

MUELLER HINTON AGAR II

Powdered and ready to use improved
antimicrobial susceptibility testing medium

Typical formula * (g/l)

Beet Extract	2.0
Acid Digest of Casein	17.5
Starch	1.5
Agar Bios Special	17.0

*Adjusted or supplemented to meet performance standard

Directions for powdered medium

Suspend 38g in 1000ml of cold distilled water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes.

Final pH 7.3 ± 0.1

DIRECTIONS FOR READY TO USE MEDIUM IN FLASKS

Dissolve the contents of the bottle in a temperature controlled water batch (100°C). Cool to 45-50°C, mix well and distribute into sterile Petri dishes

Final pH 7.3 ± 0.1

DESCRIPTION

Mueller Hinton Agar was originally prepared for the cultivation of gonococci and because of its low level of p-aminobenzoic acid it was used for sensitivity tests with sulphonamides.

Mueller Hinton Agar is recommended by NCCLS for antimicrobial disc susceptibility test of most common, rapidly growing bacteria, by the Kirby-Bauer diffusion method. To test strains that fail to grow satisfactorily on Mueller Hinton Agar, 5% defibrinated sheep blood is added to the melted and cooled medium. For tests with *H. influenzae* the Mueller Hinton Agar must be supplemented with 15mg/L NAD, 15mg/L bovine hematin, 5g/L yeast extract (see Haemophilus Test Agar cat. N°549901).

Biolife Mueller Hinton Agar II is prepared with selected raw materials. It contains low levels of thymine and thymidine since they affect sulphonamide and trimethoprim MIC values, and controlled levels of Ca⁺⁺ and Mg⁺⁺ to obtain aminoglycosides inhibition zones against *P. aeruginosa* and tetracycline inhibition zones against staphylococci within the ranges suggested by NCCLS M100-S12.

TECHNIQUE

Prepare Mueller Hinton Agar II in plates of 90/100mm or 140mm with a medium layers of 4mm (25ml of medium per plate of 9cm and 60ml per plate of 14cm).

To prepare the inoculum, suspend 4-5 colonies grown in a primary isolation medium in 4-5ml of Tryptic Soy Broth and incubate for 2-6 hours until the broth culture reaches the same density as the opacimetric standard prepared by adding 0.5ml of 0.1% barium chloride to 99.5ml of 0.36 N sulphuric acid. Within 15 minutes, dip a sterile swab into the broth culture, squeeze it against the walls of the tube to remove excess liquid, then streak it over the surface of the agar plates to obtain a uniform distribution of the inoculum. Leave the plates to dry then lay the paper discs pressing them onto the surface of the agar.

Incubate at 35°C for 18 hours then read the inhibition zones by taking in to consideration the zones, which are completely free of microbial growth and which have distinct borders.

Compare the zone sizes obtained to those reported on the table no. 2 of the NCCLS Document M100-S12 "Zone Diameter Interpretative Standards" to detect whether the organism is resistant, intermediate or susceptible to antimicrobial agents.

User quality assurance (37°C-24 h)

Mueller Hinton Agar II

S.aureus ATCC 25923

E.coli ATCC 25922

P.aeruginosa ATCC 27853

E.faecalis ATCC 33186

Mueller Hinton Blood Agar
S. pneumoniae ATCC 49619
Muller Hinton Haemophilus Test Medium
H. influenzae ATCC 49247
Inhibition zones within the ranges recommended by NCCLS

STORAGE

Dehydrated medium: 10-30°C
User prepared plates: 1month at 2-8°C
Ready to use plates: 2-8°C

REFERENCES

- Bauer, A.W.; Kirby, W.M,M.; Sherris, K.C. & Truck, M. (1966), Amer. Clin. Path, **45**, 493-496.
- National Committee for Clinical Laboratory Standard. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard M2 A4 - Seventh Edition. American National Standard.
- NCCLS Performance Standard for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement. NCCLS Document M100 – S12, January 2002.

PACKAGING

4017402	Mueller Hinton Agar II	500g (13.2 l)
4017404	Mueller Hinton Agar II	5kg (132 l)
541740	Mueller Hinton Agar II	20 ready to use plates (90mm)
501740P	Mueller Hinton Agar II	5 ready to use plates (140mm)
5117402	Mueller Hinton Agar II	6 x 100ml ready to use flasks
5117403	Mueller Hinton Agar II	6 x 200ml ready to use flasks