

# **En ny europeisk standardmetod för lappdiffusion på Mueller Hinton-agar**

Erika Matuschek

Klinisk mikrobiologi

Centrallasarettet, Växjö

# Skillnader EUCAST / CLSI

- Referensstammar
- Antal plattor
- QC-gränser
- Kliniska brytpunkter

# Två media

- Mueller Hinton-agar (MH)  
för Enterobacteriaceae, Pseudomonas,  
stafylokocker och enterokocker
- Mueller Hinton-agar med 5% hästblod och  
20 mg/L  $\beta$ -NAD i 5% CO<sub>2</sub> (MH-F)  
för krävande organismer (Haemophilus,  
pneumokocker och övriga streptokocker)

# Mueller Hinton-agar

- Nackdelar
  - Variation i tymin- and tymidinnivåer
  - Variation av tvåvärda katjoner ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ )
- Fördelar
  - Flera leverantörer
  - Standardiserad av CLSI stor-databas!

# Mueller Hinton med blod (MH-F)

- Hästblod / Fårblod
  - HI växte bra på MHA med 5% hästblod och 20 mg/L  $\beta$ -NAD
  - Med 5% fårblod krävdes  $> 100$  mg/L  $\beta$ -NAD
- $\beta$ -NAD från olika leverantörer
  - HI och pnc växte med 10, 20, 30 and 40 mg/L  $\beta$ -NAD (åtta olika märken)

# Inokulat och inkubering

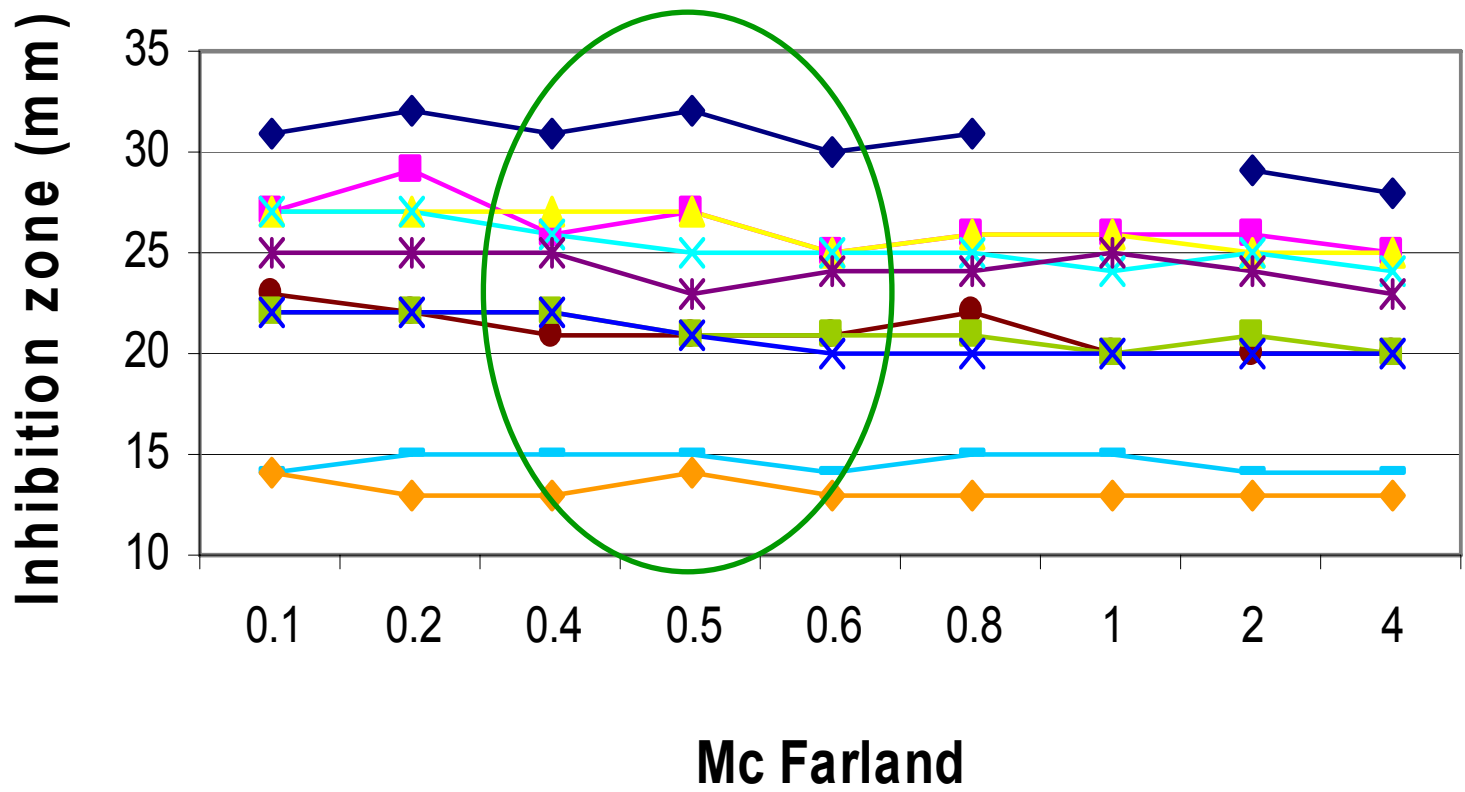
- Konfluerande inokulat  
McFarland 0,5
  - + lättare att upptäcka heteroresistens
  - mer material behövs
  - svårare att upptäcka kontaminering
- Inkubering  $18\pm 2\text{h}$  i  $35\pm 1^\circ\text{C}$   
MH i luft och MH-F i 5%  $\text{CO}_2$

# Kontroll av inokulatet

<b>McFarland 0.5</b>	<b>CFU /mL</b>
<i>E. coli</i> ATCC 25922	$4 \times 10^8$
<i>P. aeruginosa</i> ATCC 27853	$4 \times 10^8$
<i>S. aureus</i> ATCC 29213	$5 \times 10^8$
<i>S. epidermis</i> ATCC 12228	$1 \times 10^8$
<i>E. faecalis</i> ATCC 29212	$1 \times 10^8$
<i>H. influenzae</i> NCTC 8468	$2 \times 10^8$
<i>S. pneumoniae</i> ATCC 49619	$1 \times 10^8$

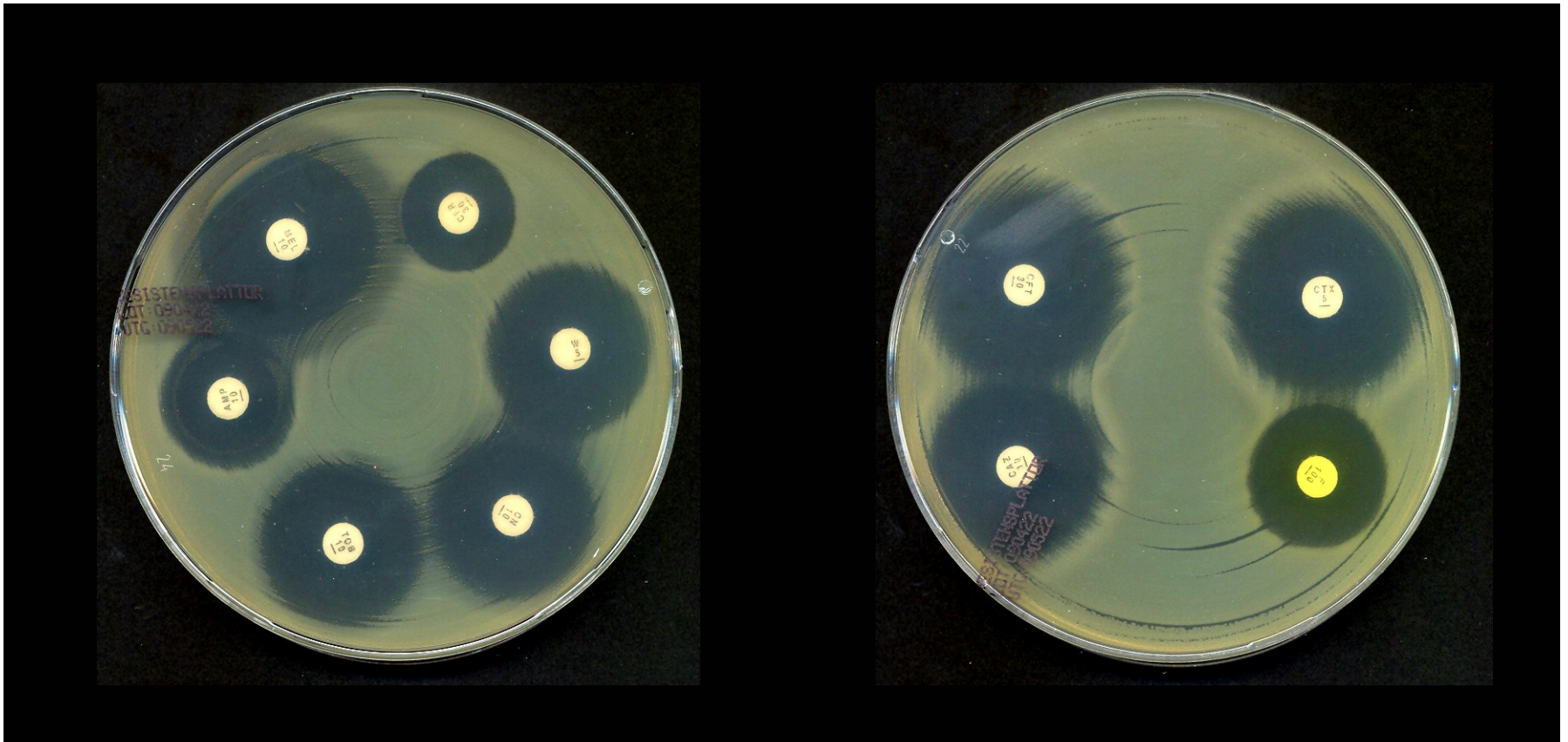
# Förhållande mellan inokulatets tjocklek och storleken på hämningszonen

*E. coli* ATCC 25922

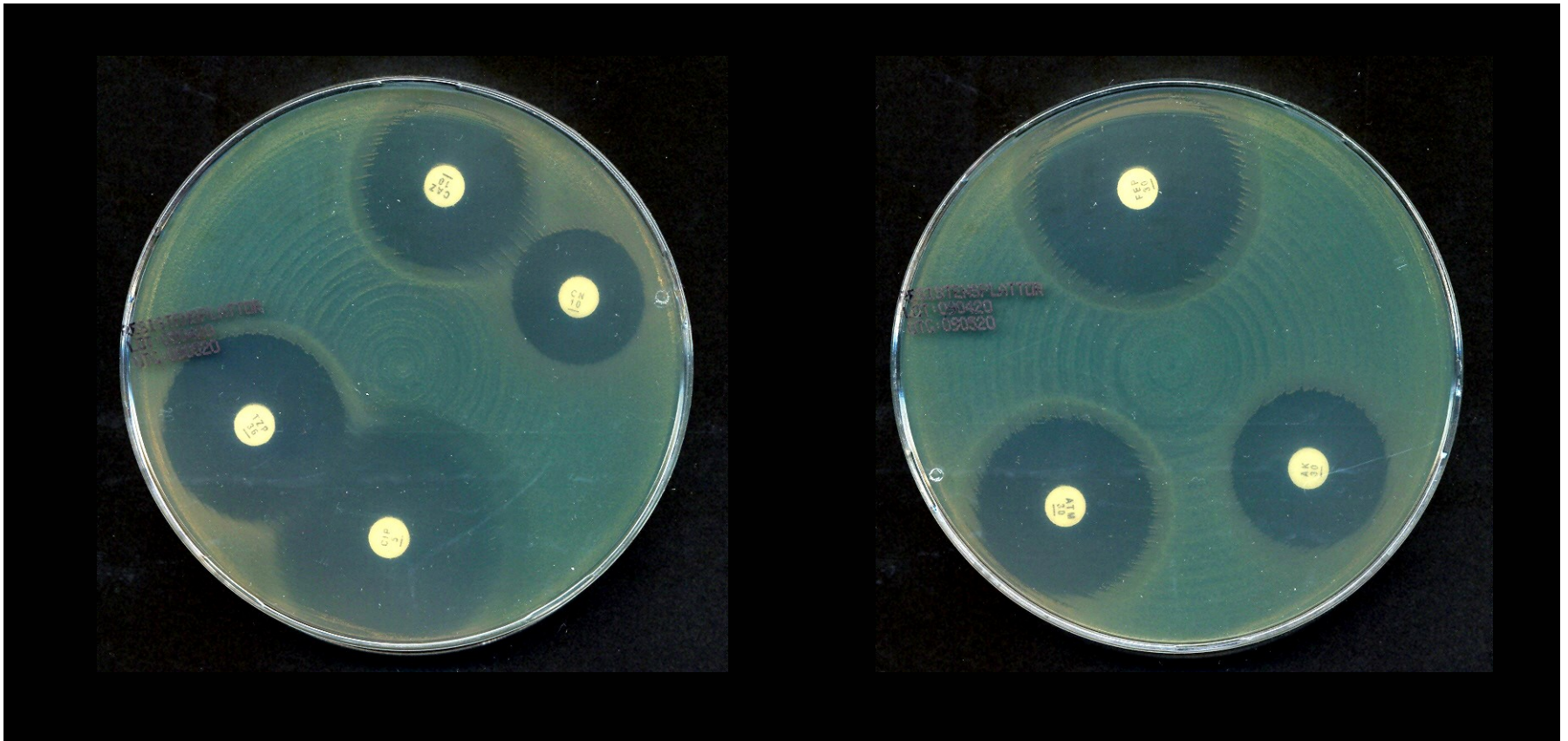


- MEM
- TSU
- NAL
- CTX
- CAZ
- TOB
- PTZ
- GEN
- CDR
- AMP

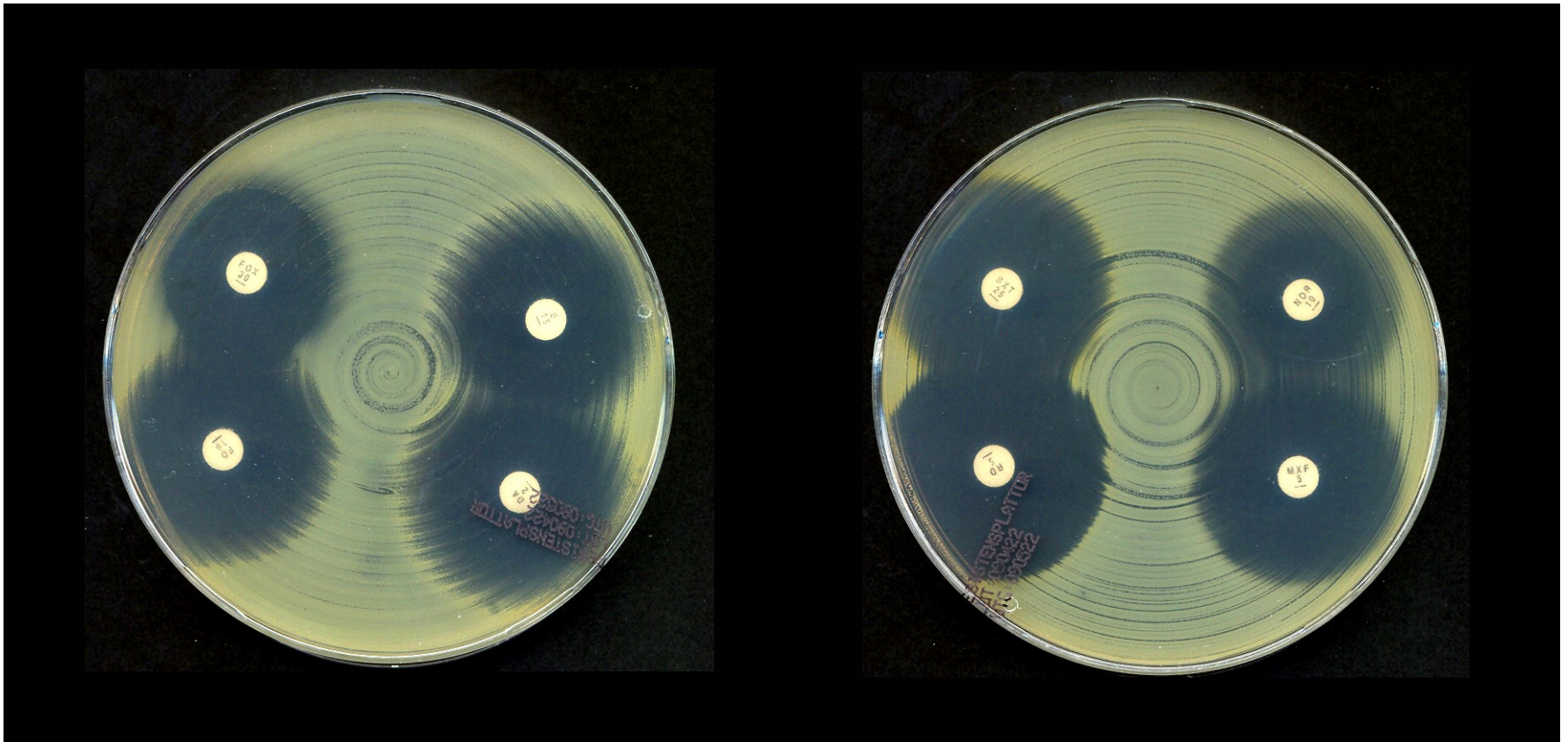
# *Escherichia coli* ATCC 25922



# *Pseudomonas aeruginosa* ATCC 27853

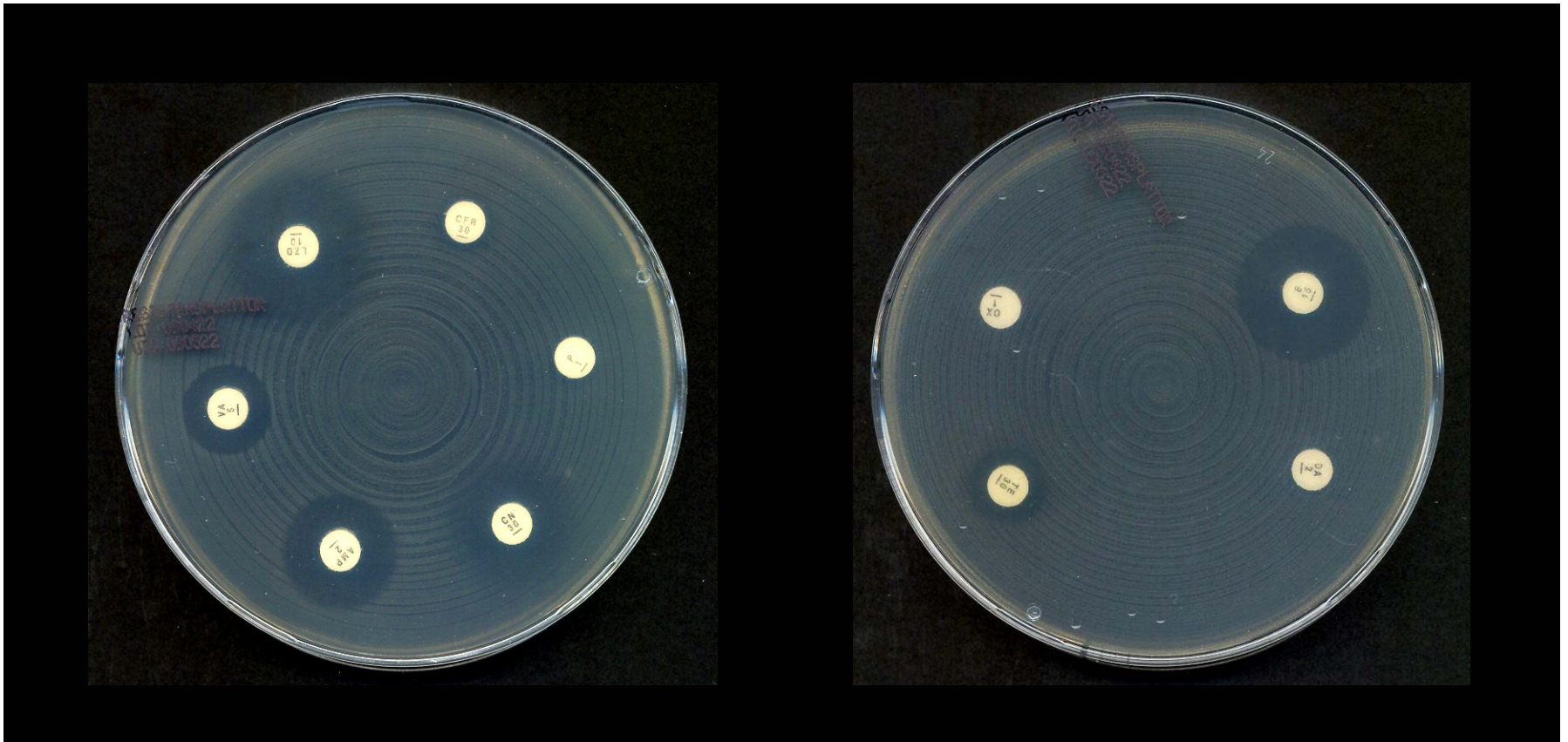


# *Staphylococcus aureus* ATCC 29213



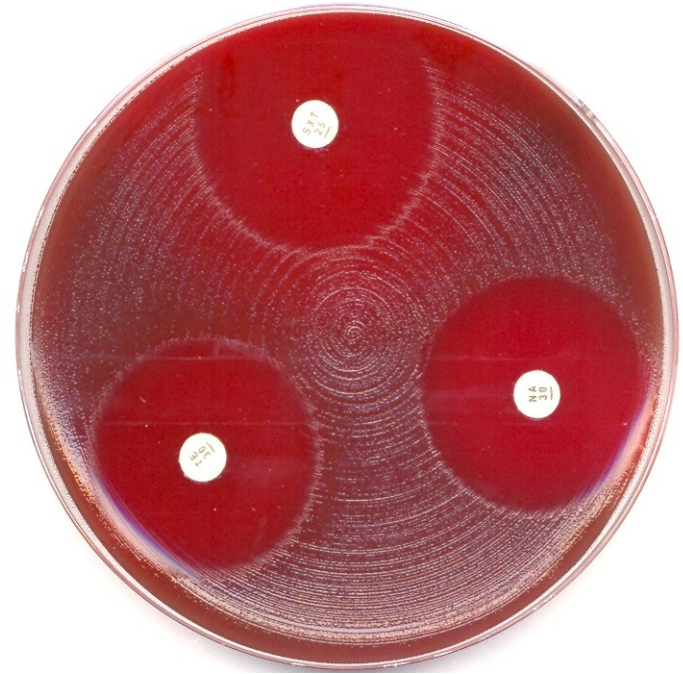
# *Enterococcus faecalis*

## ATCC 29212



# *Haemophilus influenzae*

## NCTC 8468



# *Streptococcus pneumoniae* ATCC 49619



# *Streptococcus pyogenes*

## CCUG 25571



# QC-gränser

- Varje antibiotika har testats på referensstammar vid 20 tillfällen och jämförts med "CLSI targets"
- För antibiotika och organismer som saknas i CLSI:s tabeller har vi tagit fram nya gränser

# QC-stammar

## EUCAST

*E. coli* ATCC 25922  
*P. aeruginosa* ATCC 27853  
*S. aureus* ATCC 25923  
*H. influenzae* ATCC 49247  
*S. pneumoniae* ATCC 49619  
*S. aureus* ATCC 29213  
*E. faecalis* ATCC 29212  
*H. influenzae* NCTC 8468  
*S. pyogenes* CCUG 25571

## CLSI

*E. coli* ATCC 25922  
*P. aeruginosa* ATCC 27853  
*S. aureus* ATCC 25923  
*H. influenzae* ATCC 49247  
*S. pneumoniae* ATCC 49619

# EUCAST mean / CLSI target

	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>S. aureus</i> ATCC 25923
=CLSI target	8/29	3/15	9/31
≤ 1mm från CLSI target	24/29	10/15	30/31
≤ 2mm från CLSI target	29/29	15/15	31/31

# MIC and Zone Diameter Limits for Quality Control Strains

## *Escherichia coli* ATCC 25922

Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2 h. Read complete inhibition from the back of the plates against a black background illuminated with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone size (mm)		Comments
	Target	Range <sup>1</sup>		Target	Range <sup>2</sup>	
Amikacin	1-2	0.5-4	30	23	19-26	
Amoxicillin-clavulanic acid	4/2	2/1-8/4	20/10	21	18-24	
Ampicillin	4	2-8	10	19	16-22	Ignore faint growth that appears as inner zone.
Aztreonam	0.12	0.06-0.25	30	32	28-36	
Cefadroxil	-	-	30	<b>18</b>	<b>15-21</b>	
Cefepime	0.03-0.06	0.015-0.12	30	34	31-37	
Cefotaxime	0.06	0.03-0.12	5	<b>28</b>	<b>25-31</b>	
Cefotaxime	0.06	0.03-0.12	30	32	29-35	
Cefoxitin	4	2-8	30	26	23-29	
Cefpodoxime	0.5	0.25-1	10	26	23-28	
Ceftazidime	0.12-0.25	0.06-0.5	10	<b>26</b>	<b>23-29</b>	
Ceftazidime	0.12-0.25	0.06-0.5	30	29	25-32	
Ceftibuten	0.25	0.12-0.5	30	31	27-35	
Cefuroxime	4	2-8	30	23	20-26	
Chloramphenicol	4	2-8	30	24	21-27	
Ciprofloxacin	0.008	0.004-0.015	5	35	30-40	Zone edges for fluoroquinolones are usually fuzzy.
Ertapenem	0.008	0.004-0.015	10	33	29-36	
Gentamicin	0.5	0.25-1	10	23	19-26	
Gentamicin	0.5	0.25-1	30	<b>24</b>	<b>21-27</b>	
Imipenem	0.12	0.06-0.25	10	29	26-32	
Mecillinam	0.06-0.12	0.03-0.25	10	27	24-30	
Meropenem	0.015-0.03	0.008-0.06	10	31	28-34	
Moxifloxacin	0.015-0.03	0.008-0.06	5	32	28-35	
Nalidixic acid	2	1-4	30	25	22-28	
Nitrofurantoin	8	4-16	100	<b>21</b>	<b>18-24</b>	
Nitrofurantoin	8	4-16	300	23	20-25	
Norfloxacin	0.06	0.03-0.12	10	32	28-35	Zone edges for fluoroquinolones are usually fuzzy.
Piperacillin-tazobactam	2/4	1/4-4/4	30/6	<b>24</b>	<b>21-27</b>	
Piperacillin-tazobactam	2/4	1/4-4/4	100/10	27	24-30	
Rifampicin	8	4-16	5	9	8-10	
Tetracycline	1	0.5-2	30	22	18-25	
Tigecycline	0.12	0.03-0.25	15	24	20-27	
Tobramycin	0.5	0.25-1	10	22	18-26	
Tobramycin	0.5	0.25-1	30	<b>24</b>	<b>21-27</b>	
Trimethoprim	1	0.5-2	5	25	21-28	
Trimethoprim-sulfamethoxazole	≤0.5/9.5	≤0.5/9.5	1.25/23.75	26	23-29	

# Enterobacteriaceae

Penicillins <sup>1</sup>	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)			Notes (numbers for comments on MIC breakpoints, letters for comments on disk diffusion)
	S ≤	R >		S ≥	I	R <	
							1. For aminopenicillin breakpoints, the resistant breakpoint of R>8 mg/L ensures that all isolates with resistance mechanisms are reported resistant. The wide range of dosages and intravenous vs. oral administration significantly affect therapeutic efficacy. The unspecified S breakpoint enables the user to categorise wild type Escherichia coli and Proteus mirabilis S or I to the aminopenicillins. This will depend on dosing, route of administration and on whether the infection is systemic or affects the urinary tract only.
Benzylopenicillin	-	-					
Ampicillin	Note <sup>1</sup>	8	10				
Ampicillin-sulbactam <sup>2</sup>	Note <sup>1</sup>	8	10-10				2. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L
Amoxicillin	Note <sup>1</sup>	8	10				
Amoxicillin-clavulanate <sup>3</sup>	Note <sup>1</sup>	8	30-10				3. For susceptibility testing purposes, the concentration of clavulanate is fixed at 2 mg/L
Piperacillin	8	16	100				
Piperacillin-tazobactam <sup>4</sup>	8	16	100/10				4. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L
Ticarcillin	8	16	75				
Ticarcillin-clavulanate <sup>3</sup>	8	16	75-10				See note 3
Phenoxymethylpenicillin	-	-					
Mecillinam <sup>5</sup>	8	8	10				5. Breakpoints apply to uncomplicated, lower urinary tract infections only.
Oxacillin	-	-					
Cloxacillin	-	-					
Dicloxacillin	-	-					
Flucloxacillin	-	-					

Cephalosporins <sup>1</sup>	MIC breakpoint		Disk content (µg)	Zone diameter breakpoint (mm)			Notes (numbers for comments on MIC breakpoints, letters for comments on disk diffusion)
	S ≤	R >		S ≥	I	R <	
							1. The cephalosporin breakpoints for Enterobacteriaceae will detect resistance mediated by most ESBLs and other clinically important beta-lactamases in Enterobacteriaceae. However, some ESBL-producing strains may appear susceptible or intermediate with these breakpoints. Laboratories may want to use a test which specifically screens for the presence of ESBL.
Cefadroxil			30				
Cefazolin	-	-					
Cefepime	1	8	30				
Cefotaxime	1	2	30				
Cefoxitin			10				
Ceftazidime	1	8	30				
Ceftibuten			30				
Ceftriaxone	1	2	30				
Cefuroxime <sup>2</sup>	8	8	30				2. The non-species related S/I breakpoint of 4 mg/L divides the wild type MIC distributions of relevant Enterobacteriaceae. To avoid this, the S/I-breakpoint has been increased to 8 mg/L. The breakpoint pertains to a dosage of 1.5 g x 3 and to E.coli and Klebsiella spp only.

# Pågående arbete...

- Histogram
- MIC-värden
  - Referensstammer
  - Validering av MH-F som substrat för Etest
  - Zon/MIC-korrelat
    - SIR-brytpunkter kalibrerade mot EUCAST
    - kliniska MIC-brytpunkter
- Problematiska antibiotika / organismer
- Andra krävande organismer  
(GC, MNC, anaerober)