

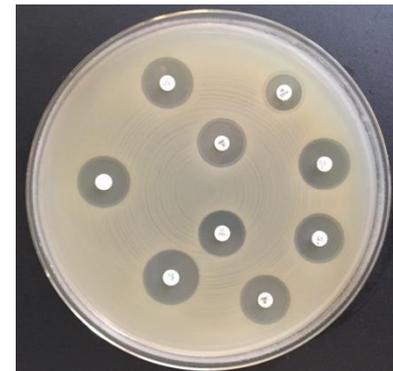


EUCAST evaluation of dehydrated Mueller Hinton media

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EUCAST disk diffusion

- Accuracy of susceptibility test result are dependent of:
 - Adherence to methodology
 - Quality of products (disks and media)
 - Storage and handling of disks and media
- Antimicrobial disks and Mueller-Hinton media can be obtained from several manufacturers.
- In 2014-2017, EDL evaluated 16 antimicrobial disks from nine manufacturers. Large variation in disk quality was observed.*



* Åhman *et al*, CMI 2019;25(3):346-352

EUCAST media evaluation study

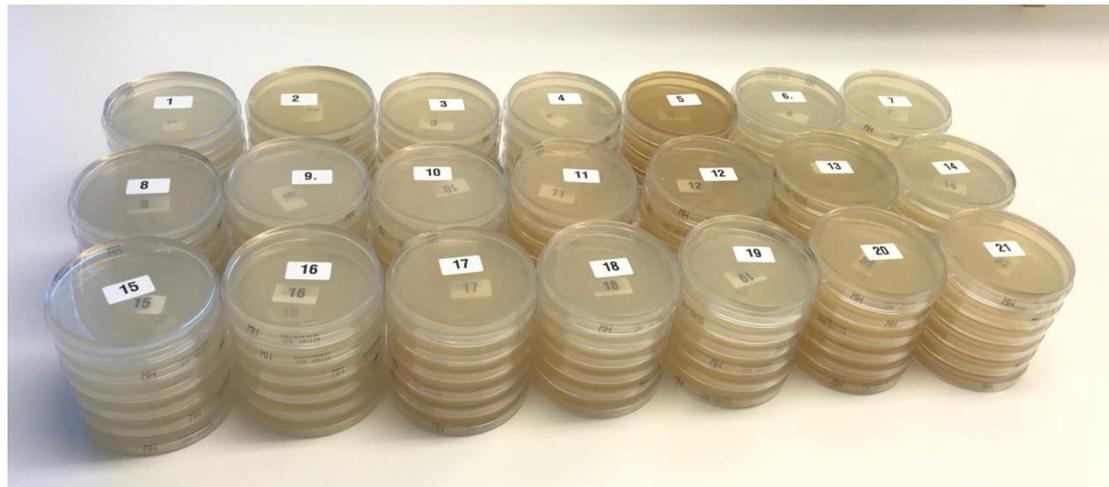
- Purpose:
 - To evaluate the performance of dehydrated Mueller-Hinton agar powders from different manufacturers using EUCAST methodology and quality control (QC) criteria.

- Mueller-Hinton agar powder
 - 21 brands
 - 17 manufacturers

Manufacturer	Product name	Product name
Acumedia	MH agar	
Alpha Biosciences	MH agar	
Becton Dickinson	BBL MH II agar	Difco MH agar
Biolab	MH II agar	
Biolife	MH agar II	
bioMérieux	MH E agar (MHE)	
Bio-Rad	MH agar	
E&O Laboratories	MH agar	
Hardy Diagnostics	CRITERION MH agar	
HiMedia	MH agar	MH agar No. 2
Lab M	MH agar	
Mast Group	MH agar	
Merck	MH agar	MH agar CLSI
Oxoid	MH agar	
Remel	MH agar	
Sifin	MH agar	
Sigma	MH agar	MH agar 2

Media preparation

- Agar plates prepared in-house at one site, according to each manufacturer's instructions.
 - the same Petri dishes, agar volume, autoclaving etc.
- Agar depth and pH was measured for all MH brands.
- Plates were labelled 1-21 to be tested blindly



Agar depth and pH

- Agar depth was measured on 2 plates (3 spots each) per MH brand.
- pH was measured (surface electrode) 5 times per MH brand.

Manufacturer	Product name	Agar depth (mm)	pH
Acumedia	Mueller-Hinton Agar	4.2	7.21
Alpha Biosciences	Mueller-Hinton Agar	4.1	7.51
Becton Dickinson	Difco Mueller-Hinton Agar	4.1	7.36
Becton Dickinson	BBL Mueller-Hinton II Agar	4.1	7.35
Biolab	Mueller-Hinton II Agar	4.1	7.63
Biolife	Mueller-Hinton Agar II	4.1	7.30
bioMérieux	Mueller-Hinton E Agar (MHE)	4.1	7.20
Bio-Rad	Mueller-Hinton Agar	4.1	7.28
E&O Laboratories	Mueller-Hinton Agar	4.2	7.37
Hardy Diagnostics	CRITERION Mueller-Hinton Agar	4.1	7.31
HiMedia	Mueller-Hinton Agar	4.2	7.30
HiMedia	Mueller-Hinton Agar No. 2	4.0	7.18
Lab M	Mueller-Hinton Agar	4.1	7.28
Mast Group	Mueller-Hinton Agar	4.2	7.28
Merck	Mueller-Hinton Agar	4.1	7.34
Merck	Mueller-Hinton Agar CLSI	4.0	7.16
Oxoid	Mueller-Hinton Agar	4.3	7.23
Remel	Mueller-Hinton Agar	4.3	7.26
Sifin	Mueller-Hinton Agar	4.1	7.27
Sigma	Mueller-Hinton Agar	4.2	7.27
Sigma	Mueller-Hinton Agar 2	4.3	7.25

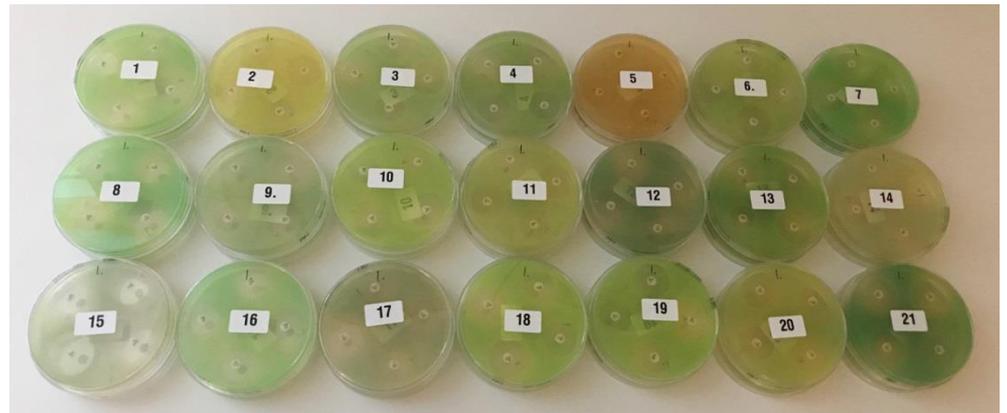
pH high
pH low

Allowed range

3.5-4.5 7.2-7.4

Study design

- Disk diffusion according to EUCAST methodology against four non-fastidious QC strains.
- Each disk-strain combination was tested in triplicate using three separate inoculum suspensions.
- Each suspension was applied to the 21 different MH agars in parallel.
- One reader of results



P. aeruginosa ATCC 27853

Antimicrobial disks and QC strains

- Disk from different antimicrobial classes
 - From a manufacturer with good quality in our previous study

Antimicrobial agent	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>S. aureus</i> ATCC 29213	<i>E. faecalis</i> ATCC 29212
Ampicillin 2 µg				•
Ampicillin 10 µg	•			
Piperacillin-tazobactam 30-6 µg		•		
Cefotaxime 5 µg	•			
Cefoxitin 30 µg			•	
Ceftazidime 10 µg	•	•		
Imipenem 10 µg		•		•
Meropenem 10 µg	•	•		
Ciprofloxacin 5 µg	•	•		
Norfloxacin 10 µg			•	•
Gentamicin 10 µg	•	•	•	
Gentamicin 30 µg				•
Tobramycin 10 µg		•		
Erythromycin 15 µg			•	
Tetracycline 30 µg			•	
Tigecycline 15 µg	•		•	•
Linezolid 10 µg			•	•
Trimethoprim-sulfamethoxazole 1.25-23.75 µg	•		•	•

- 3 repeats per QC strain and antimicrobial agent

Data analysis

- Results were rated depending on how mean values (30 per agar) from triplicate tests of each QC strains related to the respective QC criteria in EUCAST QC Tables v. 9.0.

Rating	Explanation
0	Mean value within target ± 1 mm
-1	Mean value >1 mm but within ± 2 mm from target
-3	Mean value >2 mm from target but within QC range
-5	Mean value outside QC range

- The total percentage of individual zone diameters
 - On QC target ± 1 mm
 - Outside QC ranges

Total rating and rating per QC strain

MH agar	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>S. aureus</i> ATCC 29213	<i>E. faecalis</i> ATCC 29212	Total rating
1	-2	-1	-13	-6	-22
2	-2	-1	-6	-5	-14
3	-1	-3	-4	-5	-13
4	-2	-4	-6	0	-12
5	-6	-3	-15	-16	-40
6	-1	-2	-3	-4	-10
7	-2	-1	-14	-4	-21
8	0	-1	-1	-2	-4
9	0	-4	-5	-8	-17
10	-1	-2	-5	-4	-12
11	-2	-6	-6	-6	-20
12	-3	-12	-8	-9	-32
13	0	-2	-7	-16	-25
14	-1	-13	-4	-9	-27
15	-7	-17	-9	-22	-55
16	-2	-2	-15	-6	-25
17	-1	-2	-7	0	-10
18	-5	-3	-6	-10	-24
19	-7	-2	-17	-5	-31
20	-6	-2	-7	-3	-18
21	-3	-4	-3	-1	-11

No of mean values

8

7

8

7

Results

Total rating**	Manufacturer	Product name	Product code	Percent zones on QC target +1 mm	Percent zones outside QC range	Agents outside range, high	Agents outside range, low	pH
-4	Bio-Rad	MH Agar	64884	86	0			7.28
-10	Biolife	MH Agar II	4017402	81	1.1	TS		7.30
-10	Oxoid	MH Agar	CM0337	78	1.1	TS		7.23
-11	Sigma	MH Agar 2	97580	81	0			7.25
-12	Becton Dickinson	BBL MH II Agar	211438	73	0			7.35
-12	Hardy Diagnostics	CRITERION MH Agar	C6421	71	0			7.31
-13	Becton Dickinson	Difco MH Agar	225250	70	3.3	AM		7.36
-14	Alpha Biosciences	MH Agar	M13-150	71	3.3	FQ		7.51
-17	E&O Laboratories	MH Agar	KM0183	82	8.9	CA, FQ, AM	TS	7.37
-18	Sigma	MH Agar	70191	57	3.3	CS		7.27
-20	HiMedia	MH Agar	M173	56	0			7.30
-21	bioMérieux	MHE Agar	.*	64	3.3	TS		7.20
-22	Acumedia	MH Agar	7101	63	3.3	AM		7.21
-24	Remel	MH Agar	R454082	64	6.7	AM	TS	7.26
-25	Lab M	MH Agar	LAB039	69	6.7	AM	TS	7.28
-25	Merck	MH Agar CLSI	105435	66	6.7	AM, TS		7.16
-27	Mast Group	MH Agar	DM170	59	8.9	CA, FQ	TS	7.28
-31	Sifin	MH Agar	TN1162	60	6.7	AM, TS		7.27
-32	HiMedia	MH Agar No. 2	GM1084	50	6.7	CA, AM		7.18
-40	Biolab	MH II Agar	MHT20500	52	10	PC, MA, TE	TS	7.63
-55	Merck	MH Agar	105437	44	23	CS, CA, FQ, AM	TE	7.34

* This brand can under normal circumstances be obtained only as pre-poured agar plates.

Abbreviations

PC - Penicillins
 CS - Cephalosporins
 CA - Carbapenems
 TE - Tetracyclines

AM - Aminoglycosides
 MA - Macrolides
 FQ - Flouroquinolones
 TS - Trimethoprim-sulfamethoxazole

pH high
 pH low

Conclusions

- Excellent results for MH agar from 6 manufacturers
 - $\geq 99\%$ of the readings within QC ranges
 - $\geq 70\%$ on target ± 1 mm
- Very poor results for MH agar from 2 manufacturers
 - 10 and 23% of readings out of QC range
- Zone diameters out of range were most common for
 - Trimethoprim-sulfamethoxazole
 - Aminoglycosides
 - Fluoroquinolones

Quality of Mueller Hinton agar

Manufacturers and users have a shared responsibility for quality:

- Manufacturer responsibility:
 - Ensure that dehydrated media have the correct formula to produce results within EUCAST QC ranges
- Laboratory responsibility:
 - Media preparation (according to the manufacturers instructions)
 - Agar depth (4 ± 0.5 mm)
 - pH (7.2-7.4)
 - Shelf-life (determined locally)
 - Storage (4-8°C) and handling

Laboratories must ensure that each batch of MH agar performs in accordance with EUCAST recommendations!

Order a sample for evaluation, before accepting a new powder batch from the manufacturer!

