

90922 Mueller Hinton Broth 2, Cation-Adjusted (M-H 2 Broth; Mueller Hinton II Broth)

Mueller Hinton Broth 2 cation adjusted is intended for use in quantitative procedures for susceptibility testing of rapidly growing aerobic and facultatively anaerobic bacteria isolated from clinical specimens.

Composition:

Ingredients	Grams/Litre
Casein acid hydrolysate	17.5
Beef extract	3.0
Starch	1.5

Final pH 7.3 +/- 0.2 at 25°C

Store prepared media below 8°C and protected from direct light. Store dehydrated powder in a dry place, in tightly sealed containers at 2-25°C.

Appearance: Light yellow, homogeneous, hygroscopic powder.
Gelling: Firm
Color and Clarity: Pale to light yellow coloured, clear solution without any precipitate.

Directions:

Suspend 22 g in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense and sterilize by autoclaving at 10-15 lbs pressure (115-121°C) for 10 minutes. DO NOT OVERHEAT. Mix well before pouring.

Note: This medium is supplemented with appropriate salts to provide 20-25 mg/l of calcium and 10-12.5 mg/l of magnesium and as additionally required to suit performance parameters.

Principle and Interpretation:

The Mueller Hinton Broth is used for determining minimal inhibitory concentrations (MICs) used in the determination of sulfonamide resistance of gonococci and other organisms. The originally medium was developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* (1). Mueller Hinton medium is recommended by the FDA, World Health Organization (WHO) and NCCLS for testing most commonly encountered aerobic and facultative anaerobic bacteria in food and clinical material for antimicrobial susceptibility testing (2,4). The medium shows good batch-to-batch reproducibility, it is low in sulfonamide, trimethoprim, and tetracycline inhibitors and yields satisfactory growth of most non-fastidious pathogens. Müller Hinton Medium can also be used as well to determine the potency of an antibiotic. Fleming used a serial dilution technique to find the minimal concentration of penicillin that prevented growth of a test organism in a broth (7).

Ericsson and Sherris describe various methods for susceptibility testing and the relationship of dilution and diffusion methods (8). Rammelkamp and Maxon used the tube dilution test to determine the in vitro antimicrobial susceptibility of bacteria isolated from clinical specimens (9).

Casein acid hydrolysate and beef extract provide amino acids and other nitrogenous substances, minerals, vitamins, carbon to support the growth of microorganisms. To determine the MIC values with *Enterococcus faecalis* and sulfamethoxazoletrimethoprim a special low content of thymine and thymidine in the amino acid sources is required. Starch neutralise toxic substances that may be present in the medium and have inhibitory effect on the antibiotics and the growth of organisms. Also any toxic metabolites produced by organisms are absorbed from the starch. During autoclaving a small amount of dextrose is hydrolysed from starch, which is a source of energy. Calcium and magnesium salts are added to adjust the ion concentrations to the recommended values by the protocol described in the NCCLS standard M7 to give the correct MIC values with aminoglycosides and *Pseudomonas aeruginosa* (2). This simple medium is not well suited for fastidious organisms like *Streptococcus pneumoniae* and so the addition of 2-5% lysed horse blood is recommended for susceptibility testing of *S. pneumoniae*.

Antimicrobial agents are prepared in serial two-fold dilutions in Mueller Hinton Broth 2 (Cation-Adjusted) and are inoculated with the test culture to give a final concentration of 5×10^5 CFU/mL. After incubation at 35°C, the presence of turbidity indicates growth of the organism. The lowest concentration of antimicrobial agent showing no growth is the MIC value. Testing for susceptibility of a certain organism is made by comparing the MIC to those in the MIC



interpretive standards in NCCLS standard M7 (2,10). Various factors have been identified as influencing broth dilution susceptibility tests. These include the medium, inoculum concentration, pH, the antimicrobial potency, antimicrobial stability and mechanisms of resistance by the test organisms (4,8,11). The tube dilution test (broth dilution) involves exposing bacteria to decreasing concentrations of antimicrobial agents in liquid media, usually by serial two-fold dilution. The mixture, consisting of microorganisms, nutrient medium and antimicrobial agent, is incubated at 35°C for 16-20 hours. The MIC concentration is the amount of antibiotic needed to inhibit the growth of an organism in vitro and the achievable concentrations in the blood, urine, cerebrospinal fluid or bile, under various dosage conditions. It has been suggested that in the treatment of systemic infections, the drug dosage should yield a peak concentration at the site of infection that is two to four times greater than the MIC value, while for urinary tract infections, a peak urine concentration of 10-20 times the MIC value should be achieved (12). However, effective antimicrobial therapy also depends on many other factors (13).

Some microorganisms when tested against trimethoprim/sulfamethoxazole or sulfonamides alone do not always give clear-cut end points of MIC. In the case of doubling dilutions of trimethoprim/sulfamethoxazole, there may be a minor growth. This shows an obvious inhibition of growth. This can be seen as well on the small pellets (usually less than 1 mm in diameter) in the wells with lower antibiotic concentration, or an obvious reduction of turbidity and a slight but detectable graduation in the size of the pellets. In these cases, the MIC end point should be taken at the lowest concentration of antimicrobial agent beyond which there is no further inhibition visible. An organism can be susceptible, intermediate or resistant for a given antimicrobial agent depending on the MIC value. More information about standard MIC values with various antibiotics can be found in NCCLS document M100 (M7) or may be obtained from the drug manufacturer (2).

Note: NCCLS periodically publish informational supplements to NCCLS Document M7, containing revised tables of antibiotics and interpretive standards. These tables should be consulted for current recommendations. The newest protocol and informational supplements can be ordered from the National Committee for Clinical Laboratory Standards, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898. Telephone: (610) 688-1100.

Cultural characteristics after 16-20 hours at 35 ± 2°C.

Organisms (ATCC)	Inoculum [CFU]	Growth
<i>Staphylococcus aureus</i> (25923)	50-100	+++
<i>Escherichia coli</i> (25922)	50-100	+++
<i>Enterococcus faecalis</i> (29212)	50-100	+++
<i>Pseudomonas aeruginosa</i> (27853)	50-100	+++

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