TECHNICAL DATA SHEET

MUELLER-HINTON AGAR

ANTIBIOTIC SENSITIVITY

1 INTENDED USE

Mueller Hinton Agar is recognized by all experts as being the reference medium for the study of the susceptibility of bacteria to antibiotics and sulfamides. It is also useful for the isolation of Neisseria and is an excellent base medium for the preparation of blood agars.

The typical composition responds to that defined in the standards NF U47-106 and NF U47-107.

2 HISTORY

In work concerning the development of a transparent medium capable of resisting autoclaving, Mueller and Hinton selected the complex medium of Gordon and Hine in an attempt to determine the essential components. The authors found that starch could replace pea extract in terms of nutritive value as well as protective agent acting against toxic substances present in the medium. They subsequently found that pancreatic digest of meat could be replaced by acid hydrolysate of casein, thereby favoring the growth of gonococci and meningococci. In 1966, Bauer, Kirby, Shervis and Turck recommended Mueller Hinton medium for the study of the antibiotic susceptibility of bacteria using the disk method.

3 PRINCIPLES

The choice of ingredients is determined in order to obtain a very low quantity of thymine and thymidine (substances known to inhibit the antibacterial activity of trimethoprim), and a very low quantity of para-aminobenzoic acid (PABA) and its structural analogues (which antagonize the activity of sulfonamides).

As a result of the influence of calcium and magnesium on the sensitivity of *Pseudomonas* strains to aminoglycosides, Reller *et al*. recommended that the ion concentrations be included within the following limits :

- calcium : 50-100 mg/liter
- magnesium : 20-35 mg/liter.

The Kirby-Bauer method is based on the diffusion of antibiotics impregnated in previously dried paper disks, deposited on the surface of the agar. When applied to the surface of the agar, the disks absorb a sufficient quantity of water to dissolve the antibiotic, which then diffuses into the medium according to physical laws of diffusion of molecules through a gel. In this way, a concentration gradient of antibiotic forms around each disk. At the same time as the antibiotics diffuse, the bacteria inoculated on the surface of the agar multiply. During the logarithmic phase of growth, bacterial multiplication is more rapid than the diffusion of the antibiotic and uninhibited bacterial cells continue to multiply until growth can be visualized.

No growth appears when the antibiotic is present at inhibiting concentrations. It now becomes possible to measure the diameter of the inhibition zone, which is indirectly proportional to the minimal inhibitory concentrations found by the dilution method. Tables exist for interpreting the results in order to determine if the bacteria are sensitive or resistant to the antibiotic tested.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Acid hydrolysate of casein ..... 17,5 g
- Meat extract..... 2,0 g
- Soluble starch ..... 1,5 g
- Bacteriological agar..... 17,0 g

pH of the ready-to-use media at 25 °C : 7,3 ± 0,2.

5 PREPARATION

- Dissolve 38,0 g of dehydrated media (BK048) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes or vials.
- Sterilize in an autoclave at 115 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.
- The thickness of the agar must imperatively be at least 4 mm thick.
- Dry 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.

✓ **Reconstitution :**
38,0 g/L

✓ **Sterilization :**
15 min at 115 °C

Note
The water used to prepare the medium must be of high quality, since the levels of calcium and magnesium in the medium are precisely adjusted.

6 INSTRUCTIONS FOR USE

Antibiotic sensitivity testing (standard Kirby & Bauer method) :

- Use a pure culture of the strain from Tryptone soy broth that presents an opacity which is equivalent to the standard opacity of a barium sulfate suspension (density of 0.5 on the MacFarland scale).
- Take a culture using a sterile swab.
- Inoculate the agar. Pass the swab 2 to 3 times over the entire surface of the agar in order to obtain a homogeneous inoculum.
- Allow the plates to dry for 10 minutes before depositing the disks.
- Place the disks using slight pressure to insure good adhesion to the agar. They should be situated at least 15 mm from the edge of the dish and sufficiently far apart so the inhibition zones do not overlap.
- Incubate at 37 °C for 18 to 24 hours.

✓ **Inoculation :**
Entire surface

✓ **Incubation :**
18 to 24 h at 37 °C

Notes :
The Kirby and Bauer method is recognized as fu²rnishing the most reliable and most reproducible results. Other methods may also be used, however, provided that inoculum and inoculation method are first studied and standardized.

For other methods, refer to the appropriate standards or protocols.

7 RESULTS

Measure the inhibition zones.
Refer to the table for interpreting inhibition zones furnished by the suppliers of antibiotic disks in order to establish the correlation between the inhibition zone and the minimal inhibitory concentration (M.I.C.).

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : whitish powder, free-flowing and homogeneous.
Prepared media : amber agar.

Typical culture response after 24 hours of incubation at 37 °C :

Microorganisms		Growth
Escherichia coli	WDCM 00013	Good
Staphylococcus aureus	WDCM 00034	Good
Pseudomonas aeruginosa	WDCM 00025	Good
Enterococcus faecalis	WDCM 00087	Good

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
The expiration date is indicated on the label.

Prepared media in tubes or vials (*) : 180 days at 2-8 °C.
Prepared media in plates (*) **:** 30 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK048HA

11 BIBLIOGRAPHY

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12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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ANNEX 1 : PHOTO SUPPORT

Mueller-Hinton Agar

Antibiotic sensitivity test

Results :

Growth obtained after 24 hours of incubation at 37 °C.