



# Quality control in antimicrobial susceptibility testing

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# Routine quality control

- Control of materials and equipment
  - Agar plates
  - Antimicrobial disks
  - Incubators
  - Densitometer
- Control of the procedures
  - Inoculum and inoculation
  - Incubation time
  - Reading of results
  - etc.

# Quality control strains

Organism	Number	Characteristics
<i>E. coli</i>	ATCC 25922	Susceptible, wild type
<i>P. aeruginosa</i>	ATCC 27853	Susceptible, wild type
<i>S. aureus</i>	ATCC 29213	Weak $\beta$ -lactamase producer
<i>E. faecalis</i>	ATCC 29212	Susceptible, wild type
<i>S. pneumoniae</i>	ATCC 49619	Reduced susceptibility to benzylpenicillin
<i>H. influenzae</i>	ATCC 49766	Susceptible, wild type
<i>Campylobacter jejuni</i>	ATCC 33560	Susceptible, wild type

## For control of the inhibitor component in $\beta$ -lactamase- $\beta$ -lactamase inhibitor disks

<i>E. coli</i>	ATCC 35218	TEM-1 $\beta$ -lactamase
<i>K. pneumoniae</i>	ATCC 700603	SHV-18 ESBL
<i>K. pneumoniae</i>	ATCC BAA-2814	KPC-3, SHV-11 and TEM-1
<i>S. aureus</i>	ATCC 29213	$\beta$ -lactamase

# QC of $\beta$ -lactam- $\beta$ -lactamase inhibitor combinations

- Control the active component with a susceptible QC strain.
- Control the inhibitor with specific  $\beta$ -lactamase-producing QC strains.
  - *E. coli* ATCC 35218: clavulanic acid, tazobactam
  - *K. pneumoniae* ATCC 700603: avibactam, tazobactam
  - *K. pneumoniae* ATCC BAA-2814: relebactam, vaborbactam
- Both tests should be part of the routine QC.

# QC testing procedure

- Perform QC daily, or at least four times per week.
  - Follow the same testing procedure as for clinical isolates.
- Include antimicrobial agents which are part of routine panels.
- QC results should be read and evaluated before reporting AST results for clinical isolates.
- Evaluate results against criteria in EUCAST QC Tables.

# QC ranges and targets

## *Escherichia coli* ATCC 25922

(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

See EUCAST Breakpoint Tables for short descriptions of MIC and disk diffusion methodology.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target <sup>1</sup>	Range <sup>2</sup>		Target <sup>1</sup>	Range <sup>3</sup>
Amikacin	1-2	0.5-4	30	22-23	19-26
Amoxicillin	4	<b>2-8</b>	-	-	-
Amoxicillin-clavulanic acid <sup>4,5</sup>	4	2-8	20-10	21	18-24 <sup>6</sup>
Ampicillin	4	2-8	10	18-19	15-22 <sup>6</sup>
Ampicillin-sulbactam <sup>5,7</sup>	<b>2</b>	<b>1-4</b>	10-10	21-22	19-24 <sup>6</sup>
Aztreonam	0.125	0.06-0.25	30	32	28-36
Cefadroxil	-	-	30	<b>17</b>	<b>14-20</b>
Cefalexin	8	4-16	30	<b>18</b>	<b>15-21</b>
Cefepime	0.03-0.06	0.016-0.125	30	34	31-37
Cefixime	0.5	0.25-1	5	<b>23</b>	<b>20-26</b>
Cefotaxime	0.06	0.03-0.125	5	<b>28</b>	<b>25-31</b>

### Range

Set to allow for random variation

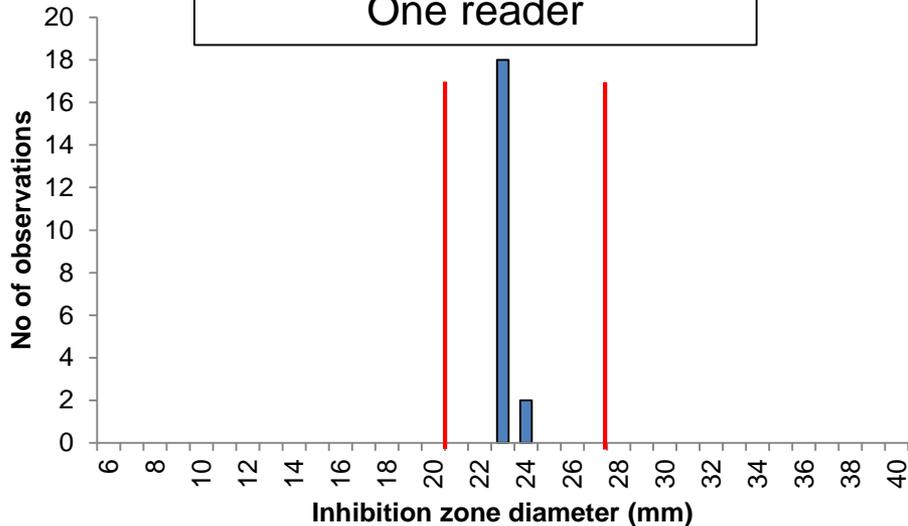
### Target

Mean values from repeated measurements should optimally be on target  $\pm 1$  mm (mode MIC on target)

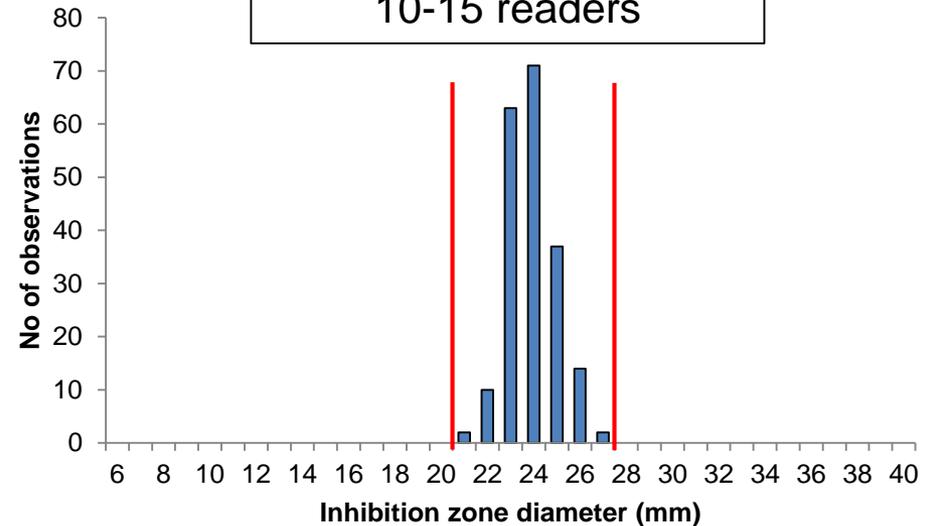
# Product accuracy vs. day-to-day variation

Example *E. coli* ATCC 25922 and piperacillin-tazobactam 30-6 µg

20 disks from one cartridge  
One test occasion  
One reader



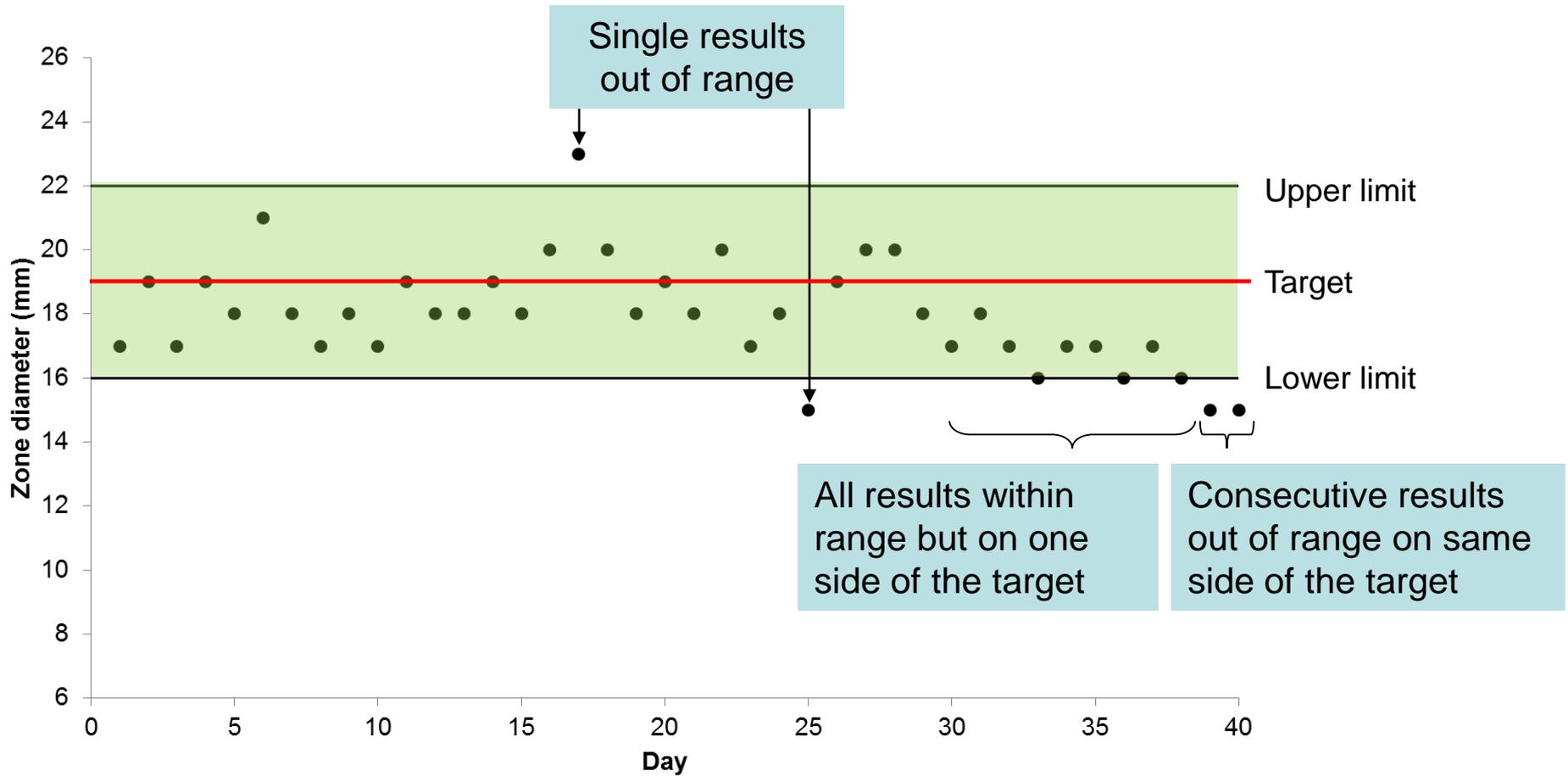
Routine data Växjö 2017  
200 test occasions  
10-15 readers



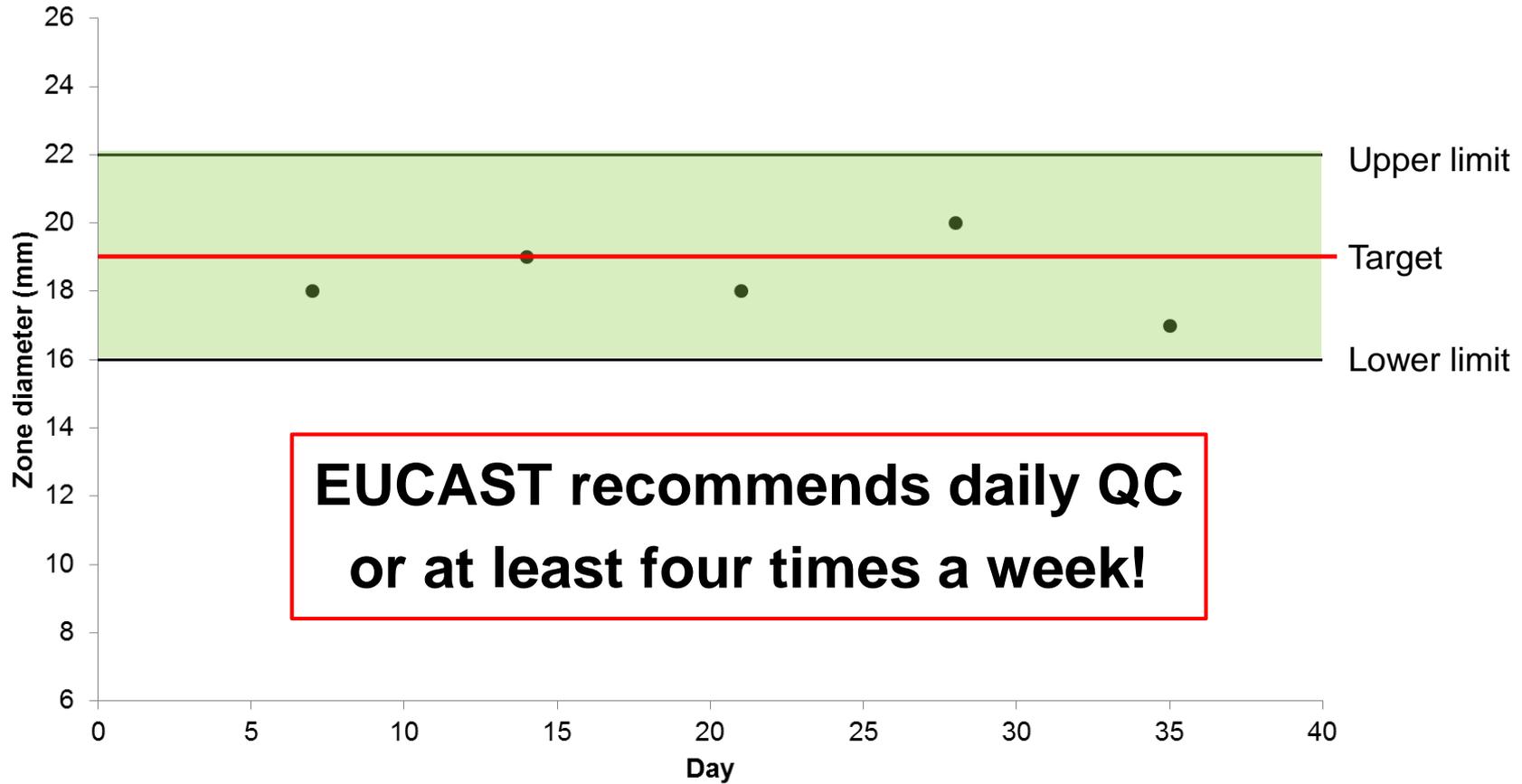
- Routine QC data

- Results randomly distributed within the QC range
- Mean value close to the target value
- Day-to-day variation due to small differences in
  - Inoculum preparation and plate inoculation
  - Incubation time and temperature
  - Reading of results

# Monitoring Laboratory QC results

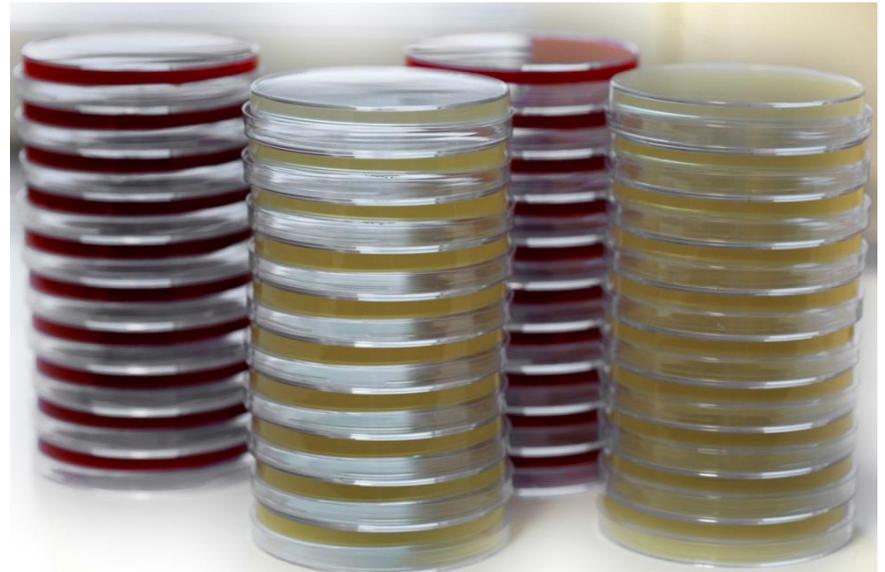


# Daily vs. weekly QC



# Potential sources of errors in disk diffusion

- Disks
- Media
- Not adhering to methodology
- Equipment
- QC strain



# Storage of antimicrobial disks

- Store disks according to manufacturers recommendations.
- Store disks in use in sealed containers with an indicating desiccant and protected from light.
- Allow disks to reach room temperature before opening containers, to prevent condensation.
- Do not use disks beyond the expiry date.

# Antimicrobial disks and media

## EUCAST evaluation of disk and media from different manufacturers

- **2014: Antimicrobial disks from 9 manufacturers**  
The quality of antimicrobial discs from nine manufacturers-EUCAST evaluations in 2014 and 2017. Åhman *et al*, CMI. 2019 Mar;25(3):346-352.
- **2019: Mueller-Hinton dehydrated media from 21 brands**  
EUCAST evaluation of 21 brands of Mueller-Hinton dehydrated media for disc diffusion testing. Åhman *et al*. CMI. 2020 Oct;26(10):1412.e1-1412.e5.
- **2020: Mueller-Hinton pre-poured plates from 10 brands**  
Evaluation of 10 brands of pre-poured Mueller-Hinton agar plates for EUCAST disc diffusion testing. Åhman *et al*. Accepted for publication in CMI.

# Disks from 9 manufacturers

Antimicrobial disk	Oxoid	Mast	BD	SirScan	Bio-analyse	Bio-Rad	Lio-filchem	Abtek	HiMedia
Benzylpenicillin 1 unit			L	H	H				NA
Amoxicillin-clavulanic acid 20-10 µg						H		L	H
Piperacillin-tazobactam 30-6 µg				H				L	NA
Oxacillin 1 µg			L	L	L		L		H
Mecillinam 10 µg				H	H			L	H
Cefotaxime 5 µg								NA	NA
Cefoxitin 30 µg						H*	H	NA	L*
Ceftazidime 10 µg								L	L
Meropenem 10 µg	H	H		H	H	H	H*	L	H
Ciprofloxacin 5 µg		L	L			L		L	H
Norfloxacin 10 µg				L				L	H*
Pefloxacin 5 µg			L	NA			L	NA	H
Gentamicin 10 µg			H	NA				L	H
Tobramycin 10 µg						NA	H		H*
Erythromycin 15 µg			L	L	L*		L	L	H
Tetracycline 30 µg		L	L*	L*	L		L	L	
% within range	100%	100%	99.0%	93.5%	93.1%	92.7%	90.2%	89.3%	66.7%
% out of range	0.0%	0.0%	1.0%	6.5%	6.9%	7.3%	9.8%	10.7%	33.3%

Mean value within  $\pm 1$  mm of the target value  
 Mean value  $>1$  mm but within  $\pm 2$  mm of the target value  
 Mean value  $>2$  mm from target value but still within the QC range  
 Mean value out of the QC range

NA = Not Available  
 H = High, mean value  $>1$  mm above target  
 L = Low, mean value  $>1$  mm below target  
 \* One or more readings out of QC range

# 21 brands of dehydrated Mueller-Hinton media

Total rating	Manufacturer	Product name	Percent zones on QC target $\pm 1$ mm	Percent zones outside QC range	Agents outside range, high	Agents outside range, low
-4	Bio-Rad	MH Agar	86	0		
-10	Biolife	MH Agar II	81	1.1	TS	
-10	Oxoid	MH Agar	78	1.1	TS	
-11	Sigma	MH Agar 2	81	0		
-12	Becton Dickinson	BBL MH II Agar	73	0		
-12	Hardy Diagnostics	CRITERION MH Agar	71	0		
-13	Becton Dickinson	Difco MH Agar	70	3.3	AM	
-14	Alpha Biosciences	MH Agar	71	3.3	FQ	
-17	E&O Laboratories	MH Agar	82	8.9	CA, FQ, AM	TS
-18	Sigma	MH Agar	57	3.3	CS	
-20	HiMedia	MH Agar	56	0		
-21	bioMérieux	MHE Agar	64	3.3	TS	
-22	Acumedia	MH Agar	63	3.3	AM	
-24	Remel	MH Agar	64	6.7	AM	TS
-25	Lab M	MH Agar	69	6.7	AM	TS
-25	Merck	MH Agar CLSI	66	6.7	AM, TS	
-27	Mast Group	MH Agar	59	8.9	CA, FQ	TS
-31	Sifin	MH Agar	60	6.7	AM, TS	
-32	HiMedia	MH Agar No. 2	50	6.7	CA, AM	
-40	Biolab	MH II Agar	52	10	PC, MA, TE	TS
-55	Merck	MH Agar	44	23	CS, CA, FQ, AM	TE

## Abbreviations

PC - Penicillins; CS - Cephalosporins; CA - Carbapenems; FQ - Flouroquinolones; AM - Aminoglycosides; MA - Macrolides; TE - Tetracyclines; TS - Trimethoprim-sulfamethoxazole

# 10 brands of pre-poured Mueller-Hinton plates

Total rating	Manufacturer	Product name	Percent zones on QC target $\pm 1$ mm	Percent zones outside QC range	Agents outside range, high	Agents outside range, low
-11	Becton Dickinson	BBL MH II Agar	70	0.0		
-14	bioMérieux	MHE	66	2.2	TS	
-17	Hardy Diagnostics	MH Agar	60	0.0		
-22	Oxoid	MH Agar	56	3.3	TS	
-23	E&O Laboratories	MH Agar	58	5.6	CA	TE
-25	Bio-Rad	MH Agar	48	1.1	AM	
-30	Biolife	MH Agar II	50	7.8	AM, TS	
-40	Liofilchem	MH II Agar	50	8.9	AM, TS	
-54	HiMedia	MH Agar No. 2	34	18	PC, CS, CA, FQ, AM, TS	
-61	HiMedia	MH Agar	27	20	PC, CS, CA, FQ, AM, TS	

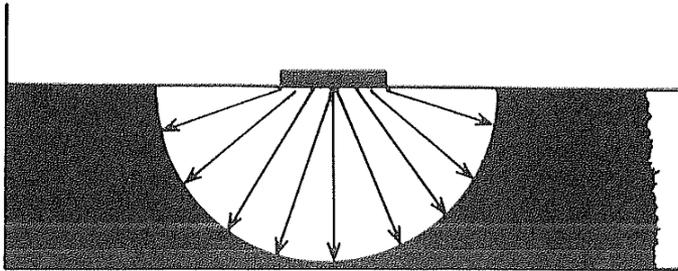
The quality of pre-poured MH plates were generally poorer than for in-house prepared plates of the same brand in our previous study!

#### Abbreviations

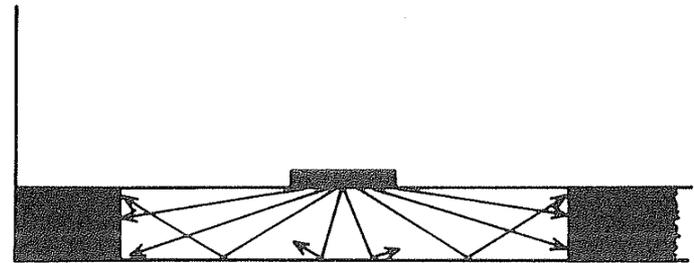
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# Agar depth

- The diffusion of the antimicrobial agent is affected by the agar depth



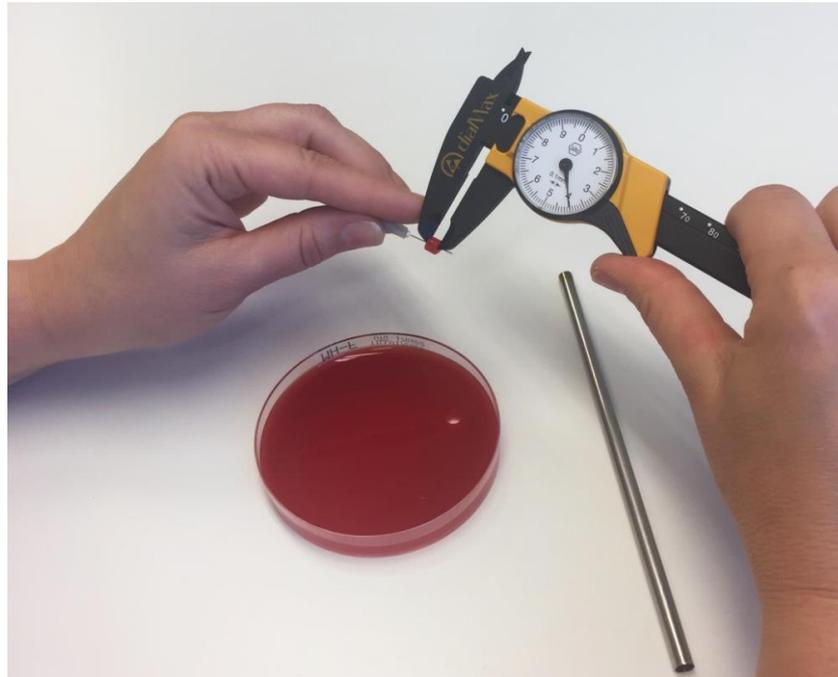
**Correct agar depth**



**Agar too thin**

- Agar depth  $< 4$  mm: Larger zone diameters
- Agar depth  $> 4$  mm : Smaller zone diameters

# Measuring agar depth



Adjust the volume if the agar depth is repeatedly above or below 4.0 mm!

# Drying and storage of agar plates

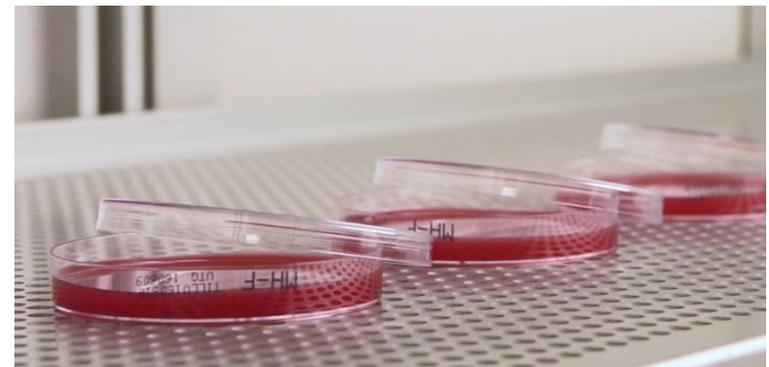
- Store plates at 4-8°C
- Make sure agar plates are at room temperature prior to inoculation.
- No drops of water should be visible on the surface of the agar or inside the lid.
- Excess moisture may cause fuzzy zone edges and/or haze within zones.



# Drying and storage of agar plates

- **In-house production:**
  - Dry newly produced plates in room temperature over night to remove excess humidity, before storing them in the fridge.
- **Plates stored in plastic bags or sealed containers:**
  - Unpack plates to be used within a short time and store in ventilated racks in the fridge.

- If necessary, dry plates
  - 20-25°C overnight or
  - 35°C for 15 min  
with the lid removed



# QC of broth microdilution

- Problems with short concentration range in BMD panels
- Susceptible QC strains may give MICs below the lowest concentrations
  - Truncated MICs can not be evaluated.
- Test several QC strains to get results within the concentration range for as many agents as possible
- Add specific QC strains to control the inhibitor component

## External controls

- Test clinical isolates with known reference MICs
  - For example UKNEQAS isolates.
- Compare MIC distributions with reference distributions on the EUCAST website.

# Examples QC BMD

## Haemophilus panel

### *H. influenzae* ATCC 49766

AMC	AMO	AMP	CTX	CRO	CXM	CIP	DOX	IMI	MER	PTZ	TET	TSU
0.5	0.5	≤0.25	≤0.015	≤0.015	1	≤0.015	0.5	0.5	≤0.03	≤0.008	0.5	≤0.06
≤0.25	0.5	≤0.25	≤0.015	≤0.015	1	≤0.015	0.5	0.5	≤0.03	≤0.008	0.5	≤0.06
0.5	0.5	≤0.25	≤0.015	≤0.015	1	≤0.015	0.5	0.5	≤0.03	≤0.008	0.5	≤0.06

Target 0.25 0.25 0.125 0.008 0.004 0.5 0.008 0.5 0.5 0.06 - 0.5 0.03  
 Range 0.125-0.5 0.125-0.5 0.06-0.25 0.004-0.016 0.002-0.008 0.25-1 0.004-0.016 0.25-1 0.25-1 0.03-0.12 - 0.25-1 0.016-0.06

### *S. pneumoniae* ATCC 49619

AMC	AMO	AMP	CTX	CRO	CXM	CIP	DOX	IMI	MER	PTZ	TET	TSU
≤0.25	≤0.25	≤0.25	0.06	0.06	≤0.25	1	≤0.12	≤0.25	0.06	0.5	0.25	0.25
≤0.25	≤0.25	≤0.25	0.06	0.06	≤0.25	0.5	≤0.12	≤0.25	0.06	0.5	0.25	0.25
≤0.25	≤0.25	≤0.25	0.06	0.06	≤0.25	0.5	≤0.12	≤0.25	0.06	0.5	0.25	0.5

Target 0.06 0.06 0.12 0.06 0.06 0.5 - 0.03-0.06 0.06 0.06-0.12 - 0.12-0.25 0.25-0.5  
 Range 0.03-0.12 0.03-0.12 0.06-0.25 0.03-0.12 0.03-0.12 0.25-1 - 0.016-0.12 0.03-0.12 0.03-0.25 - 0.06-0.5 0.12-1

### *E. coli* ATCC 25922

AMC	AMO	AMP	CTX	CRO	CXM	CIP	DOX	IMI	MER	PTZ	TET	TSU
8	8	4	0.12	0.12	8	≤0.015	1	≤0.25	≤0.03	4	1	≤0.06
8	8	4	0.06	0.12	8	≤0.015	1	≤0.25	≤0.03	2	1	≤0.06
8	4	4	0.06	0.06	8	≤0.015	1	≤0.25	≤0.03	4	1	≤0.06

Target 4 4 4 0.06 0.06 4 0.008 1 0.125-0.25 0.015-0.03 2 1 ≤0.5  
 Range 2-8 2-8 2-8 0.03-0.12 0.03-0.12 2-8 0.004-0.015 0.5-2 0.06-0.5 0.008-0.06 1-4 0.5-2

On target
Upper limit
Lower limit
Out of range

# QC of gradient tests

- No EUCAST recommendations for gradient tests.
- Manufacturers are responsible for calibrating the product against reference methodology (broth microdilution).
- Users should follow the manufacturers instructions for storage, handling and testing.
  - Media, inoculum, incubation
- Use EUCAST QC strains and criteria
  - Do not use criteria provided by the manufacturer (package inserts)
- Susceptible QC strains will only control the lower end of the concentration range.
- Add specific QC strains to control the inhibitor component

# Contact us for support!



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