BD Mueller Hinton Fastidious Agar (MH-F)

INTENDED USE

BD Mueller Hinton Fastidious Agar (MH-F) is used for antimicrobial susceptibility testing of clinical isolates of fastidious organisms as standardized by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[1] This medium is composed of Mueller Hinton Agar supplemented with 5% mechanically defibrinated horse blood and 20 mg/L ß-NAD. Current EUCAST guidelines recommend the use of MH-F for fastidious microorganisms including *Streptococcus pneumoniae*, *Haemophilus* ssp., *Moraxella catarrhalis*, *Campylobacter jejuni* and *coli*, Viridans group streptococci, Streptococcus groups A, B, C and G, *Listeria monocytogenes*, *Pasteurella multocida* as well as *Corynebacterium* spp.[2]

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

For antimicrobial susceptibility testing of fastidious organisms (including disc diffusion methodology and implementation, instructions for reading) the current procedures recommended by EUCAST should be consulted.[2]

In short, for the antimicrobial susceptibility test procedure based on the widely-used Kirby-Bauer Method[3], a confluent inoculum of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specified amounts of antibiotic or other antimicrobial agents are then placed on the surface of the medium, the plate is incubated and zones of inhibition around each antibiotic disc are measured. The determination as to whether the organism is susceptible (S) or resistant (R) to an agent is made by comparing zone sizes obtained to those listed in the EUCAST Breakpoint Tables.[4]

Low concentrations of thymine-thymidine and controlled levels of calcium and magnesium in the Mueller Hinton base restricts the growth around the discs and enable more accurate measurements of the zones of inhibition.[5-8] Un-supplemented Mueller Hinton Agar, although adequate for susceptibility testing of rapidly growing aerobic pathogens, is not adequate for more fastidious organisms which require specific growth supplements. The composition of Mueller Hinton Fastidious Agar with defibrinated horse blood and NAD enables the growth of fastidious bacteria while guaranteeing minimum interference from the constituents of the formula in the result of the antimicrobial susceptibility test. Mueller Hinton Fastidious Agar is a common test medium for most fastidious microorganism as described above and makes the usage of separate media for antimicrobial susceptibility testing of fastidious microorganism unnecessary.[1]

REAGENTS

BD Mueller Hinton Fastidious Agar (MH-F)

Formula* Per Liter Purified Water

Meat Extract	2.0 g
Acid Hydrolysate of Casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Horse Blood, mechanically defibrinated	5 %
ß-NAD	0.02 g
pH 7.3 ± 0.1	

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

PRECAUTIONS

IVD For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration. Excessive shrinkage of this medium due to desiccation may lead to false susceptibility results.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8°C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times. Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8°C.

USER QUALITY CONTROL

For user quality control, the EUCAST recommendations should be consulted.[9] Inoculate representative samples with the following strains on each medium (for details, see **Specimen Types** and **Test Procedure**). Incubate the plates, preferably in an inverted position, according to the below indicated temperature, time and atmospheric condition.

Table 1: Expected results for quality control strains according to the EUCAST guidelines[9]

Strain	Antimicrobial agent	Range (mm)	Incubation
***Streptococcus pneumoniae* ATCC™ 49619**	Erythromycin (15 µg)	26 - 32	16-20 h, 35±1°C, CO_2 atmosphere
	Oxacillin (1 µg)	8 - 14	
	Norfloxacin (10 µg)	18 - 24	
	Meropenem (10 µg)	30 - 38	
	Trimethoprim-Sulfamethoxazole (1.25-23.75 µg)	18 -26	
***Haemophilus influenzae* ATCC™ 49766**	Ampicillin (2 µg)	19 - 25	16-20 h, 35±1°C, CO_2 atmosphere
	Cefuroxime (30 µg)	26 - 34	
	Chloramphenicol (30 µg)	31 - 37	
	Trimethoprim-Sulfamethoxazole (1.25-23.75 µg)	27 - 35	
***Campylobacter jejuni* ATCC™ 33560** (DSM 4688)	Ciprofloxacin (5 µg)	34 - 42	24 h, 41±1°C, microaerophilic atmosphere
	Erythromycin (15 µg)	27 - 35	
	Tetracycline (30 µg)	30 - 38	
Uninoculated	Red to burgundy, opaque		

PROCEDURE

Materials Provided

BD Mueller Hinton Fastidious Agar. Microbiologically controlled.

Materials Not Provided

1. 0.9% saline (5 ml amounts) for preparation of standard inoculum.
2. Barium sulfate comparison standard (0.5 ml of 0.048 M $BaCl_2$ [1.175% w/v $BaCl_2 2H_2O$] to 99.5 ml of 0.18 M [0.36 N] H_2SO_4 [1% v/v]) or
3. A photometric device for adjusting the turbidity of the inoculum suspension to be equivalent to the McFarland 0.5 standard.
4. As an alternative to the above materials (1-3), the **BD Prompt™ Inoculation System** (volumetric inoculum preparation device) can be used.[10]
5. Control culture - *Streptococcus pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 49766 and *Campylobacter jejuni* ATCC 33560/DSM 4688.
6. Paper discs impregnated with specified amounts of antimicrobial agents, such as **BD Sensi-Disc™** susceptibility test discs.

7. Disc dispensing device, such as the **BD Sensi-Disc** Self-Tamping 6-place dispenser.
8. Ruler or another device for measuring zone size in millimeters.
9. An incubator that produces an atmosphere containing 5% CO_2, or another device that produces a similar CO_2-enriched atmosphere.
10. Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types
This product is used for susceptibility testing of pure cultures that have been isolated from clinical specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure
This methodology describes the direct colony suspension method as recommended by EUCAST.[2]

1. Assure that a pure, fresh (=overnight) culture from a non-selective medium is available. For routine susceptibility tests, the inoculum may be prepared by making a direct saline suspension of several morphologically similar colonies.
2. Adjust the inoculum to the density of a McFarland 0.5 standard using a photometric device or visually to be equivalent to the barium sulfate standard (McFarland 0.5 standard).
3. Alternative methods of inoculum preparation involving devices that permit direct standardization of inocula without adjustment of turbidity, such as the **BD Prompt™ Inoculation System**, have been found to be acceptable for routine testing purposes.[10]
4. *Streptococcus pneumoniae* is, preferably, suspended from a blood agar plate to the density of a McFarland 0.5 standard. When *Streptococcus pneumoniae* is suspended from a chocolate agar plate, the inoculum must be equivalent to a McFarland 1.0 standard.
5. Use the inoculum optimally within 15 min of adjusting the turbidity. The suspension must always be used within 60 min of preparation. Dip a sterile swab into the properly diluted inoculum and rotate it firmly several times against the upper inside wall of the tube to express excess fluid and avoid over-inoculation.
6. Inoculate onto **BD Mueller Hinton Fastidious Agar** by streaking the entire agar surface of the plate three times, rotating the plate 60° between streaking to obtain even inoculation.
7. Apply the discs of the dried plate within 15 min of inoculation using aseptic precautions. Deposit a maximum of six discs on the plate. After discs have been placed on the agar, tamp them with a sterile needle or forceps to make complete contact with the medium surface. This step is not necessary if the discs are deposited using the **BD Sensi-Disc** self-tamping dispensers.
8. Within 15 minutes after the discs are applied, invert the plates (preferably) and place them in the incubator. Incubation conditions are summarized in Table 2.

Table 2: Incubation conditions for different fastidious organisms according to EUCAST[2]

Organism	Incubation condition
Streptococcus groups A,B, C and G	35±1°C in 4-6% CO_2 in air for 16-20h
Streptococcus pneumoniae	35±1°C in 4-6% CO_2 in air for 16-20h
Viridans group streptococci	35±1°C in 4-6% CO_2 in air for 16-20h
Haemophilus spp.	35±1°C in 4-6% CO_2 in air for 16-20h
Moraxella catarrhalis	35±1°C in 4-6% CO_2 in air for 16-20h
Listeria monocytogenes	35±1°C in 4-6% CO_2 in air for 16-20h
Pasteurella multocida	35±1°C in 4-6% CO_2 in air for 16-20h
Corynebacterium spp.	35±1°C in 4-6% CO_2 in air for 16-20h. Isolates with insufficient growth after 16-20h incubation are reincubated immediately and inhibition zones read after a total of 40-48h incubation
Campylobacter jejuni and *coli*	41±1°C in microaerobic environment for 24h. Some *C. coli* isolates may not have sufficient growth after 24h incubation. These are reincubated immediately and inhibition zones read after a total of 40-48h incubation

Reading of Results

1. After incubation, confluent growth should be visible. If only isolated colonies grow, the inoculum was too light and the test should be repeated.
2. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disc, to the nearest whole millimeter, using sliding calipers, or a ruler, with lid removed and held about 30 cm from the eye.
3. The endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies which can be detected with difficulty near the edge of the obvious zone of inhibition.
4. In case of double zones, the inner zone should be measured unless otherwise specifically stated.[2,4,9,11]
5. For hemolytic streptococci, read inhibition of growth and not inhibition of hemolysis. β-hemolysis is usually free from growth, whereas α-hemolysis and growth usually coincide.

Interpretation of Results

Interpret zone diameters by reference to breakpoint tables.[2] Results obtained with specific organisms may then be reported as resistant or susceptible. Additional information about specific growth characteristics, interpretation and other guidance documents can be obtained from www.eucast.org.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Mueller Hinton Fastidious Agar is designed for testing the susceptibility of fastidious organisms recommended by EUCAST.[1] Breakpoint tables for the interpretation of sensitivity are updated anually[4] and the most recent version should be consulted for proper interpretation of the obtained results.

Performance Results

Internal Performance Evaluation

Performance of **BD Mueller Hinton Fastidious Agar** was internally validated using the recommended quality control (QC) strains[9] (see Table 3) and 151 additional previously characterized strains (see Table 4) including *Corynebacterium* spp., Viridans group streptococci, *Listeria monocytogenes*, *Moraxella catarrhalis, Streptococcus* groups A, B, C and G, *Haemophilus* ssp. and *Streptococcus pneumoniae*.

Table 3 summarizes the validated antimicrobial agents for the QC strains. Unless stated otherwise, the determined inhibition zone sizes for the validated antimicrobial agents were within the specified EUCAST inhibition zone diameter ranges.[9] For *H. influenzae* ATCC 49766, Amoxicillin-clavulanic acid showed inhibition zones outside the ranges recommended by EUCAST.[9] For *S. pneumoniae* ATCC 49616, Cefepime, Cefpodixime and Cefuroxime showed inhibition zones outside the diameter ranges recommended by EUCAST.[9]

Antimicrobial susceptibility testing of the 151 additional characterized fastidious bacteria (see Table 4) indicated satisfactory growth after recommended incubation times allowing for adequate reading of inhibition zones and determination of respective antimicrobial resistance according to the EUCAST breakpoints[4].

Table 3: Validated antimicrobial agents and quality control strains. Unless stated otherwise, the inhibition zone diameters were within the respective EUCAST ranges.[9] Divergent inhibition zones are specified

Antimicrobial agent	Disc content (µg)	*H. influenzae* ATCC 49766	*S. pneumoniae* ATCC 49616	*C. jejuni* ATCC 33560
Ampicillin	2	✓	✓	
Amoxicillin-clavulanic acid	2-1	20-30 mm[1]		
Benzylpenicillin	1 unit	✓	✓	
Cefaclor	30		✓	
Cefepime	30	✓	35-39 mm[1]	
Cefixime	5	✓		
Cefotaxime	5	✓	✓	
Cefpodixime	10	✓	33-39 mm[1]	
Ceftarolin	5	-	-	
Ceftibuten	30	✓		
Ceftriaxone	30	✓	✓	
Cefuroxime	30	✓	33-38 mm[1]	
Chloramphenicol	30	✓	✓	
Ciprofloxacin	5	✓	✓	✓
Clindamycin	2		✓	
Doripenem	10	✓	✓	
Ertapenem	10	✓	✓	
Erythromycin	15	✓	✓	✓
Imipenem	10	✓	✓	
Levofloxacin	5	✓	✓	
Linezolid	10		✓	
Meropenem	10	✓	✓	
Minocycline	30	✓	✓	
Moxifloxacin	5	✓	✓	
Nalidixic acid	30	✓		
Nitrofurantoin	100		✓	
Norfloxacin	10		✓	
Ofloxacin	5	✓	✓	
Oxacillin	1		✓	
Rifampicin	5	✓	✓	
Teicoplanin	30		✓	
Telithromycin	15	✓	✓	
Tetracycline	30	✓	✓	✓
Tigecycline	15		✓	
Trimethoprim-Sulfamethoxazole	1.25-23.75	✓	✓	
Vancomycin	5		✓	

✓ Indicates zones of inhibition within the EUCAST range.[9]

[1] Average inhibition zone ranges are outside the recommended EUCAST QC ranges.[9] Please consult the most recent QC recommendations by EUCAST for comparison.

Table 4: Overview of validated fastidious organisms and antimicrobial agents

Antimicrobial agent	Disc content (µg)	Total no. of strains: 151																	
		H. influenzae	*H. parainfluenzae*	*H. aprophilus*	*S. pneumoniae*	Group A, B, C, G Streptococci	Viridans Streptococci	*M. catarrhalis*	*L. monocytogenes*	*C. pseudodiphtheriticum*	*C. striatum*	*C. jeikeium*	*C. amycolatum*	*C. xerosis*	*C. urealytikum*	*C. afermentans*	*C. jejuni*	*C. coli*	*P. multocida*
		19	6	1	19	41	22	7	9	4	3	3	2	1	2	1	6	4	3
Ampicillin	2		✓				✓		✓										✓
Amoxicillin-clavulanic acid	2-1		✓					✓											✓
Benzylpenicillin	1 unit		✓			✓	✓		✓				✓						✓
Cefaclor	30				✓														
Cefazolin	30						✓												
Cefepime	30		✓				✓	✓											
Cefixime	5		✓					✓											
Cefotaxime	5		✓				✓	✓											✓
Cefpodixime	10		✓					✓											
Ceftarolin	5																		
Ceftibuten	30		✓																
Ceftriaxone	30		✓				✓	✓											
Cefuroxime	30		✓				✓	✓											
Chloramphenicol	30		✓		✓	✓		✓											
Ciprofloxacin	5		✓		✓			✓					✓				✓		✓
Clindamycin	2				✓	✓	✓						✓						
Doripenem	10		✓					✓											
Ertapenem	10		✓					✓											
Erythromycin	15		✓		✓	✓		✓	✓								✓		
Gentamicin	10												✓						
Imipenem	10		✓					✓											
Levofloxacin	5		✓		✓	✓		✓											✓
Linezolid	10				✓	✓							✓						
Meropenem	10		✓					✓	✓										
Minocycline	30		✓		✓	✓		✓											
Moxifloxacin	5		✓		✓	✓		✓					✓						
Nalidixic acid	30		✓					✓											✓
Nitrofurantoin	100					✓													
Norfloxacin	10				✓	✓													
Ofloxacin	5		✓		✓			✓											
Oxacillin	1				✓														
Rifampicin	5		✓		✓	✓							✓						
Teicoplanin	30				✓	✓	✓												
Telithromycin	15		✓		✓	✓		✓											
Tetracycline	30		✓		✓	✓		✓					✓				✓		✓
Tigecycline	15					✓													
Trimethoprim	5					✓													
Trimethoprim-Sulfamethoxazole	1.25-23.75		✓		✓	✓		✓	✓										✓
Vancomycin	5				✓	✓	✓						✓						

External Performance Evaluation

In an external performance evaluation, 169 characterized clinical isolates were tested on BD Mueller Hinton Fastidious Agar. A simultaneous comparison of the susceptibility results with another available Mueller Hinton Fastidious medium indicated an equivalency rate of 99.8% for the determined category of resistance (S, susceptible, R, resistant, or I, intermediate, respectively). BD MH-F supported satisfactory growth of all tested organisms when incubated at the recommended incubation time.

Table 5: Tested clinical isolates on BD Mueller Hinton Fastidious Agar (MH-F) during the external performance evaluation

Strain	No. of strains tested	No. of antibiotics tested
Haemophilus influenzae	28	24
Streptococcus pneumoniae	32	27
Campylobacter jejuni	31	3
Streptococcus groups A, B, C and G	10	9
Viridans group streptococci	10	14
Moraxella catarrhalis	11	9
Pasteurella multocida	4	9
Pasteurella canis	1	9
Listeria monocytogenes	10	5
Campylobacter coli	10	3
Corynebacterium spp.	10	9
Haemophilus spp.	12	6
Total no. of strains tested	**169**	

Limitations of the procedure

The disc diffusion susceptibility test is designed for use with pure cultures only. A Gram stain and a presumptive identification of the isolate are recommended before the susceptibility test is prepared.

With some organism-antimicrobial agent combinations, the inhibition zone may not have a sharply demarcated edge (fuzzy zone edges were observed with *S. pneumoniae*), which could lead to incorrect interpretation. Please consult the EUCAST reading guide for detailed information.[11] Various factors have been identified as influencing disc diffusion susceptibility tests. These include the medium, agar depth, disc potency, inoculum concentration, age of inoculum, and pH.[12]

Incorrect inoculum concentration may produce incorrect results. Zones of inhibition may be too small if the inoculum is too heavy and they may be too large and difficult to measure if the inoculum is too light. Therefore, it is strongly recommended to follow the EUCAST guidelines on handling of the inoculum and the inoculated plates to minimize the potential risk of incorrect results due to improper handling.[1] Improper storage of antimicrobial discs may cause a loss of potency and a falsely resistant result. Excessive shrinkage of the medium due to improper storage may lead to falsely susceptible results.

In vitro susceptibility of an organism to a specific antimicrobial agent does not necessarily mean that the agent will be effective in vivo. Consult appropriate references for guidance in the interpretation of results.[12,13]

REFERENCES

1. Matuschek, E., Brown, D.F. and Kahlmeter, G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clin Microbiol Infect. 2014; 20(4): 255-66.

2. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing. *Search for latest version at http://www.eucast.org.*
3. Bauer, A.W., Kirby, W.M.M, Sherris, J.C., and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 1966: 45:493-496.
4. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, 2016. http://www.eucast.org.
5. Koch, A.E. and Burchall, J.J. Reversal of the antimicrobial activity of trimethoprim by thymidine in commercially prepared media. Appl. Microbiol. 1971; 22:812-817.
6. Ferone, R., Bushby, S.R.M., Burchall, J.J., Moore, W.D., and Smith, D. Identification of Harper-Cawston factor as thymidine phosphorylase and removal from media of substances interfering with susceptibility testing to sulfonamides and diaminopyrimidines. Antimicrob. Agents Chemother. 1975; 7:91-98.
7. Reller, L.G., Schoenknecht, F.D., Kenny, M.A., and Sherris, J.C. Antibiotic susceptibility testing of Pseudomonas aeruginosa: selection of a control strain and criteria for magnesium and calcium content in media. J. Infect. Dis. 1974: 130:454-463.
8. D'Amato, R.F., and Thornsberry, C. Calcium and magnesium in Mueller-Hinton agar and their influence on disk diffusion susceptibility results. Current Microbiol. 1979: 2:135-138.
9. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 6.0, 2016. http://www.eucast.org.
10. Baker, C.N., Thornsberry, C. and Hawkinson R.W. Inoculum standardization in antimicrobial susceptibility testing: evaluation of overnight agar cultures and the rapid inoculum standardization system. J. Clin. Microbiol. 1983: 17:450-457.
11. The European Committee on Antimicrobial Susceptibility Testing. Reading guide. EUCAST disk diffusion method for antimicrobial susceptibility testing. *Search for latest version at http://www.eucast.org.*
12. Washington, J.A., and Woods G.L. 1995. Antibacterial susceptibility tests: dilution and disk diffusion methods. p. 1327-1341. In Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., and Yolken, R.H. (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C. 1995.
13. Neumann, M.A., Sahm, D.F., Thornsberry, C., McGowan, J.E., Jr. Cumitech 6A, New developments in antimicrobial agent susceptibility testing: a practical guide. Coordinating ed., J.E. McGowan, Jr. American Society of Microbiology, Washington, D.C. 1991.

PACKAGING / AVAILABILITY

BD Mueller Hinton Fastidious Agar (MH-F)

Cat. No.	Description
REF 257491	Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

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