

EUCAST MIC broth microdilution of *Streptococcus* spp. and *Haemophilus influenzae*. Evaluation of broth with and without β -NAD and with different concentrations of lysed horse blood.

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Introduction

For broth microdilution (BMD) of fastidious organisms, EUCAST recommends MH-F broth (Mueller-Hinton (MH) broth with 5% lysed horse blood (LHB) and 20 mg/L β -NAD). The β -NAD is added to allow good growth of *H. influenzae*. The ISO standard for BMD, 20776-1, recommends MH broth with 2.5-5% LHB for streptococci, but there is no recommendation for *H. influenzae*.

Methods

Three Mueller-Hinton broths with different supplements were prepared according to the manufacturer's instructions, MH-F 5.0, MH-F 2.5 and MH-LHB (Table 1). The horse blood was lysed by freezing (-20°C) and thawing (4 repetitions) and clarified by centrifugation. The three broths were produced on the same day using the same lot of broth powder, horse blood and β -NAD. BMD was performed according to the ISO standard 20776-1 (except for using different broths) with a final inoculum of 5×10^5 CFU/mL on custom Sensititre panels (TREK Diagnostics/Thermo Fisher Scientific). Sealed panels were incubated in ambient air at 35°C for 16-20 h. MIC values were read manually using a mirror. The panels included agents from several antimicrobial classes: 24 agents for streptococci and 13 agents for *H. influenzae*. Tests were performed for *H. influenzae* (n=25), *S. pneumoniae* (n=25), β -haemolytic streptococci (n=18) and viridans group streptococci (n=7), selected to represent a wide range of MIC values for important agents. *S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247 were used for quality control.

Table 1. Supplements used for the different Mueller-Hinton broths.

Broth	Lysed horse blood (LHB) (%)	β -NAD (mg/L)
MH-F 5.0 (EUCAST recommended broth)	5.0	20
MH-F 2.5	2.5	20
MH-LHB	2.5	-

Objective

The objective of this study was to investigate i) the effect on streptococci of the presence of β -NAD in the broth and ii) the effect on *H. influenzae* and streptococci of varying the LHB concentration.

Table 2. Number of readings for MH-F 2.5 and MH-F 5.0 within ± 1 dilution of MH-LHB for *Streptococcus* spp.¹

Supplemented Mueller-Hinton broth	MH-F 2.5			MH-F 5.0		
	-1	0	+1	-1	0	+1
Differences in dilutions \rightarrow Antimicrobial agent						
Benzylopenicillin	3	45	2	2	43	5
Ampicillin	0	49	1	0	45	5
Amoxicillin	0	46	4	3	41	6
Amoxicillin-clavulanic acid	0	49	1	1	46	3
Piperacillin-tazobactam	2	43	5	4	41	5
Cefepime	1	49	0	3	47	0
Cefotaxime	1	45	4	1	42	7
Ceftriaxone	1	46	3	1	46	3
Cefuroxime	3	46	1	5	42	3
Imipenem	4	44	2	3	43	4
Meropenem	3	45	2	2	41	7
Ciprofloxacin	3	46	1	9	39	2
Levofloxacin	3	45	2	6	44	0
Moxifloxacin	1	45	4	4	45	1
Vancomycin	0	48	2	0	48	2
Azithromycin	1	44	5	5	44	1
Clarithromycin	0	49	1	2	48	0
Erythromycin	0	47	3	2	46	2
Clindamycin	1	49	0	3	46	1
Tetracycline	2	45	3	4	45	1
Doxycycline	3	46	1	4	45	1
Linezolid	2	47	1	9	40	1
Rifampicin	5	40	5	7	38	5
Trimethoprim-sulfamethoxazole	1	49	0	2	47	1
TOTAL (No)	40	1107	53	82	1052	66
TOTAL (%)	3.3	92.3	4.4	6.8	87.7	5.5

¹ *S. pneumoniae* (n=25), β -haemolytic n=18) and viridans group (n=7) streptococci.

Results

For streptococci, all MICs with MH-F 5.0 and MH-F 2.5 were within ± 1 dilution of MH-LHB, with 2159 of 2400 values (90%) being identical. MICs were not systematically lower or higher than MICs with MH-LHB (Table 2). MH-F 2.5 showed the highest proportion of MICs being identical to MH-LHB. This implies that adding β -NAD to the broth had a very small or no effect, but that there might be some effect of varying concentration of LHB.

For *H. influenzae*, MH-LHB was not included in the evaluation, since β -NAD is needed for sufficient growth. All MICs with MH-F 2.5 were within ± 1 dilution of MH-F 5.0 (EUCAST recommended media), with 301 of 325 values (93%) being identical. The number of MICs being one dilution lower with MH-F 2.5 compared with MH-F 5.0 (6.1%) was larger than the number being higher (1.2%). This is probably due to poorer growth of *H. influenzae* in broth with 2.5 compared with 5% LHB. Lower MICs with MH-F 2.5 were most common for cephalosporins, carbapenems and trimethoprim-sulfamethoxazole.

Conclusions

The results from this study indicate that adding β -NAD to the broth for BMD had a very small or no effect on MIC values for streptococci (but has previously been shown to be crucial for *H. influenzae*). Decreasing the concentration of lysed horse blood from 5.0 to 2.5% influenced the MICs for *H. influenzae*. Therefore, the standard MH-F broth, with 5% lysed horse blood and 20 mg/L β -NAD, is needed to perform BMD according to EUCAST recommendations using a common broth for streptococci and *H. influenzae*.