ericsson methodology.

Formula* in g/L

Peptone	17,50
Beef infusion solids	2,00
Starch	1,50
Agar	17,00

Final pH 7,3 ± 0,1 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 38 g of powder to 1 L of distilled water and let it soak. Bring to the boil to dissolve the medium completely. Sterilize in the autoclave at 121°C for 15 minutes.

Description

Mueller-Hinton Agar was originally designed for the primary isolation of meningococci and gonococci.

With the addition of blood it becomes an optimal medium for the growth of *Neisseria*. It is also more effective if reheated and turned into Chocolate Agar. **It should not be re-melted or reheated once blood has been added to it**.

Technique

For the culture of *Neisseria* the best results are obtained if incubation is carried out in a moist chamber with a CO_2 enriched atmosphere. If an anaerobic jar is not available, this environment can be obtained by placing the plates in a candle jar. The atmosphere inside the container is 5 to 8% CO_2 enriched.

Mueller-Hinton Agar has proved to be one of the most efficient media for use in anti-bacterial susceptibility testing. Without the addition of blood it can be used for sulfonamide sensitivity testing since it is free from most of its antagonists (nucleotides, etc.). If this type of assay is conducted, the zones of inhibition should be examined after 12-18 hours, before overgrowth occurs, since after 24 hours it can interfere with the sulphonamide sensitivity test. Using a small inoculum will help the early formation of zones of inhibition. The inoculum should be 100 to 300-fold smaller than that used in the testing of other antibiotics.

In 1970 the WHO proposed this medium for antibacterial sensitivity testing, and it has been widely used since then. Sensitivity testing can be conducted by a variety of techniques, both on solid and liquid media. The most commonly used method in routine work is that derived from Kirby-Bauer and recommended by the American Association of Clinical Pathologists.

The Kirby-Bauer method is a precise, semi-quantitative testing system. It uses Mueller-Hinton Agar and disks with a high antibiotic concentration. The inoculum is first standardized using a MacFarland standard, then the plate is inoculated with a swab dipped in the standardized suspension, and finally the disks are arranged properly equidistant from each other on the plate and then incubated (see the table).

Some authors suggest that the inoculum should be modified by introducing a double layer of inoculated medium. This system undoubtedly provides sharper and more defined zones of clearing of inhibition. Plates are incubated at 37°C overnight and then the zones of inhibition are measured. Results are reported in terms of Resistant, Moderately Resistant (Intermediate) and Sensitive strains (See table).

The Ericsson technique, which has been adopted in most European countries, uses a standardized culture medium (Mueller-Hinton), a standardized quantity per plate (25 mL on 9 cm diameter plates) and standardized inoculum concentration.

The fresh culture suspension used is incubated for 18 hours is in liquid medium and is then diluted accordingly. So as to ensure the appropriate amount of growth on the agar.

Suggested Dilutions:

- *Enterobacteria*- Pseudomonas: dilution of 1/300.
- *Staphylococcus* - *Enterococcus*: dilution of 1/300.
- *Streptococcus* - *Haemophilus*: dilution of 1/10.

The plate is seeded by flooding its surface. The excess inoculum is removed with a sterile pipette and the antibiotic disks are arranged properly on the plate. A pre-diffusion period of 30-60 minutes is allowed prior to incubation so that the antibiotic can slowly diffuse before growth. After incubation at 37°C for 12-18 hours, the zones of inhibition are measured and the Assay Regression Curves referenced. Results are reported in terms of Sensitive or Resistant or as Minimum Inhibitory Concentration (MIC) values.

The Ericsson technique undoubtedly offers more precision and reliability than the Kirby-Bauer. Nevertheless, the Kirby method, which is semi-quantitative, is much simpler and easier to perform in everyday practice.

The Ericsson technique is highly recommended where high efficacy and precision are required.

Mueller-Hinton medium plates can be stored refrigerated in plastic bags for a month without affecting the results of the sensitivity testing. However, they should not be used if the medium shows any signs of dehydration.

Scharlau's Mueller-Hinton Agar fulfils the WHO requirements for microbial sensitivity tests and the basic characteristics are verified in every batch. Nevertheless some variation in results can sometimes occur. Please note the following factors that can be a source of variability:

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on the medium.

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2. Factors such as: inoculum size, rate of growth, medium formulation and pH, length of incubation and incubation environment, disk content and drug diffusion rate, and measurement of endpoints can all affect the results.
   Therefore, strict adherence to protocol is required to ensure reliable results.
3. Disk diffusion susceptibility testing is limited to rapidly growing organisms. Drug inactivation may result from the prolonged incubation times required by slow growing organisms.
4. Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effects of sulphonamides and trimethoprim, causing zones of growth inhibition to be smaller or less distinct.
5. Variation in the concentration of divalent cations, primarily calcium and magnesium, affects results of amino-glycoside, tetracycline, and colistin tests with *Pseudomonas aeruginosa* isolates. A cation content that is too high reduces zone sizes, whereas a cation content that is too low has the opposite effect.
6. When Mueller-Hinton Medium is supplemented with blood, the zone of inhibition for oxacillin and methicillin may be 2 to 3 mm smaller than those obtained with unsupplemented agar. Conversely, sheep's blood may markedly increase the zone diameters of some cephalosporins when they are tested against enterococci.
   Sheep's blood may cause indistinct zones or a film of growth within the zones of inhibition around sulphonamide and trimethoprim disks.
7. Mueller-Hinton Medium deeper than 4 mm may cause false-resistant results, and agar less than 4 mm deep may be associated with a false-susceptibility results.
8. A pH value outside the range of 7,3 ± 0,1 may adversely affect susceptibility test results. If the pH is too low, amino-glycosides and macrolides will appear to lose potency; others may appear to have excessive activity.
   The opposite effects are possible if the pH is too high.
9. When Mueller-Hinton Medium is inoculated, no droplets of moisture should be visible on the surface or on the Petri dish cover.
10. Mueller-Hinton Medium should be inoculated within 15 minutes after the inoculum suspension has been adjusted.
11. The zone of inhibition diameters of some drugs, such as the macrolides, amino-glycosides and tetracyclines, are significantly altered by CO_2. Plates should not be incubated in an increased CO_2 atmosphere.

For further information on the performance of the antibiotic disk susceptibility test refer to the M2-A9 CLSI (formerly NCCLS) Monograph.

References

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Interpretation of inhibition zones of most common antibiotics according to the Kirby-Bauer Method

Antibiotic	Concentration	Diameter of the inhibition zone (in mm)		
		Resistant	*Moderately R.*	*Sensitive*
Ampicillin with S. aureus	10 mcg	20 or less	21 - 28	29 or more
Ampicillin	10 mcg	11 or less	12 – 13	14 or more
Bacitracin	10 iu	8 or less	9 – 12	13 or more
Cephaloridine	30 mcg	11 or less	12 - 15	16 or more
Cephalothin	30 mcg	14 or less	15-17	18 or more
Chloramphenicol	30 mcg	12 or less	13 -17	18 or more
Colistin	10 mcg	8 or less	9 - 10	11 or more
Doxycycline	30 mcg	12 or less	13 – 15	16 or more
Erythromycin	15 mcg	13 or less	14 – 17	18 or more
Gentamicin	10 mcg	12 or less	-	13 or more
Kanamycin	30 mcg	13 or less	14 – 17	18 or more
Lincomycin	2 mcg	9 or less	10 - 14	15 or more
Methicillin	5 mcg	9 or less	10 - 13	14 or more
Nalidixic, Acid	30 mcg	13 or less	14 – 18	19 or more
Neomycin	30 mcg	12 or less	13 - 16	17 or more
Nitrofurantoin	300 mcg	14 or less	15 - 16	17 or more
Novobiocin	30 mcg	17 or less	18 - 21	22 or more
Oleandomycin	15 mcg	11 or less	12 - 16	17 or more
Penicillin G	10 iu	20 or less	21 – 28	29 or more
Polymyxin B	300 iu	8 or less	9 - 11	12 or more
Streptomycin	10 mcg	11 or less	12 - 14	15 or more
Sulphonamide	300 mcg	12 or less	13 - 16	17 or more
Tetracycline	30 mcg	14 or less	15 - 18	19 or more
Vancomycin	30 mcg	9 or less	10 - 11	12 or more

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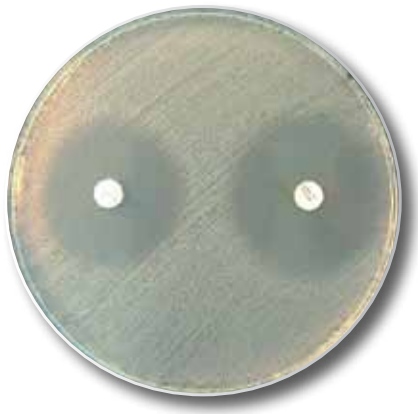
Quality control

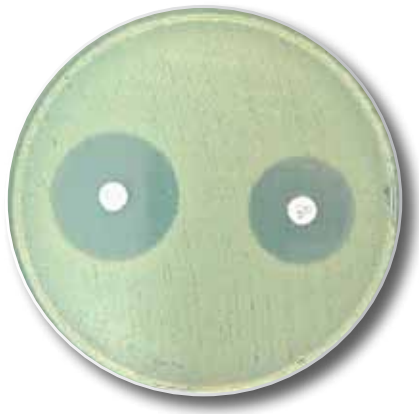
Incubation temperature: 35°C ± 2,0
Incubation time: 18 - 24 h
Inoculum: Inoculate the entire agar surface and add antibiotic disks according to CLSI guidelines.

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Good	-
Escherichia coli ATCC 25922	Good	-
Pseudomonas aeruginosa ATCC 27853	Good	-
Escherichia coli ATCC 35218	Good	-
Enterococcus faecalis ATCC 29212	Good	-

Pseudomonas aeruginosa ATCC 27853



Staphylococcus aureus ATCC 25923



Escherichia coli ATCC 25922

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