Bacteriële gevoeligheidsbepalingen: algemene principes en technieken

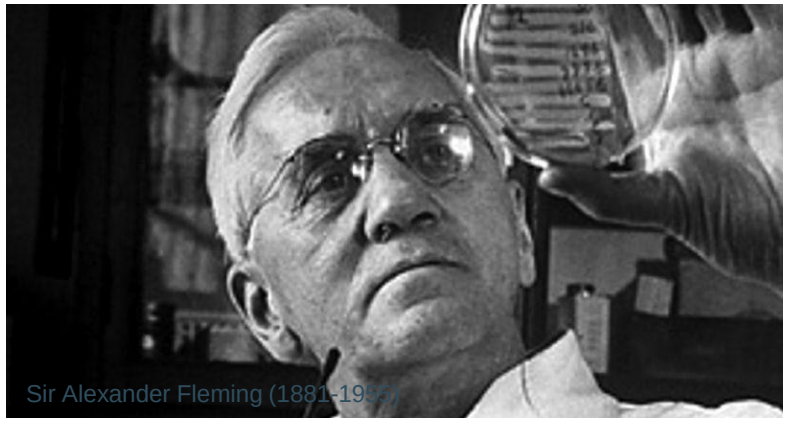
Stefanie Desmet

28/01/2025

- Historiek
- Methoden voor gevoeligheidsbepalingen
- Interpretatie van antibiogram

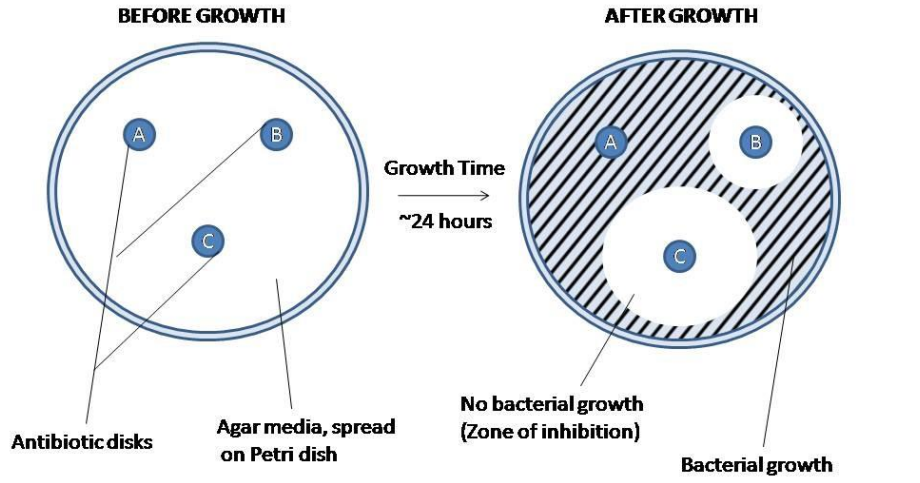
Historiek gevoeligheidsbepalingen (AST)

- 1924
  - Ditch test
    - In agar plaat groef waar oplossing met te testen substantie (antiseptica) werd toegevoegd waarna groei van te testen stam werd onderzocht
- 1929
  - broth dilutie test gebaseed op turbiditeitsmeting


Sir Alexander Fleming (1881-1955)

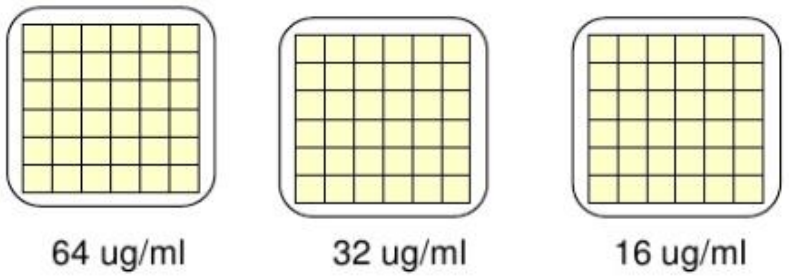
Historiek gevoeligheidsbepalingen

- Jaren ‘40
  - **Diffusie methoden**
    - absorberend papier dat antimicrobiële oplossing bevat (Heatley, Vincent & Vincent, Moh)
    - tabletten met penicilline (Hoyt & Levine)

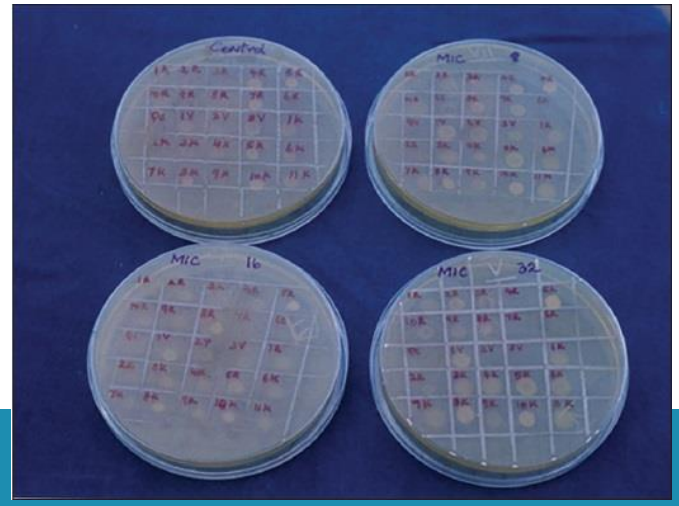


Historiek gevoeligheidsbepalingen

- Jaren ‘40
  - **Diffusie methoden**
    - absorberend papier dat antimicrobiële oplossing bevat (Heatley, Vincent & Vincent, Moh)
    - tabletten met penicilline (Hoyt & Levine)
  - **Agar dilutie** technieken (Smith & Reymann)
    - Antibiotica in agar verwerken
    - Eén concentratie antibioticum per plaat



incubation

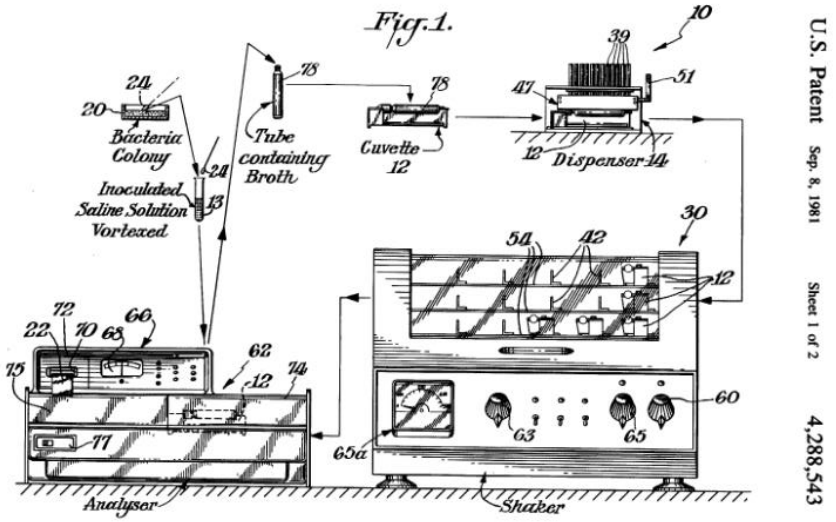


Historiek gevoeligheidsbepalingen

- Jaren ‘50
  - Nood aan standaardisatie (vele variabelen)
- Jaren ‘ 70
  - Standaardisatie **disk diffusie**:
    - NCCLS disc diffusion standards
    - Europa: 6 verschillende systemen (Zweeds, Nederlands, Duits, Frans, Brits en NCCLS)

Historiek gevoeligheidsbepalingen

- 1974
  - Eerste semi-geautomatiseerde AST methode
    - Autobac disc elution system (Pfizer Diagnostics)



Historiek gevoeligheidsbepalingen

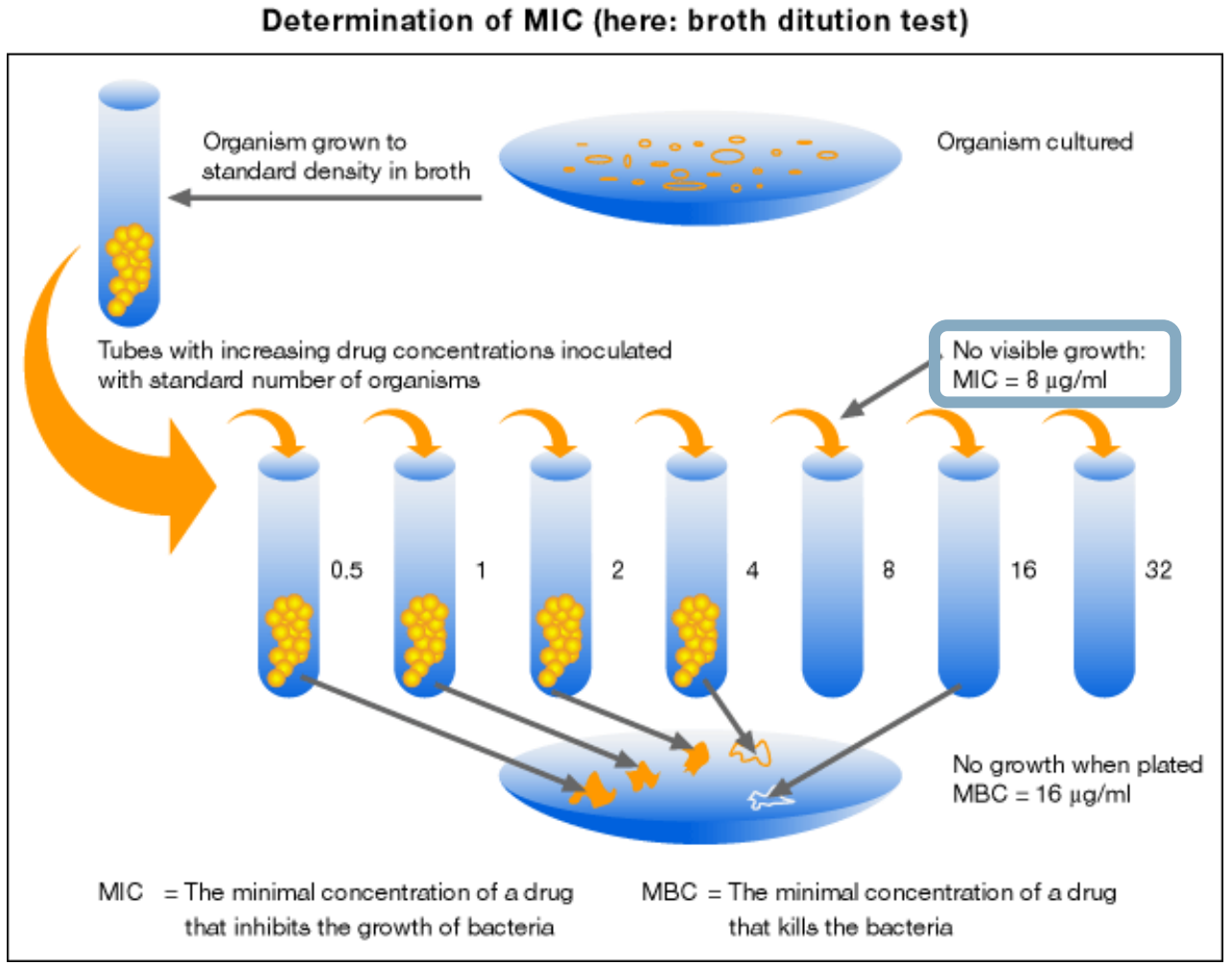
- 1977
  - Abbott MS-2 System
  - AMS system (McDonnell Douglas Corporation)
    - Gedehydrateerde reagentia in plastieken kaarten
    - Voorloper VITEK® system bioMérieux (1989 op de markt)

Historiek gevoeligheidsbepalingen

- Vanaf jaren 70
  - Gestandaardiseerde microtiter platen met antibiotica
    - Micro-Media Systems
    - Sensititre
    - BBL Sceptor
    - Micronaut
    - Microscan
      - Basis microtiter systeem tot meer complexe systemen

- Jaren ‘90 - begin 2000
  - geautomatiseerde systemen gebaseerd op microdilutie principe

Minimale inhibitorische concentratie (MIC)



overnacht incubatie
+/- 37°C

Methoden voor gevoeligheidsbepalingen

- **Referentie methoden**

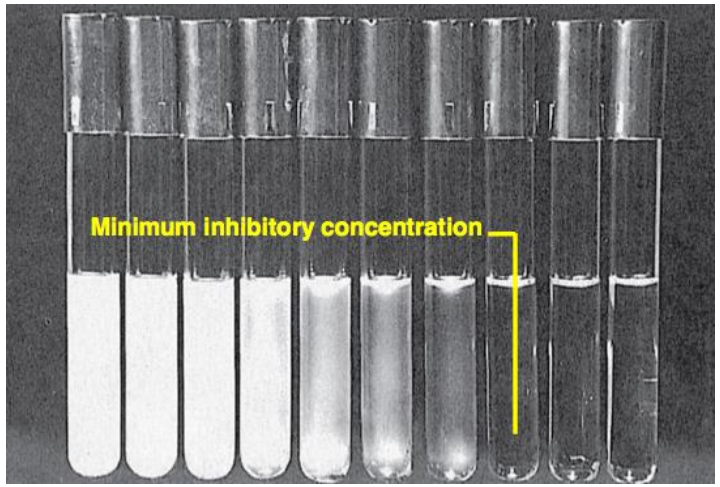
(reagentia kunnen verkregen worden vanuit verschillende bronnen en kunnen gemaakt worden in het laboratorium zonder een nood aan een complex manufacturing proces)

  - Broth macrodilutie (CLSI)
  - **Broth microdilutie** (CLSI, EUCAST, ISO)
  - Agar dilutie (CSLI)
  - Disk diffusie (EUCAST)

- **Commerciële methoden**
  - meestal geautomatiseerd en mechanisch (Vitek, Microscan, Phoenix, Sensititre)
  - Gradiënt diffusie testen (Etest, Epsilometer)

Broth dilutie methoden

- Macrodilutie (CLSI) (volume >=1mL)
  - Arbeidsintensief
  - Niet geschikt voor routine gevoeligheidsbepaling



- Microdilutie (internationale standaard)
  - CLSI en ISO/EUCAST
  - Ook commerciële ingevroren of gevriesdroogde platen (oa. Sensititre, Micronaut)

Broth microdilutie

Sensititre (Thermo Scientific)

Microscan Autoscan (Beckmann Coulter)

Broth microdilutie

Voorbeeld van een 96 well plaat in routine gebruikt voor gevoeligheidsbepaling van reserve antibiotica bij gram-negatieven

Plate Code: **DKMGN** Date: **12-Oct-16**

	1	2	3	4	5	6	7	8	9	10	11	12
A	MERO	MERO	MERO	MERO	MERO	MERO	MERO	MERO	AMI	AMI	AMI	AMI
	0.12	0.25	0.5	1	2	4	8	16	4	8	16	32
B	GEN	GEN	GEN	GEN	GEN	AZT	AZT	AZT	AZT	AZT	AZT	AZT
	0.5	1	2	4	8	0.5	1	2	4	8	16	32
C	CIP	CIP	CIP	CIP	CIP	CIP	P/T4	P/T4	P/T4	P/T4	P/T4	P/T4
	0.06	0.12	0.25	0.5	1	2	1/4	2/4	4/4	8/4	16/4	32/4
D	AUGC	AUGC	AUGC	AUGC	AUGC	C/T	C/T	C/T	C/T	C/T	C/T	C/T
	4/2	8/2	16/2	32/2	64/2	0.5/4	1/4	2/4	4/4	8/4	16/4	32/4
E	COL	COL	COL	COL	COL	COL	FOT	FOT	FOT	FOT	FOT	TOB
	0.25	0.5	1	2	4	8	0.5	1	2	4	8	1
F	TGC	TGC	TGC	TGC	TGC	SXT	SXT	SXT	SXT	TOB	TOB	TOB
	0.25	0.5	1	2	4	1/19	2/38	4/76	8/152	2	4	8
G	TAZ	TAZ	TAZ	TAZ	TAZ	TAZ	CZA	CZA	CZA	CZA	CZA	NEG
	0.5	1	2	4	8	16	0.5/4	1/4	2/4	8/4	16/4	
H	IMI	IMI	IMI	IMI	IMI	IMI	ETP	ETP	ETP	ETP	ETP	POS
	0.5	1	2	4	8	16	0.12	0.25	0.5	1	2	

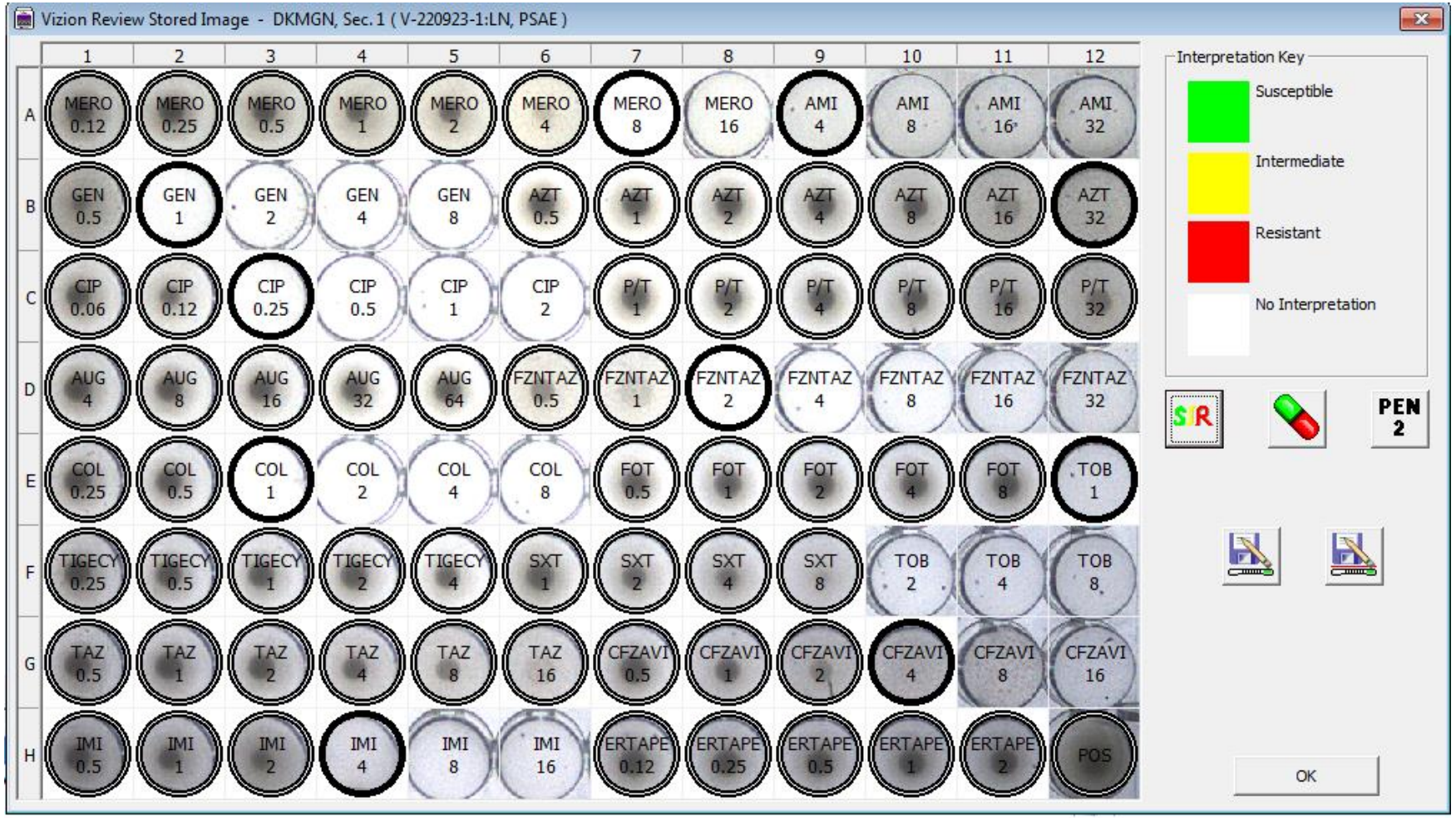
READ METHOD: VIZION/MANUAL

ANTIMICROBICS

MERO	Meropenem
GEN	Gentamicin
CIP	Ciprofloxacin
AUGC	Amoxicillin / clavulanic acid constant 2
COL	Colistin
TGC	Tigecycline
TAZ	Ceftazidime
IMI	Imipenem
AZT	Aztreonam
C/T	Ceftolozane/tazobactam 4
SXT	Trimethoprim / sulfamethoxazole
P/T4	Piperacillin / tazobactam constant 4
FOT	Cefotaxime
CZA	Ceftazidime/avibactam
ETP	Ertapenem
AMI	Amikacin
TOB	Tobramycin
NEG	Negative Control
POS	Positive Control

Broth microdilutie

Voorbeeld van een resultaat van 96 well plaat in routine gebruikt voor gevoeligheidsbepaling van reserve antibiotica bij gram-negatieven



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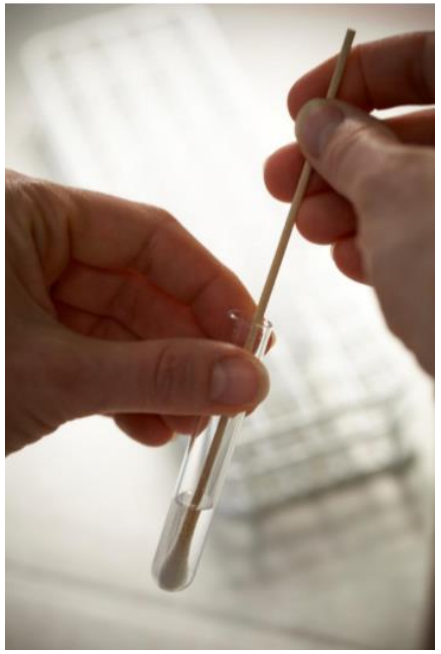
Disk diffusie

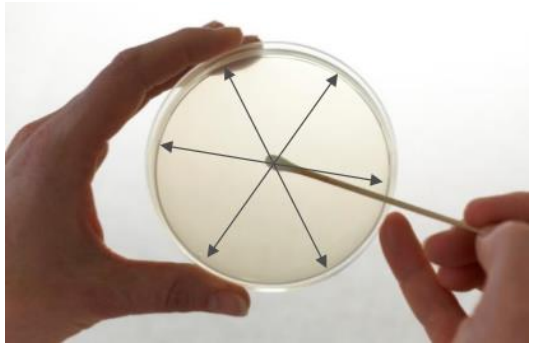
- Disk
  - Papieren disk

  opgelet met bewaring!
  - Rosco Neosensitab
- Agar
  - Mueller-Hinton agar
  - Mueller-Hinton agar met supplementen
    - MH + 5% schapen bloed
    - MH + 5% gedefibrineerd paarden bloed + β-NAD + (=MH-F(astidious))
- Inoculatie
- Aflezing

Inoculatie disk diffusie

- Pik enkele overnacht gegroeide kolonies (niet selectief medium) met steriele katoen wisser of entoog
- Suspendeer de kolonies in saline
- Zorg voor suspensie met densiteit van 0,5 McFarland (+/- 10^8 CFU/mL)
- Verwijder overvloedig vloeistof door swab tegen de rand van de tube te draaien
- Verspreid suspensie gelijkmatig over de plaat door in drie richtingen te inoculeren





Inoculatie disk diffusie

Inoculatie Disk diffusie

Te laag inoculum → opnieuw inzetten

Inoculatie disk difussie

- MH platen moeten op kamertemperatuur zijn voor inoculatie
- 15-15-15 minuten regel
    - Gebruik het inoculum binnen de **15 minuten** na preparatie (zeker binnen 60 minuten)
    - Plaats de disks binnen **15 minuten** na het inoculeren van de platen
    - Incubeer de platen binnen **15 minuten** na het aanbrengen van de disks

- Zie EUCAST website voor instructiefilm http://www.eucast.org/videos_from_eucast/#c19324
- Zie CLSI en EUCAST breekpunt tabellen voor details per bacterie

Aflezing disk diffusie

- Aflezing na 16-20u incubatie
  - 24u voor vancomycine en oxacilline van respectievelijk entorokokken en stafylokokken
  - Andere uitzonderingen: trage groeiers,, campylobacter
- Meet de zones
  - Reflecterend licht (niet tegen het licht houden of geen vergrootglas gebruiken, tenzij anders gespecifieerd)
    - MH
      - Meet via achterkant van de plaat tegen zwarte achtergrond
    - MH-F of MH bloed
      - Verwijder deksel en meet zones voorkant

Aflezing disk diffusie

- Aflezing van zone: punt van complete inhibitie van de groei afgelezen op 30 cm van het naakte oog

E. coli
Ciprofloxacin

S. aureus
Erythromycin

CoNS
Trimethoprim

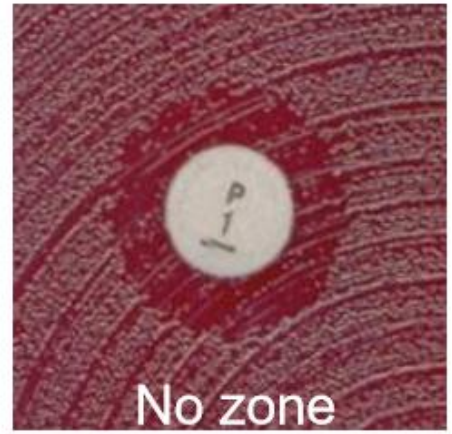
S. pneumoniae
Rifampicin

Ingroeiende kolonies

- Controleer zuiverheid
- Voer subcultuur uit
- Hertest indien noodzakelijk
- Indien geen contaminant: hou rekening met deze kolonies bij aflezing

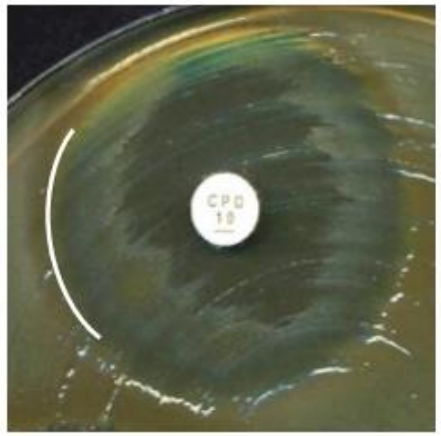
E. coli with ESBL

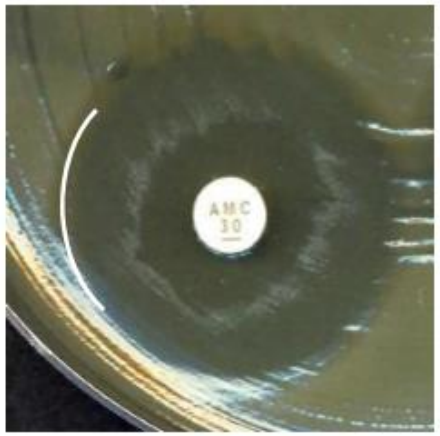
H. influenzae with PBP mutations



Zwerming

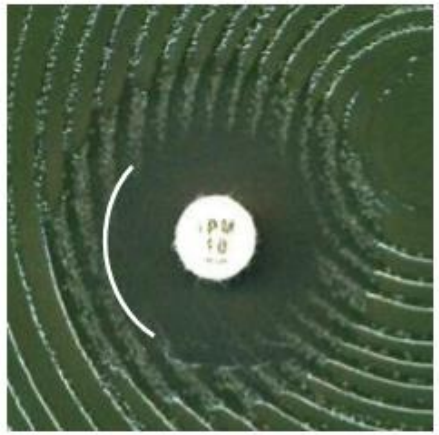
- Lees de inhibitie van groei af en niet de zwerming (vaakst gezien bij *Proteus* sp.)





Dubbele zones

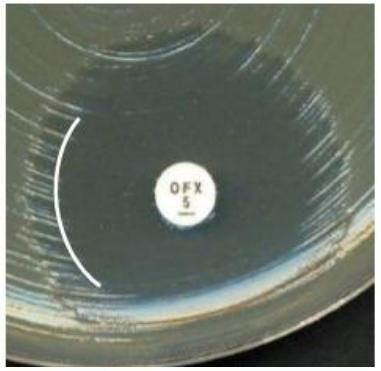
- Controleer zuiverheid
- Hertest
- Indien geen contaminant: lees de binnenste zone



Fuzzy zone randen

Plaat tegen donkere achtergrond op 30 cm van het oog. Niet in het licht houden voor aflezing.

Enterobacteriaceae



Staphylococcus species

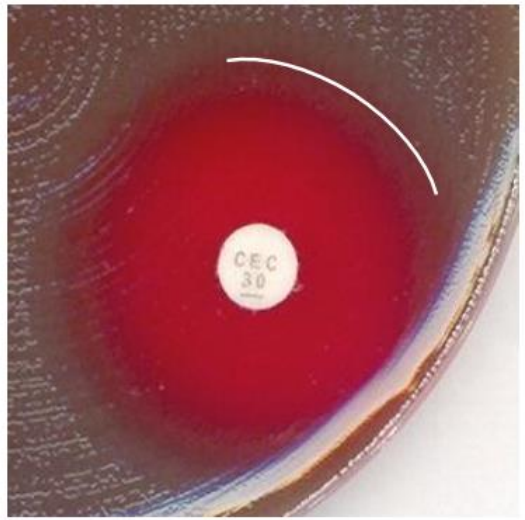
Groei of hemolyse?

S. pyogenes

Streptococcus group C

Groei of hemolyse?

There is usually growth in the whole area of α-haemolysis.



For some organisms, there is additional α-haemolysis without growth. Tilt the plate to differentiate between haemolysis and growth!

Trimethoprim-sulfamethoxazole

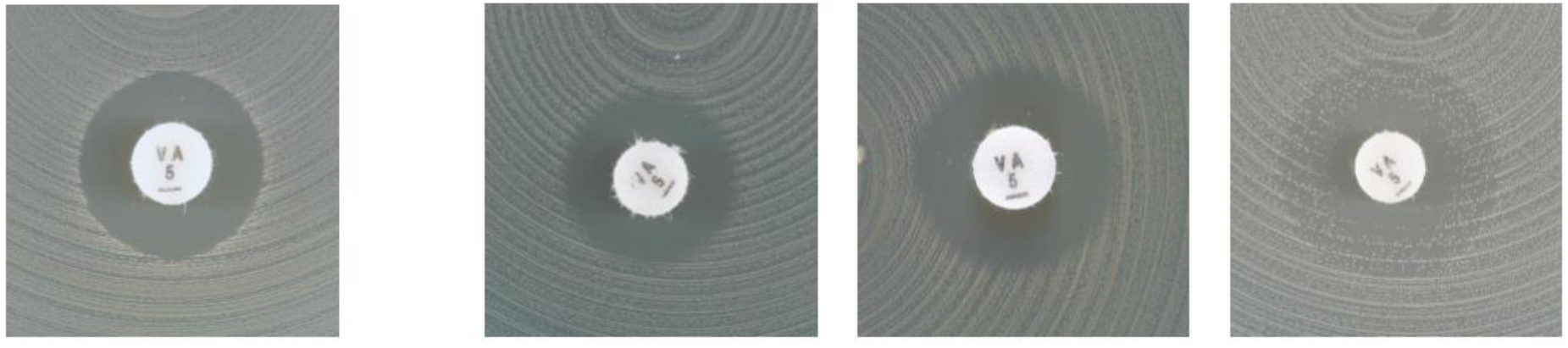
- *Stenotrophomonas maltophilia*

Ignore growth and read an inhibition zone if any zone edge can be seen.
= Susceptible if zone diameter ≥ 16 mm

Growth up to the disk and no sign of inhibition zone = Resistant

Vancomycine enterokokken

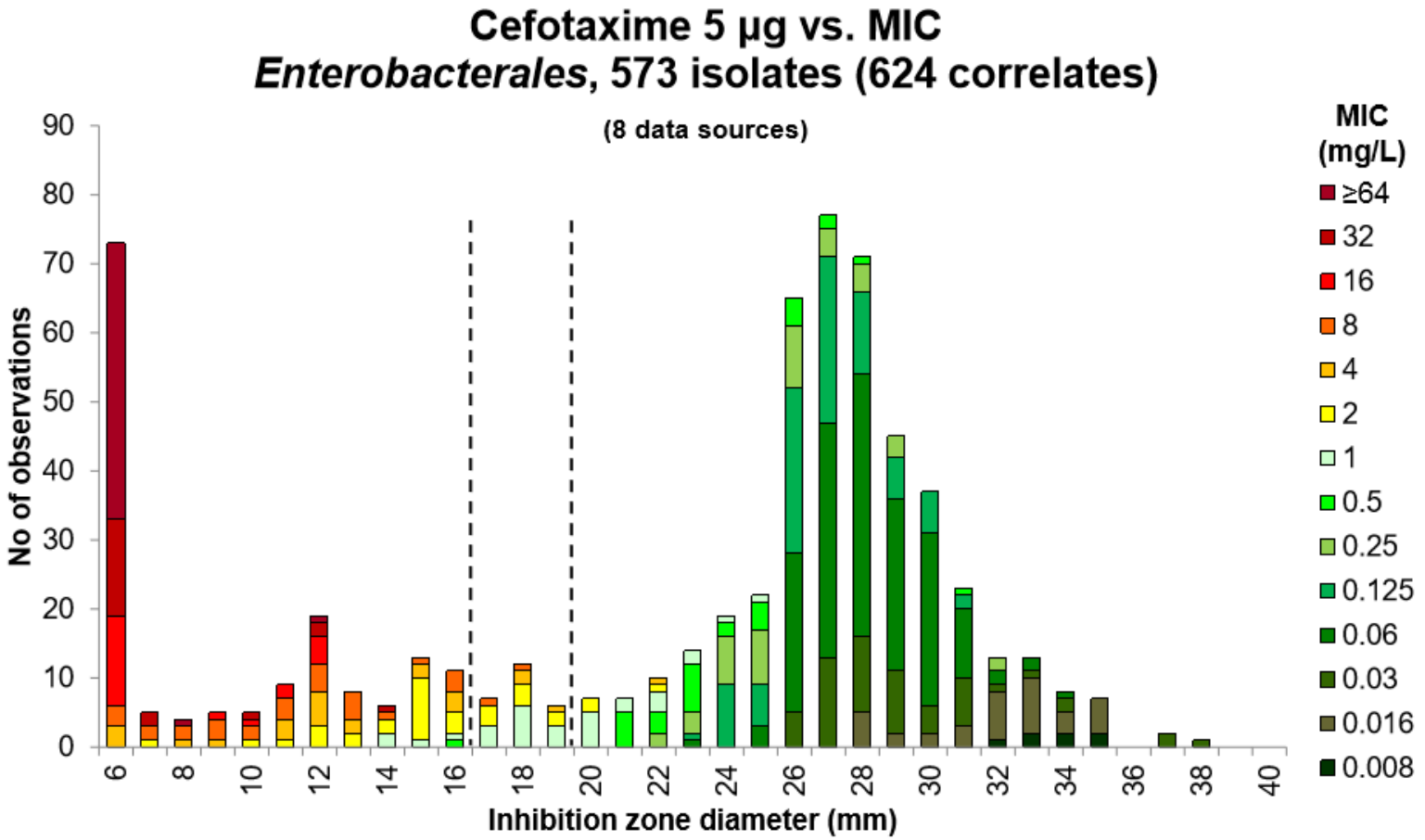
- Fuzzy zone randen en ingroeiende kolonies ➔ indicatie vancomycine resistentie



E. faecalis
non-VRE

E. faecium
VRE

Correlatie zonediameter versus MIC



Breakpoints	
MIC	S≤1, R>2 mg/L
Zone diameter	S≥20, R<17 mm

https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_criteria/Validation_2020/Enterobacterales_v_8.0_January_2020.pdf

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Original article

EUCAST evaluation of 21 brands of Mueller–Hinton dehydrated media for disc diffusion testing

J. Åhman*, E. Matuschek, G. Kahlmeter

EUCAST Development Laboratory, Växjö, Sweden

Table 3
Results for 21 different brands of Mueller-Hinton (MH) dehydrated media for disk diffusion testing.

Total rating[a]	MH agar brand	Disc diffusion results				Cation content (mg/L)[c]					pH	Agar depth (mm)
		% zones on QC target ±1 mm	% zones outside QC range	Agents[b] outside range, high	Agents[b] outside range, low	Ca	Mg	Zn	Mn	Fe		
−4	Bio-Rad MH Agar	86	0			43	9.6	0.47	<0.21	0.45	7.28	4.1
−10	Biolife MH Agar II	81	1.1	TS		43	19	0.53	<0.21	0.68	7.30	4.1
−10	Oxoid MH Agar	78	1.1	TS		24	15	0.39	<0.21	0.92	7.23	4.3
−11	Sigma MH Agar 2	81	0			19	8.4	0.50	<0.21	0.63	7.25	4.3
−12	BD BBL MH II Agar	73	0			21	20	0.89	<0.21	0.75	7.35	4.1
−12	CRITERION MH Agar	71	0			35	11	0.43	<0.21	0.59	7.31	4.1
−13	BD Difco MH Agar	70	3.3	AM		18	7.0	0.69	<0.21	0.65	7.36	4.1
−14	Alpha Biosciences MH Agar	71	3.3	FQ		40	6.7	7.8	<0.21	0.77	7.51	4.1
−17	E&O Labs MH Agar	82	8.9	CA/FQ/AM	TS	23	14	0.62	<0.21	0.66	7.37	4.2
−18	Sigma MH Agar	57	3.3	CS		19	8.5	0.54	<0.21	0.48	7.27	4.2
−20	HiMedia MH Agar	56	0			15	9.3	0.36	<0.21	8.8	7.30	4.2
−21	bioMérieux MHE Agar	64	3.3	TS		40	13	2.0	<0.21	0.57	7.20	4.1
−22	Acumedia MH Agar	63	3.3	AM		28	12	2.9	<0.21	0.65	7.21	4.2
−24	Remel MH Agar	64	6.7	AM	TS	27	17	1.1	<0.21	0.44	7.26	4.3
−25	Lab M MH Agar	69	6.7	AM	TS	14	64	0.52	<0.21	0.84	7.28	4.1
−25	Merck MH Agar acc. to CLSI	66	6.7	AM/TS		31	15	2.0	<0.21	0.54	7.16	4.0
−27	Mast MH Agar	59	8.9	CA/FQ	TS	8.8	7.7	0.61	<0.21	0.62	7.28	4.2
−31	Sifin MH Agar	60	6.7	AM/TS		29	15	1.9	<0.21	0.47	7.27	4.1
−32	HiMedia MH Agar No. 2	50	6.7	CA/AM		6.8	4.0	0.57	<0.21	1.3	7.18	4.0
−40	Biolab MH II Agar	52	10	PC/MA/TE	TS	17	38	0.56	<0.21	0.81	7.63	4.1
−55	Merck MH Agar	44	23	CS/CA/FQ/AM	TE	7.4	4.2	0.66	23	<0.34	7.34	4.1

[a] Based on how mean values (30 per agar) from triplicate tests of four QC strains relates to the respective QC criteria (EUCAST QC Tables v. 9.0): 0 points if mean value on target ±1 mm. -1 point if mean value on target ±2 mm (but not ±1 mm). -3 points if mean value >2 mm from target but within range. -5 points if mean value outside range.
[b] **Antimicrobial agents**: PC = Penicillins (ampicillin, piperacillin-tazobactam). CS = Cephalosporins (cefotaxime, cefoxitin, ceftazidime). CA = Carbapenems (imipenem, meropenem). FQ = Flouroquinolones (ciprofloxacin, norfloxacin). AM = Aminoglycosides (gentamicin, tobramycin). MA = Macrolides (erythromycin). TE = Tetracyclines (tetracycline, tigecycline). TS = Trimethoprim-sulfamethoxazole.
[c] For MH requirements in DIN, FDA, ISO and WHO, see Table 1.

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Original article

The quality of antimicrobial discs from nine manufacturers—EUCAST evaluations in 2014 and 2017

J. Åhman*, E. Matuschek, G. Kahlmeter

EUCAST Development Laboratory, Växjö, Sweden

	STUDY 2017									
Antimicrobial disk	Abtek	BD	Bio-analyse	Bio-Rad	HiMedia	Liofil-chem	Mast	Oxoid	SirScan	
Benzylpenicillin 1 unit		L							NA	
Amoxicillin-clav. 20-10 µg	L			H	L**	L				
Piperacillin-tazo. 30-6 µg	L		H		L**		H			
Oxacillin 1 µg				H	H					
Mecillinam 10 µg										
Cefotaxime 5 µg										
Cefoxitin 30 µg		L	H		L					
Ceftazidime 10 µg	L				L*					
Meropenem 10 µg					L*					
Ciprofloxacin 5 µg										
Pefloxacin 5 µg					H	L				
Norfloxacin 10 µg										
Gentamicin 10 µg					H		H			
Tobramycin 10 µg					H					
Erythromycin 15 µg										
Tetracycline 30 µg					L					
Total number of readings	120	120	120	120	120	120	120	120	111	**1071**

[1]Data reanalysed due to changes in QC criteria since 2014.

Mean value within ± 1 mm of the target value
Mean value >1 mm but within ± 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
* Single reading out of QC range
** Variation ≥4 mm for consecutive tests

Fig. 2. Results for discs from nine manufacturers versus EUCAST quality control targets and ranges.

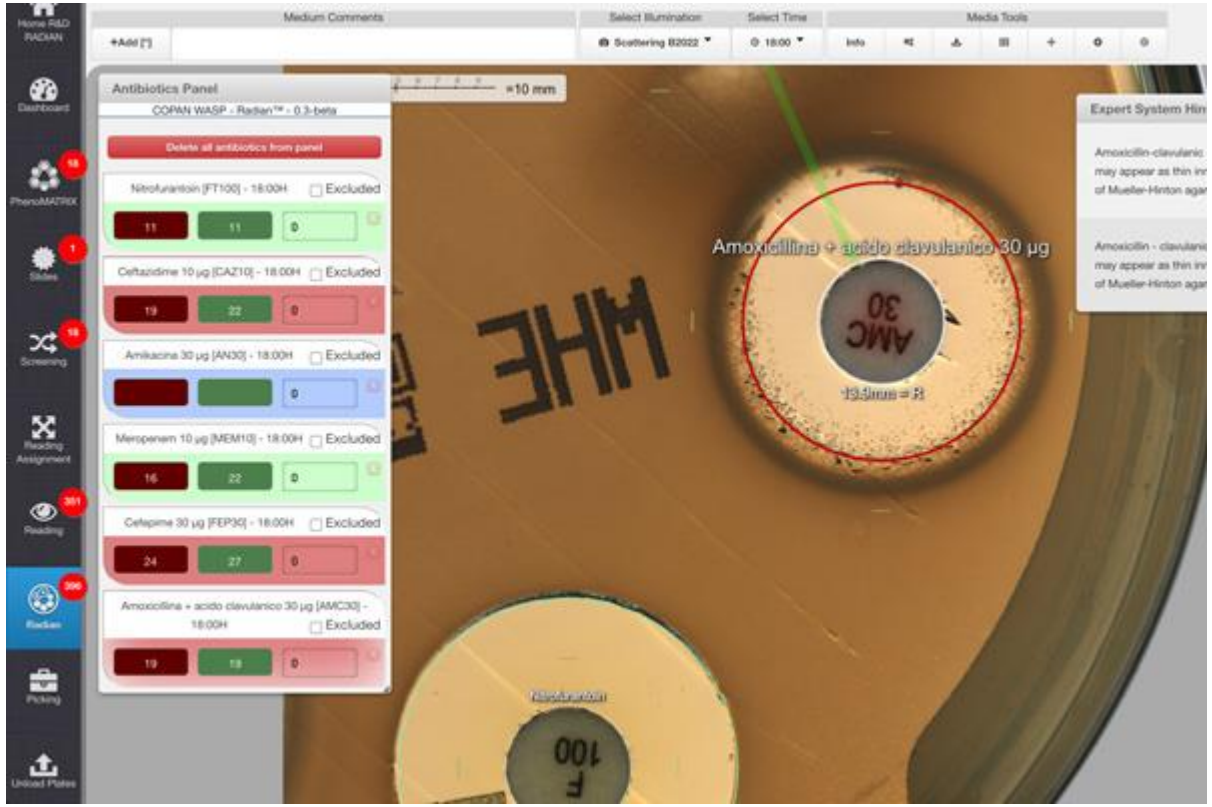
Disk diffusie

Voordelen

- Technisch eenvoudig
- Reproduceerbaar
- Relatief goedkoop
- Flexibel naar selectie van antibiotica

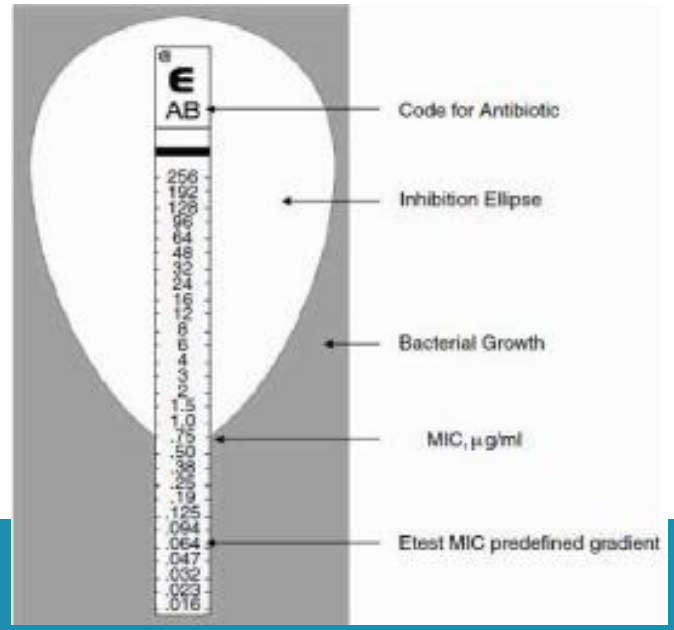
Nadelen

- Niet gestandaardiseerd voor moeilijk groeiende bacteriën
- Niet bruikbaar voor enkele antibiotica/microorganisme combinaties
  - Colistine
    - Slechte diffusie in agar
  - Vancomycine bij stafylokokken
    - Met disk diffusie geen onderscheid tussen vancomycine intermediaire en resistente isolaten
- Enkel kwalitatief resultaat (geen informatie over MIC (noodzakelijk bijvoorbeeld bij behandeling van endocarditis)
- Semi-geautomatiseerd

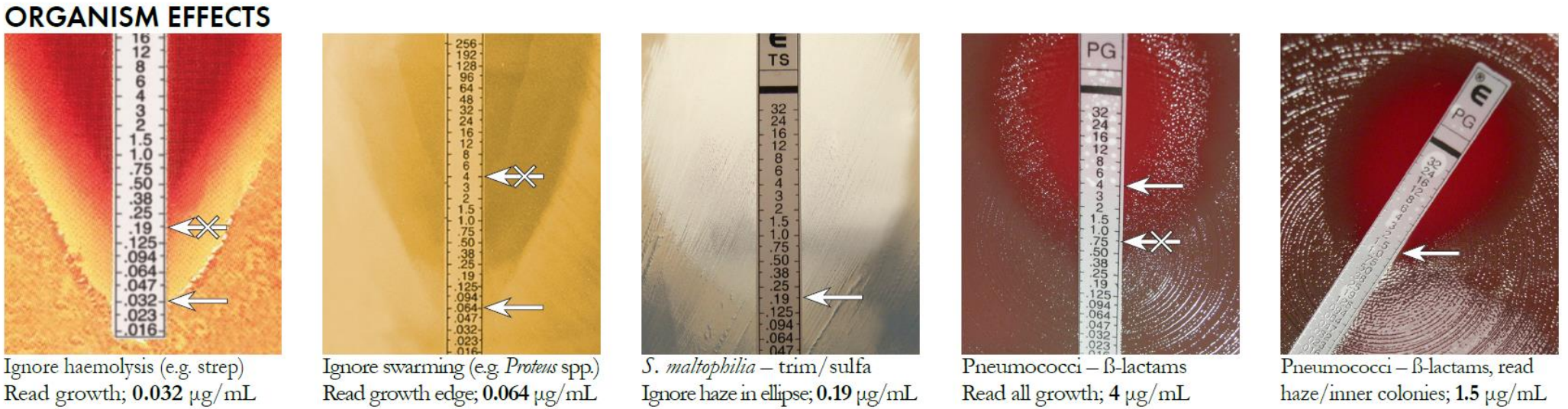

Medium Comments
Select Illumination
Select Time
Media Tools
Antibiotics Panel
COPAN WASP - Radian™ - 0.3-beta
Delete all antibiotics from panel
Nitrofurantoin [FT100] - 18:00H
Excluded
Ceftazidime 10 µg [CAZ10] - 18:00H
Excluded
Amikacina 30 µg [AN30] - 18:00H
Excluded
Meropenem 10 µg [MEM10] - 18:00H
Excluded
Cefepime 30 µg [FEP30] - 18:00H
Excluded
Amoxicillina + acido clavulanico 30 µg [AMC30] - 18:00H
Excluded
=10 mm
Amoxicillina + acido clavulanico 30 µg
AMC 30
MHE
F 100
Expert System Hin

Gradiënt diffusie

- 1994: Etest (bioMérieux)
- Andere: M.I.C.Evaluator (Thermo Fisher Scientific)/Liofilchem MIC Test (Liofilchem)
- Plastieken strip met aan één kant antibioticum met gradient concentratie
- Inoculatie conform disk diffusie



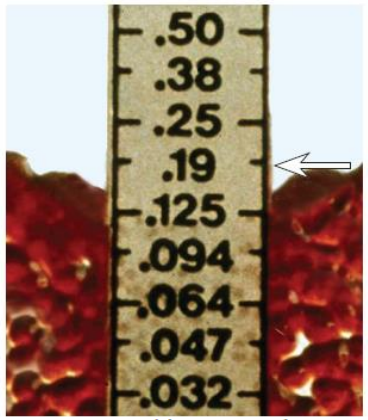
Aflezing gradiënt diffusie



Etest reading guide: http://www.biomerieux-diagnostics.com/sites/clinic/files/etest-reading-guide-aerobic-bacteria.pdf

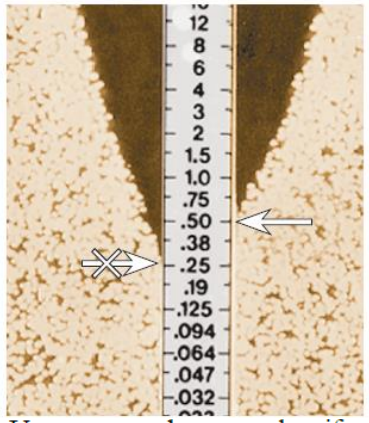
Aflezing gradiënt diffusie

TECHNICAL AND HANDLING

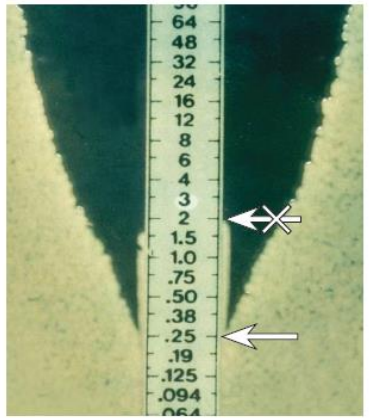


Between markings – read upper value; **0.19** µg/mL

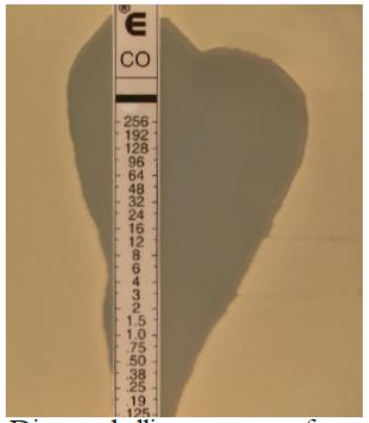
Etest strip placed upside down Invalid; repeat the test



Uneven – read upper value; if >1 dilution, repeat the test



Ignore line of growth alongside strip; **0.25** µg/mL



Distorted ellipse – wet surface, invalid; repeat the test

Gradiënt diffusie

Voordelen

- Eenvoudig af te lezen
- Flexibel naar selectie van antibiotica
- AST van moeilijk groeiende bacteriën en anaeroben door gebruik van aangerijkte media

Nadelen

- Duur
- Niet geschikt voor panel testing
- Niet geschikt voor bepaalde antibiotica oa:
  - Colistine
  - Penicilline (pneumokokken)
  - Piperacilline-tazobactam (Etest, Liophilchem)
  - Tigecycline (Etest)

Hindler et al. J. Clin Microbiol. 2013 Jun;51(6):1678-84
EUCAST warning 28 November, 2016
EUCAST warning 2015
Marcheim et al. J Clin Microbiol. 2014 May; 52(5): 1617–1621.

Geautomatiseerde systemen

- Vitek2 (bioMéreiux)
- Phoenix (Beckton Dickinson)

- Microscan WalkAway
- Sensititre

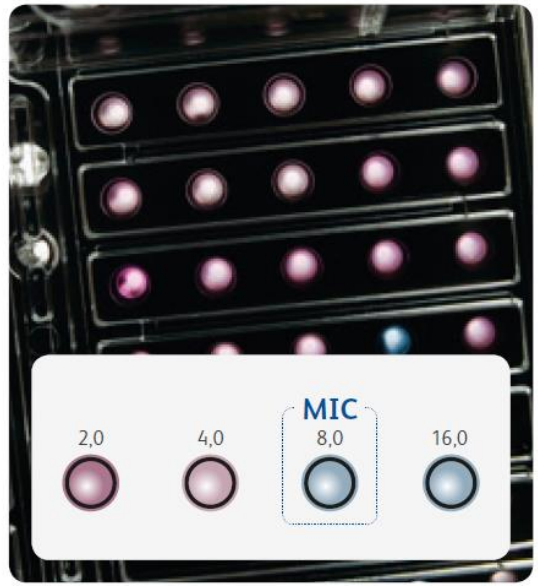
Vitek2

- In 2000 Vitek2 , 2005 Vitek2 Compact (bioMérieux)
- Dunne 64-well kaart met 1-6 concentraties van 9-20 antibiotica
- Inoculum 0,5 McFarland
- Aflezing turbiditeit om de 15 minuten
- Hands-on time: +/- 10 minuten
- Resultaat < 16uur (gemiddeld resultaat na 8.4± 2 uur[1])
- Identificatiekaarten gebaseerd op colorimetrie

[1] **Eigner U, Schmid A, Wild U, Bertsch D, Fahr A-M.** 2005. Analysis of the comparative workflow and performance characteristics of the VITEK 2 and Phoenix systems.*J Clin Microbiol* **43:**3829–3834.

Phoenix

- In 2001 in Europa (Beckton Dickinson)
- polystyreen trays met 136 wells volledig voor AST
  of ID (51 wells) en AST (85-wells voor 14-22 antibiotic)
- Phoenix AP instrument
- Aflezing om de 20 minuten
  - Redox-reactie groei : colorimetrische meting
  - Turbiditeitsmeting
- Resultaat binnen 6-16 uur (gemiddeld resultaat na 12.1 ± 2.7 u[1])



[1] **Eigner U, Schmid A, Wild U, Bertsch D, Fahr A-M.** 2005. Analysis of the comparative workflow and performance characteristics of the VITEK 2 and Phoenix systems.*J Clin Microbiol* **43:**3829–3834.

BD Phoenix™ AP Workflow – Prep and Pour

1 PREPARE INOCULUM

- Label ID tube with EpiCenter™ or LIS barcode
- Select colonies and make a heavy suspension in ID broth
- Place in rack with AST broth

2 BD PHOENIX AP

- Performs automatic nephelometry to 0.5 or 0.25 McFarland
- Adds AST indicator to AST broth
- Transfers isolate to AST broth
- Mixes both samples

3 REMOVE PROCESSED TUBES AND PLACE ON INOCULATION STATION WITH PHOENIX PANELS

- Single or batch inoculation
- Central or individual work stations

4 SCAN BARCODES INTO BD EPICENTER™ AND INOCULATE PANELS

- Provides rapid, efficient batch sample login
- Supports single-handed barcode scanning
- Provides positive sample ID for Phoenix isolate

5 OPEN BD PHOENIX DOOR AND PLACE PANEL IN ANY AVAILABLE STATION

Microscan

- Eerste systeem in 1990 (Siemens/Beckman Coulter)
- Microscan WalkAway *plus*
- Conventionele 96-well platen
- Turbidimetrie
- Reader en incubator

Automatische systemen

Voordelen

- Eenvoudig en weinig werk
- Reproduceerbaar
- Connectie met LIS en gebruik expertsysteem analyse
- Snelle resultaten

Nadelen

- Dure instrumenten en consumables
- Weinig flexibiliteit met voorgemaakte antimicrobiële panels
- Onmogelijk alle relevante bacteriën en antibiotica te testen
- Moeilijkheid voor detectie heteroresistentie en bepaalde resistentie fenotypes (induceerbare clindamycine resistentie, ESBL)
- Veel consumables/afval

Overzicht van gevoeligheidsbepalingen

Bepaling van MIC

- Broth dilutie methoden
- Geautomatiseerde systemen gebaseerd op dilutie
- Gradiënt diffusie (Etest)

Bepaling van zone diameter

- Disk diffusie methode

European Journal of Clinical Microbiology & Infectious Diseases (2023) 42:1477–1483
https://doi.org/10.1007/s10096-023-04681-y

ORIGINAL ARTICLE

Multicenter interlaboratory study of routine systems for the susceptibility testing of temocillin using a challenge panel of multidrug-resistant strains

Corentin Deckers[1] · Florian Bélik[1] · Olivier Denis[1] · Isabel Montesinos[1] · Pierre Bogaerts[1] · Jerina Boelens[2] · Laetitia Brassinne[3] · Julie Descy[4] · Stefanie Desmet[5] · Sarah Gils[6] · Bénédicte Lissoir[7] · Koen Magerman[8] · Veerle Matheeussen[9] · Cécile Meex[10] · Hector Rodriguez Villalobos[11] · Anne Marie Van den Abeele[12] · Kris Vernelen[13] · Pieter-Jan Ceyssens[14] · Te-Din Huang[1] · on behalf of the Belgian National Antibiogram Committee

Table 3 Performance of MIC-based temocillin testing methods

TMO MIC-based method (n labs)	Etest (n=3)					BD Phoenix™ (n=3)					VITEK® 2 (n=5)				
Species (total n isolates)	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	AA(%) %[95CI]	EA(%) %[95CI]	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	AA(%) %[95CI]	EA(%) %[95CI]	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	AA(%) %[95CI]	EA(%) %[95CI]
All (n=47)	95,2 [89,8-97,7]	4,0 [1,7-9,0]	0,8 [0,1-4,4]	51,8 [41,2-62,1]	88,2 [78,3-92,6]	85,4 [78,3-90,4]	2,3 [0,8-19,1]	12,3 [7,7-19,1]	54,3 [42,7-65,4]	93,5 [82,5-9,8]	87,3 [82,3-91,1]	7,2 [4,5-11,4]	5,4 [3,1-9,2]	49,3 [38,3-60,4]	90,6 [82,0-95,4]
All except PM/MM (n=43)	97,5 [92,8-99,1]	1,7 [0,4-5,9]	0,8 [0,1-4,6]	53,7 [42,9-64,0]	91,5 [83,3-95,8]	88,5 [81,6-93,0]	0,0 [0,0-3,0]	11,5 [7,6-19,3]	57,6 [45,6-68,7]	100 [91,8-100]	93,2 [88,7-95,6]	4,2 [2,1-11,7]	2,6 [1,1-5,9]	51,6 [39,4-63,6]	96,7 [88,8-99,1]
EC (n=11)	100 [89-100]	0,0 [0,0-11,0]	0,0 [0,0-11,0]	61,9 [40,8-70,2]	100 [84,5-100]	87,5 [71,9-95,0]	0,0 [0-10,7]	12,5 [4,9-28,1]	52,4 [49,7-71,6]	100 [74,1-100]	81,1 [68,6-89,4]	9,4 [4,0-20,2]	9,4 [4,0-20,2]	22,2 [9,0-45,2]	100 [82,4-100]
KP (n=15)	100 [89,0-100]	0,0 [0-10,4]	0,0 [0-10,4]	44,0 [26,6-62,9]	88,0 [70,0-95,8]	100 [91,2-100]	0,0 [0-8,8]	0,0 [0-8,8]	70,0 [48,1-85,4]	100 [83,9-100]	96,9 [89,4-99,1]	3,1 [0,8-10,5]	0,0 [0-5,6]	63,6 [42,9-80,3]	90,9 [72,2-97,4]
Non-EC/KP (n=21)	90 [79,8-95,3]	8,3 [3,0-18,1]	1,7 [0,3-8,9]	55,6 [39,6-70,5]	92,6 [83,0-97,2]	74,1 [61,2-83,6]	5,2 [1,8-14,1]	20,7 [12,2-62,4]	44,8 [28,4-62,4]	82,4 [91,8-100]	84,5 [73,2-90,2]	8,7 [4,6-15,8]	6,8 [3,3-13,3]	54,3 [38,2-69,5]	85,7 [78,6-93,7]
Non-EC/KP/PM/MM (n=17)	94,4 [84,8-98,0]	3,7 [1,0-9,8]	1,9 [0,3-9]	55,5 [39,5-70,4]	92,5 [82,4-97,1]	80 [67,0-88,8]	0 ,0 [0-7,1]	20 [11,2-33,0]	52,0 [33,5-70,0]	100 [78,5-100]	95,2 [88,2-98,1]	4,8 [1,8-11,7]	0,0 [0-4,4]	59,2 [40,7-75,4]	100 [95,6-100]

Caption: EC: *E. coli*; KP: *K. pneumoniae*; MM: *M. morganii*; PM: *P.mirabilis*; %[95CI]: Confidence Interval of 95%; TMO: temocillin; AA: Absolute agreement; CA: Categorical agreement; MD: Major discrepancy; VMD: Very Major Discrepancy;

Interpretation criteria:

VMD/MD (%)	0-3%	VMD/MD (%)	3-5%	VMD/MD (%)	>5%
CA/EA (%)	<80%	CA/EA (%)	80-90%	CA/EA (%)	90-100%

Table 4 Performance of temocillin disk diffusion methods

TMO disk diffusion (n labs)	Total disks (n=6)			BioRad (n=3)			BD (n=2)			Rosco (n=1)		
Species (total n isolates)	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	CA(%) %[95CI]	VMD(%) %[95CI]	MD(%) %[95CI]	CA (%) %[95CI]	VMD(%) %[95CI]	MD (%) %[95CI]
All (n=47)	90,9 [86,9-93,7]	2,9 [1,5-5,6]	6,2 [3,9-9,7]	90,1 [84,0-93,4]	2,1 [0,7-6,1]	7,8 [4,4-13,4]	92,0 [84,3-96,0]	1,1 [0,2-6,2]	6,9 [3,2-14,2]	91,3 [79,7-96,6]	8,7 [3,4-20,3]	0,0 [0,0-7,7]
All -PM/MM (n=43)	91,6 [87,5-94,4]	1,6 [0,6-4,0]	6,8 [4,3-10,6]	90,7 [84,4-94,6]	0,8 [0,1-4,3]	8,5 [4,8-14,6]	92,4 [84,4-96,5]	0,0 [0-10,6]	7,6 [3,5-15,6]	92,9 [78,4-96,3]	7,1 [2,4-18,6]	0,0 [0,0-8,2]
EC (n=11)	86,2 [75,7-92,5]	3,1 [0,8-10,6]	10,7 [5,3-20,6]	84,8 [69,1-93,3]	3,0 [0,5-15,3]	12,1 [4,8-27,3]	85,7 [65,3-95,0]	0 ,0 [0-15,4]	14,2 [4,9 -34,6]	90,9 [62,2-98,4]	9,0 [1,6-37,6]	0,0 [0-25,8]
KP (n=15)	92,9 [85,2-96,7]	1,1 [0,2-6,4]	6,0 [2,5-13,1]	93,3 [82,1-97,7]	0,0 [0-7,8]	6,7 [2,9-17,9]	92,0 [75,0-97,7]	0 ,0 [0-13,3]	8,0 [2,2-27,9]	92,8 [68,5-98,7]	7,1 [1,2-31,5]	0,0 [0-21,5]
Non-EC/KP (n=21)	92,0 [85,9-95,6]	4,0 [1,2-7,9]	4,0 [1,2- 7,9]	90,5 [80,7-95,6]	3,2 [0,8-10,8]	6,3 [2,5-15,2]	95,1 [83,9-98,6]	2,4 [0,4-12,6]	2,4 [0,4-12,6]	90,5 [71,1-97,3]	9,5 [2,6-28,9]	0,0 [0,0-15,5]
Non-EC/KP/PM/MM (n=17)	94,0 [87,6-97,2]	1,0 [0,2-5,4]	5,0 [2,1-11,1]	92,2 [81,5-96,7]	0,0 [0-7,0]	7,8 [3,1-,18,5]	97,0 [84,7-99,4]	0,0 [0-10,4]	3,0 [0,5-15,3]	94,1 [73,0-98,9]	5,9 [1,0-26,7]	0,0 [0,-18,4]

Caption: EC: *E. coli*; KP: *K. pneumoniae*; MM: *M. morganii*; PM: *P.mirabilis*; %[95CI]: Confidence Interval of 95%; TMO: temocillin;AA: Absolute agreement; CA: Categorical agreement; MD: Major Discrepancy; VMD: Very Major Discrepancy

Interpretation criteria:

VMD/MD (%)	0-3%	VMD/MD (%)	3-5%	VMD/MD (%)	>5%
CA (%)	<80%	CA (%)	80-90%	CA (%)	90-100%

Interpretatie gevoeligheidsbepaling

MIC

Zone diameter

klinisch breekpunt

Interpretatie en rapportering

S/I/R

Proteus mirabilis ++

S : amoxicilline-clavulaanzuur, levofloxacine, amikacine, tobramycine

I : cefuroxime

R : amoxicilline , co-trimoxazol, nitrofurantoine, colistine

Historiek van breekpunten

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

klinisch breekpunt

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Gevoeligheidscategoriën

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

afkorting	interpretatie	hoge waarschijnlijkheid op
S	gevoelig, standaard dosis	therapeutisch succes bij gebruik standaard dosering
I	gevoelig, verhoogde blootstelling	therapeutisch succes als de blootstelling aan het middel verhoogd is
R	resistent	falen van de behandeling, ook al wordt een hoge dosis gegeven

- aanpassing dosering
- verkrijgen van (voldoende hoge) concentratie op de plaats van infectie

blootstelling is een functie van
-de toedieningswijze, dosis, dosisinterval, infusietijd
-distributie, metabolisme en excretie van het antimicrobieel middel

KU LEUVEN

Escherichia coli

S : piperacilline-tazobactam, levofloxacine, amikacine, tobramycine, co-trimoxazol, nitrofurantoine

I : cefuroxime, temocilline

R : amoxicilline , amoxicilline-clavulaanzuur

I en S gecategoriseerde antibiotica zijn therapeutische opties

Enterobacterales *

EUCAST Clinical Breakpoint Tables v. 13.0, valid from 2023-01-01

Expert Rules and Expected Phenotypes

For abbreviations and explanations of breakpoints, see the Notes sheet

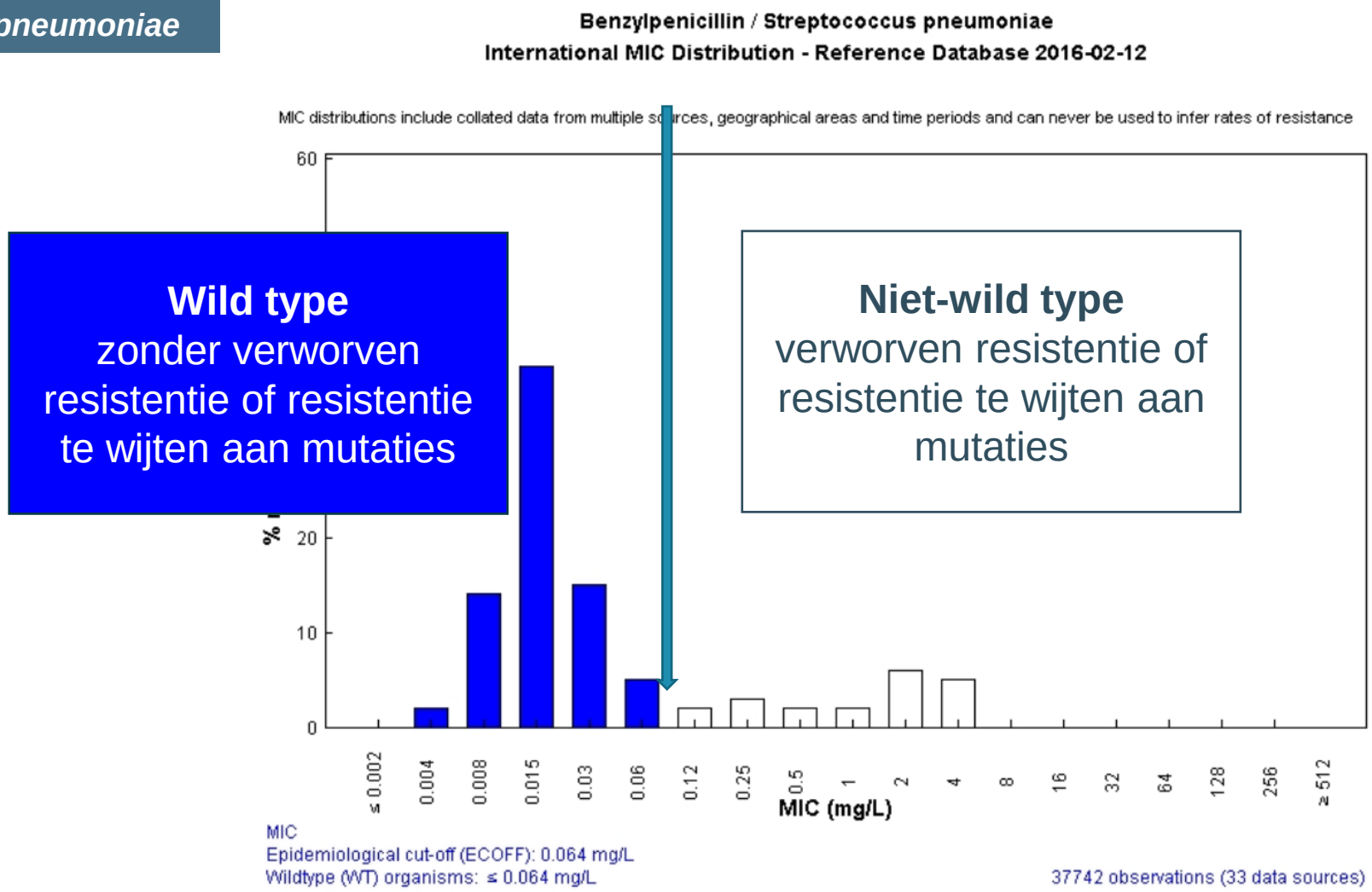
Carbapenems[1]	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem	1	2		10	24	21		**1.** Some isolates that produce carbapenemase are categorised as susceptible with the current breakpoints and should be reported as tested, i.e. the presence or absence of a carbapenemase does not in itself influence the categorisation of susceptibility. Carbapenemase detection and characterisation are recommended for public health and infection control purposes. For carbapenemase screening, a meropenem screening cut-off of >0.125 mg/L (zone diameter <28 mm) is recommended.
Ertapenem	0.5	0.5		10	25	25		
Imipenem, *Enterobacterales* except *Morganellaceae*	2	4		10	22	19		
Imipenem[2], *Morganellaceae*	0.001	4		10	50	19		**2.** The intrinsically low activity of imipenem against *Morganella morganii*, *Proteus* spp. and *Providencia* spp. requires the high exposure of imipenem.
Imipenem-relebactam, *Enterobacterales* except *Morganellaceae*	2[3]	2[3]		10-25	22	22	20-22	**3.** For susceptibility testing purposes, the concentration of relebactam is fixed at 4 mg/L.
Meropenem (indications other than meningitis)	2	8		10	22	16		**4.** For susceptibility testing purposes, the concentration of vaborbactam is fixed at 8 mg/L.
Meropenem (meningitis)	2	2		10	22	22		**A.** For isolates in the ATU, if resistant to meropenem report resistant to meropenem-vaborbactam. If not resistant to meropenem, investigate further.
Meropenem-vaborbactam	8[4]	8[4]		20-10	20	20	15-19[A]	

Monobactams	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam[1]	1	4		30	26	21		**1.** The aztreonam breakpoints for *Enterobacterales* will detect clinically important resistance mechanisms (including ESBL). Some isolates that produce beta-lactamases are susceptible to aztreonam with these breakpoints and should be reported as tested, i.e. the presence or absence of an ESBL does not in itself influence the categorisation of susceptibility. ESBL detection and characterisation are recommended for public health and infection control purposes.

Fluoroquinolones	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin, *Salmonella* spp.[1]	0.06	0.06			Note[A]	Note[A]		**1.** There is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by *Salmonella* spp. with low-level ciprofloxacin resistance (MIC >0.06 mg/L). The available data relate mainly to *Salmonella* Typhi but there are also case reports of poor response with other *Salmonella* species.
Ciprofloxacin (indications other than meningitis)	0.25	0.5	0.5	5	25	22	22-24	**2/B.** In meningitis, where low-level ciprofloxacin resistance must be excluded, either perform an MIC test, or infer susceptibility from the pefloxacin 5 µg screening test.
Ciprofloxacin (meningitis)[2]	0.125	0.125			Note[B]	Note[B]		
Pefloxacin (screen only)	NA	NA		5	24[A,B,C]	24[A,B,C]		**A.** Tests with a ciprofloxacin 5 µg disk will not reliably detect low-level resistance in *Salmonella* spp. Perform an MIC test, or infer susceptibility from the pefloxacin 5 µg screening test.
Delafloxacin, *E. coli*	0.125	0.125			Note[D]	Note[D]		**C.** The pefloxacin screening test can also be used to detect fluoroquinolone resistance mechanisms in other *Enterobacterales* such as *E. coli*, *K. pneumoniae* and *Shigella* spp.
Levofloxacin	0.5	1		5	23	19		**D.** A disk diffusion test awaits action from the responsible pharmaceutical company.
Moxifloxacin	0.25	0.25		5	22	22		
Nalidixic acid (screen only)	NA	NA			NA	NA		
Norfloxacin (uncomplicated UTI only)	0.5	0.5		10	24	24		
Ofloxacin	0.25	0.5		5	24	22		

Voorbeeld penicilline *S. pneumoniae*

Epidemiologisch breekpunt (ECOFF)



Wild type
zonder verworven resistentie of resistentie te wijten aan mutaties

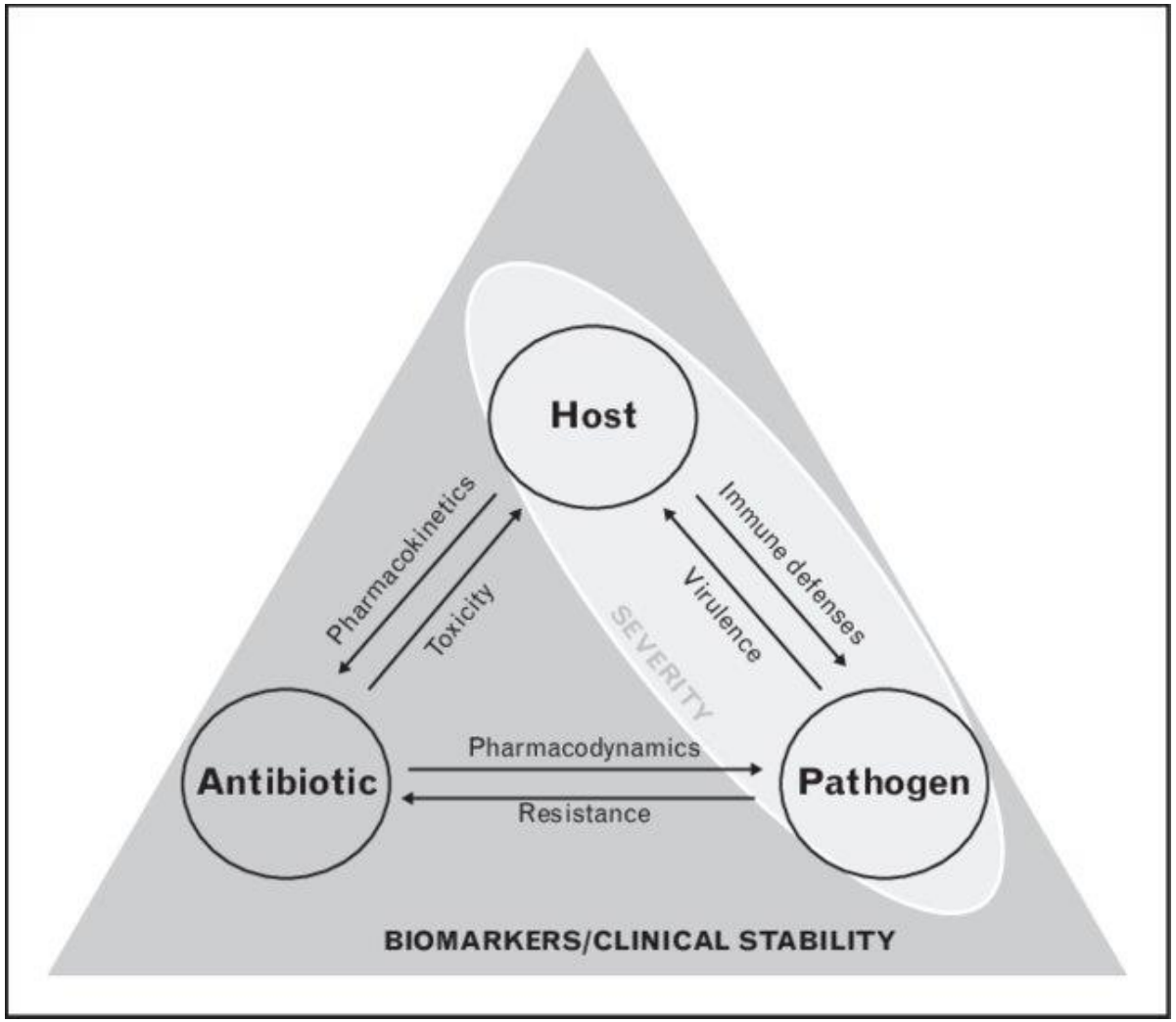
Niet-wild type
verworven resistentie of resistentie te wijten aan mutaties

KU LEUVEN

Kans op therapie succes hangt van meer af dan enkel aanwezigheid van resistentie mechanismen bij het micro-organisme

PK/PD indices
noodzakelijk blootstelling

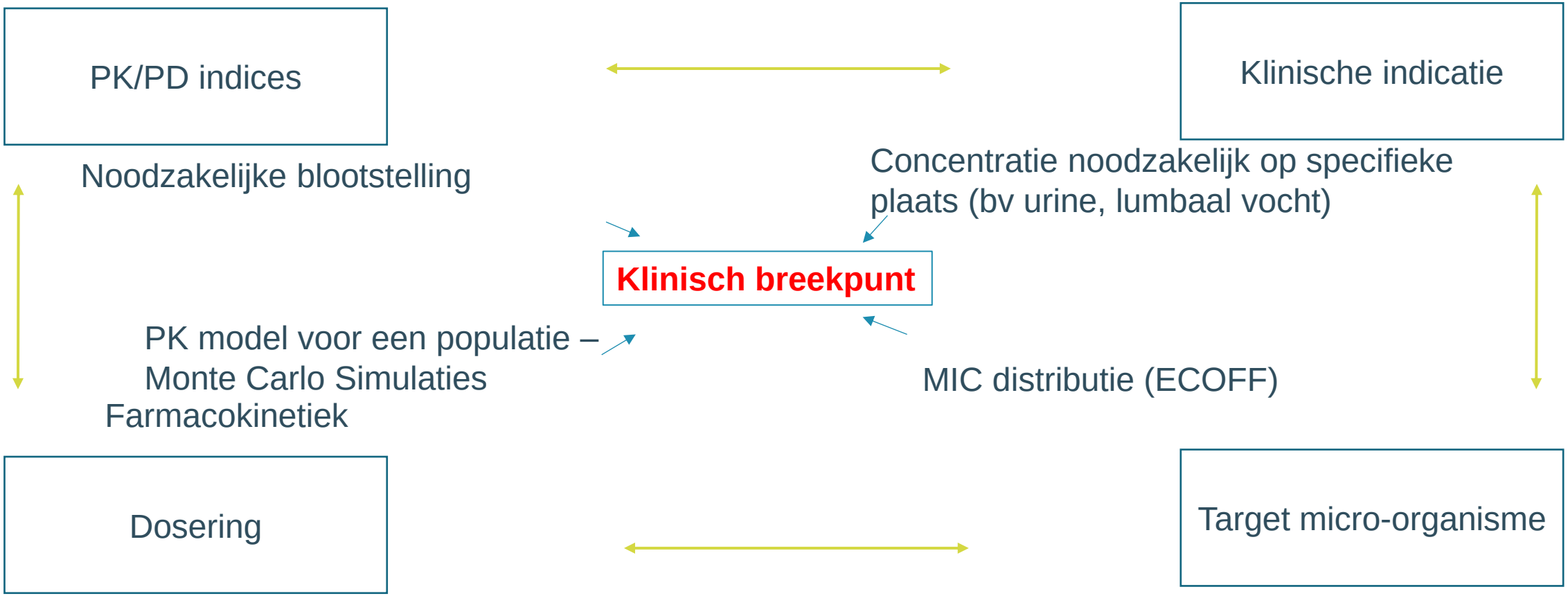
Dosering



Klinische indicatie

MIC distributie
(ECOFF)

Veel factoren zijn belangrijk om kans op therapie succes te bepalen en die factoren zijn mee opgenomen binnen de klinische breekpunten



EUCAST rationale documenten

- www.eucast.org
- Meer achtergrond:

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

search term

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04 May 2017

The European Committee on Antimicrobial Susceptibility Testing - EUCAST

EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees. EUCAST was formed in 1997. It has been chaired by Ian Phillips (1997 - 2001), Gunnar Kahlmeter (2001 - 2012), Rafael Canton 2012 - 2016) and Christian Giske (2016 -). Its scientific secretary is Derek Brown (1997 - 2016) and John Turnidge (2016 -). Its webmaster is Gunnar Kahlmeter (2001 -). From 2016, Rafael Canton is the Clinical Data Co-ordinator and Gunnar Kahlmeter the Technical Data Co-ordinator.

EUCAST deals with breakpoints and technical aspects of phenotypic in vitro antimicrobial susceptibility testing and functions as the breakpoint committee of EMA and ECDC. EUCAST does not deal with antibiotic policies, surveillance or containment of resistance or infection control. The Steering Committee is the decision making body. It is supported by a General Committee with representatives from European and other countries, FESCI and ISC. The Steering Committee also consults on EUCAST proposals with experts within the fields of infectious diseases and microbiology, pharmaceutical companies and susceptibility testing device manufacturers.

EUCAST has several subcommittees - see page Subcommittees.

Most antimicrobial MIC breakpoints in Europe have been harmonised by EUCAST. Breakpoints for new agents are set as part of the licensing process for new agents through EMA. EUCAST breakpoints are available in devices for automated susceptibility testing but with some limitations, depending on the system. A disk diffusion susceptibility test method calibrated to EUCAST MIC breakpoints is also available.

QUICK NAVIGATION

EUCAST News

07 Jun 2017
Ceftolozane-tazobactam zone diameter breakpoints for Ps. aeruginosa now available.

04 May 2017
Posaconazole RD for Candida and Aspergillus merged and updated.

19 Apr 2017
EUCAST Posters at ECCMID 2017

18 Apr 2017
EUCAST General Committee 2017 Agenda

18 Apr 2017
Maps of EUCAST uptake and website stats 2017 updated.

About Newsfeeds

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

ORIGINAL ARTICLE BACTERIOLOGY

The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach

J. W. Mouton[1], D. F. J. Brown[2], P. Apfalter[3], R. Cantón[4], C. G. Giske[5], M. Ivanova[6], A. P. MacGowan[7], A. Rodloff[8], C.-J. Soussy[9], M. Steinbakk[10] and G. Kahlmeter[11]

Clin Microbiol Infect 2012; **18:** E37–E45
10.1111/j.1469-0691.2011.03752.x

KU LEUVEN

Streptococcus pneumoniae

EUCAST Clinical Breakpoint Tables v. 13.0, valid from 2023-01-01

Expert Rules and Expected Phenotypes

For abbreviations and explanations of breakpoints, see the Notes sheet

MIC determination (broth microdilution according to ISO standard 20776-1)
Medium: Mueller-Hinton broth + 5% lysed horse blood and 20 mg/L β-NAD (MH-F broth)
Inoculum: $5x10^5$ CFU/mL
Incubation: Sealed panels, air, 35±1ºC, 18±2h
Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth. See "EUCAST Reading Guide for broth microdilution" for further information.
Quality control: *Streptococcus pneumoniae* ATCC 49619. For agents not covered by this strain, see EUCAST QC Tables.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5 from blood agar or McFarland 1.0 from chocolate agar
Incubation: 5% CO_2, 35±1ºC, 18±2h
Reading: Unless otherwise stated, read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light. See "EUCAST Reading Guide for disk diffusion" for further information.
Quality control: *Streptococcus pneumoniae* ATCC 49619. For agents not covered by this strain, see EUCAST QC Tables.

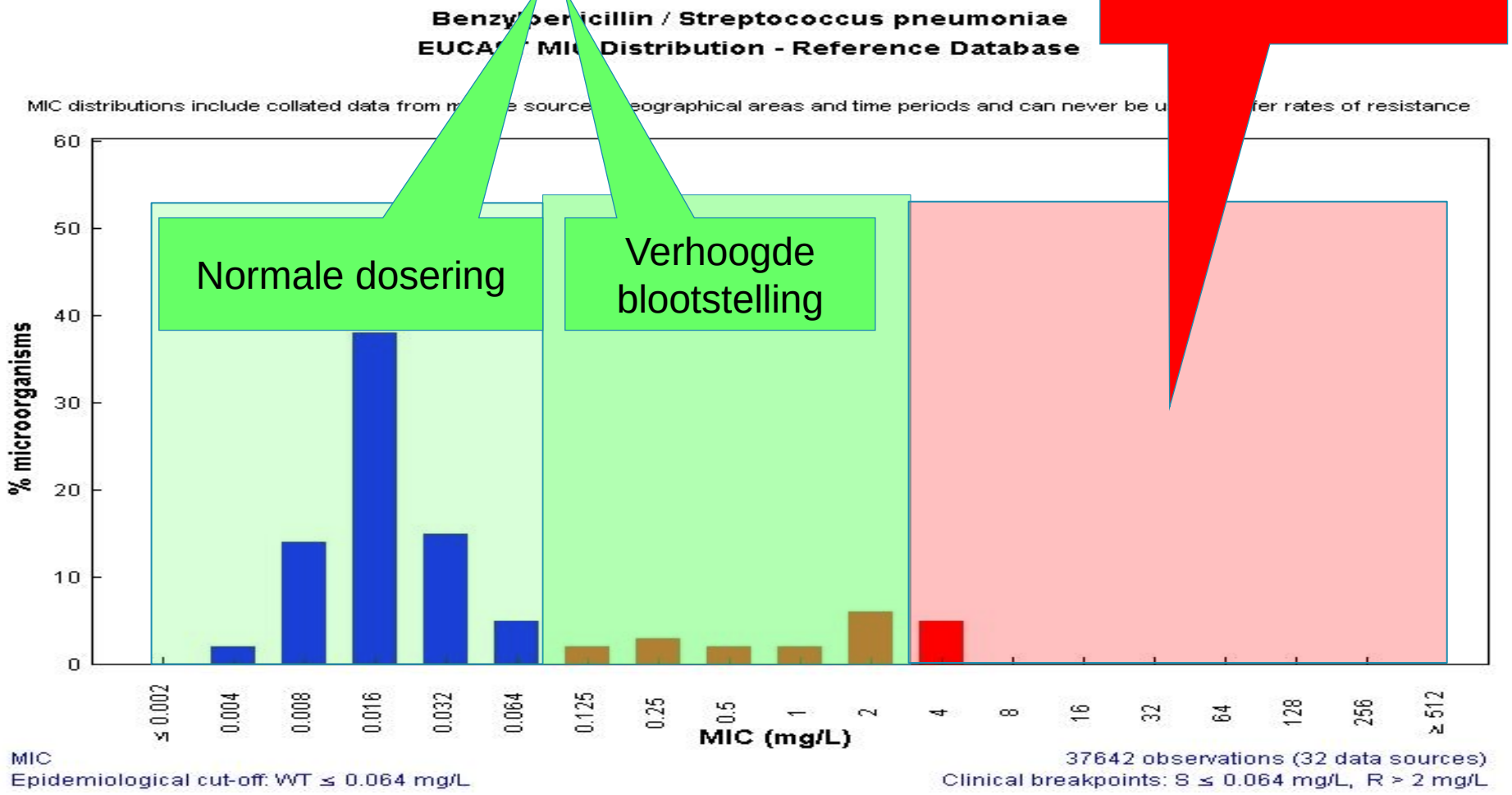
Penicillins[1]	MIC breakpoints (mg/L)			Disk content (µg)	Zone diameter breakpoints (mm)			Notes
	S ≤	R >	ATU		S ≥	R <	ATU	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin (indications other than meningitis)[2]	0.06	2			Note[A]	Note[A]		**1/A.** The oxacillin 1 µg disk diffusion screening test or a benzylpenicillin MIC test shall be used to exclude beta-lactam resistance mechanisms. When the screen is negative (oxacillin zone diameter ≥20 mm, or benzylpenicillin MIC ≤0.06

Voorbeeld
penicilline
S. pneumoniae

Klinische breekpunten

Gevoelig

Resistent



KU LEUVEN

Voorbeeld penicilline *S. pneumoniae*

21-03-2022 21:33 - bloed aeroob (set 1)

Gramkleuring	streptokokken
Cultuur	positief

Geisoleerd(e) micro-organisme(n):

Streptococcus pneumoniae

S : cefotaxime, moxifloxacine, erythromycine, clindamycine, vancomycine, tetracycline
I : penicilline, amoxicilline , levofloxacine
R : co-trimoxazol

- *I = gevoelig, verhoogde expositie. Dit betekent dat de kiem behandeld kan worden met antibiotica die als I gecategoriseerd worden, mits hoge dosis zie doseringstabel antibioticagids (https://files.uzleuven.be/antibioticagids/2019/VII/N13571.pdf).*

Dosages

EUCAST Clinical Breakpoint Tables v. 12.0, valid from 2022-01-01

EUCAST breakpoints are based on the following dosages (see section 8 in Rationale Documents). Alternative dosing regimens may result in equivalent exposure. The table should not be considered a guidance for dosing in clinical practice, and does not replace specific local, national, or regional dosing guidelines. However, if national practices significantly differ from those listed below, EUCAST breakpoints may not be valid. Situations where less antibiotic is given as standard or high dose should be discussed locally or regionally.

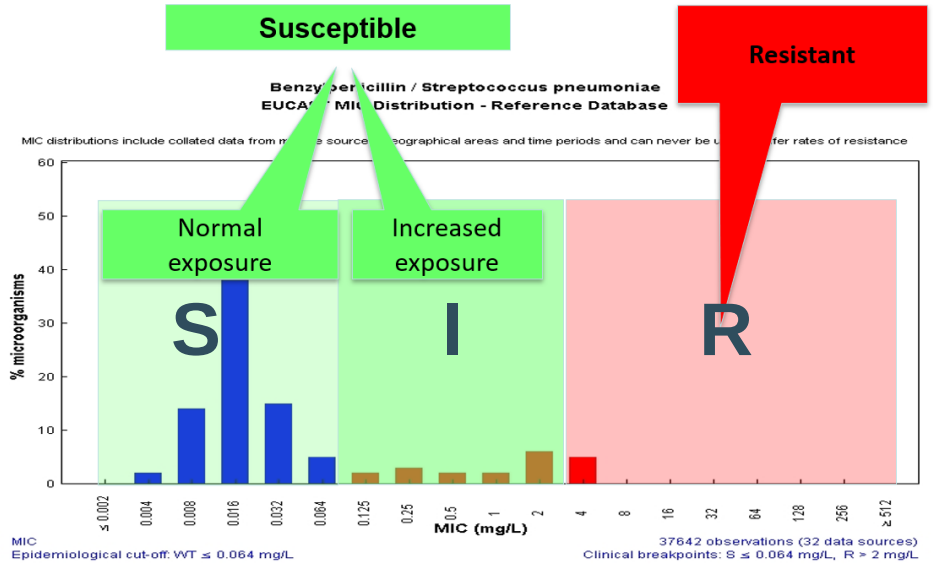
Uncomplicated UTI: acute, sporadic or recurrent lower urinary tract infections (uncomplicated cystitis) in patients with no known relevant anatomical or functional abnormalities within the urinary tract or comorbidities.

Penicillins	Standard dosage	High dosage	Uncomplicated UTI	Special situations
Benzylpenicillin	0.6 g (1 MU) x 4 iv	1.2 g (2 MU) x 4-6 iv		**Meningitis caused by *S. pneumoniae*:** For a dose of 2.4 g (4 MU) x 6 iv, isolates with MIC≤0.06 mg/L are susceptible. **Pneumonia caused by *S. pneumoniae*: breakpoints are related to dosage:** For a dose of 1.2 g (2 MU) x 4 iv, isolates with MIC ≤0.5 mg/L are susceptible. For a dose of 2.4 (4 MU) g x 4 iv or 1.2 g (2 MU) x 6 iv, isolates with MIC ≤1 mg/L are susceptible. For a dose of 2.4 g (4 MU) x 6 iv, isolates with MIC ≤2 mg/L are susceptible.
Ampicillin	2 g x 3 iv	2 g x 4 iv		**Meningitis:** 2 g x 6 iv
Ampicillin-sulbactam	(2 g ampicillin + 1 g sulbactam) x 3 iv	(2 g ampicillin + 1 g sulbactam) x 4 iv		
Amoxicillin iv	1 g x 3-4 iv	2 g x 6 iv		**Meningitis:** 2 g x 6 iv
Amoxicillin oral	0.5 g x 3 oral	0.75-1 g x 3 oral	0.5 g x 3 oral	
Amoxicillin-clavulanic acid iv	(1 g amoxicillin + 0.2 g clavulanic acid) x 3-4 iv	(2 g amoxicillin + 0.2 g clavulanic acid) x 3 iv		
Amoxicillin-clavulanic acid oral	(0.5 g amoxicillin + 0.125 g clavulanic acid) x 3 oral	(0.875 g amoxicillin + 0.125 g clavulanic acid) x 3 or	(0.5 g amoxicillin + 0.125 g clavulanic acid) x 3 oral	Amoxicillin-clavulanic acid has separate breakpoints for systemic infections and uncomplicated UTI. When amoxicillin-clavulanic acid is reported for uncomplicated UTI, the report must make clear that the susceptibility category is only valid for uncomplicated UTI.
Piperacillin	4 g x 4 iv	4 g x 4 iv by extended 3-hour infusion		High dosage for more serious infections.
Piperacillin-tazobactam	(4 g piperacillin + 0.5 g tazobactam) x 4 iv 30-minute infusion or x 3 iv by extended 4-hour infusion	(4 g piperacillin + 0.5 g tazobactam) x 4 iv by extended 3-hour infusion		A lower dosage of (4 g piperacillin + 0.5 g tazobactam) x 3 iv, 30-minute infusion, is adequate for some infections such as complicated UTI, intraabdominal infections and diabetic foot infections, but not for infections caused by isolates resistant to third-generation cephalosporins.
Ticarcillin	3 g x 4 iv	3 g x 6 iv		
Ticarcillin-clavulanic acid	(3 g ticarcillin + 0.1-0.2 g clavulanic acid) x 4 iv	(3 g ticarcillin + 0.1 g clavulanic acid) x 6 iv		
Temocillin	2 g x 2 iv	2 g x 3 iv		The 2 g x 2 iv dose has been used in the treatment of uncomplicated UTI caused by

S

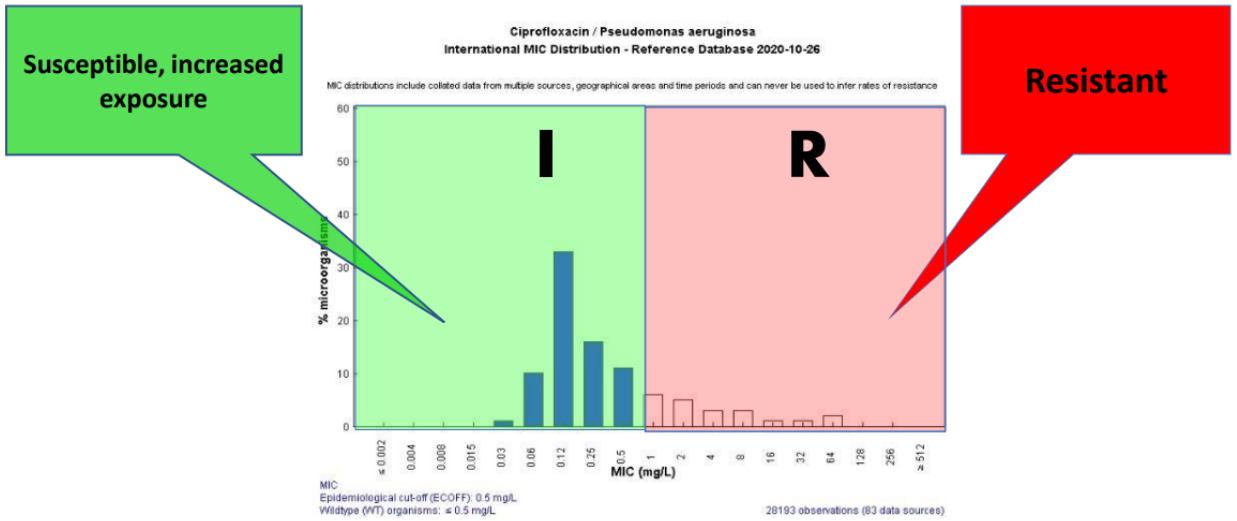
S/I

“I” gerelateerd aan ‘lagere sensitiviteit’ van bacterie aan het middel en de nood aan hogere blootstelling



te wijten aan

verworven laaggradige resistentie die zorgt voor verlaagde gevoeligheid van bacterie
- “S” en “R”, en soms “I” zijn mogelijk



species dat intrinsiek minder gevoelig is aan het antibioticum
- enkel “I” en “R” categorie – geen “S” categorie

Voorbeeld ceftazidime *Pseudomonas aeruginosa*

07-01-2022 08:00 - sputum

Macroscopisch uitzicht	mucopurulent
Gramkleuring	polynucleairen ++
	mondepitheelcellen zeldzaam
	mondflora zeldzaam

Gisten/Schimmelcultuur

V (negatief)

Cultuur

groei van:

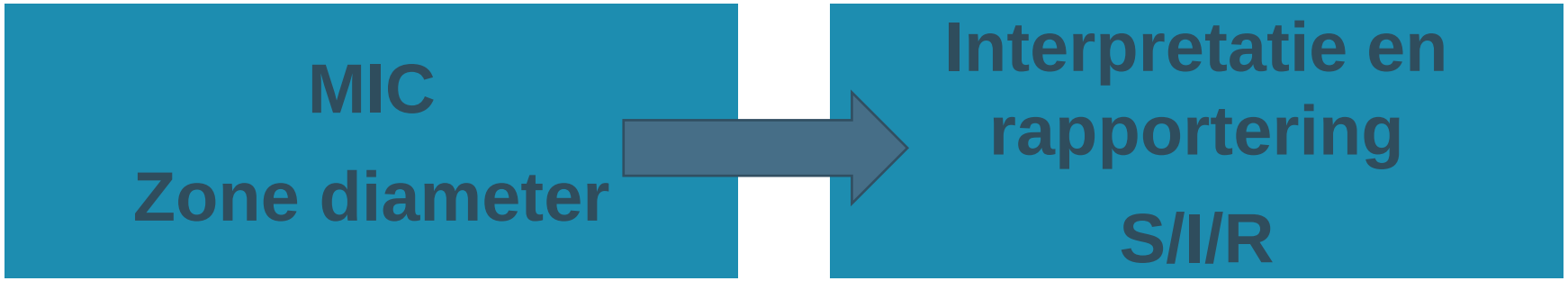
Pseudomonas aeruginosa +++

I : ceftazidime

R : piperacilline-tazobactam

Geen “S” categorie aangezien de standaard dosis onvoldoende is, steeds hoge dosis ceftazidime (3x2g) nodig.

Interpretatie gevoeligheidsbepaling



Klinische breekpunten

Expert regels

Intrinsieke resistenties

Resistentiemechanismen

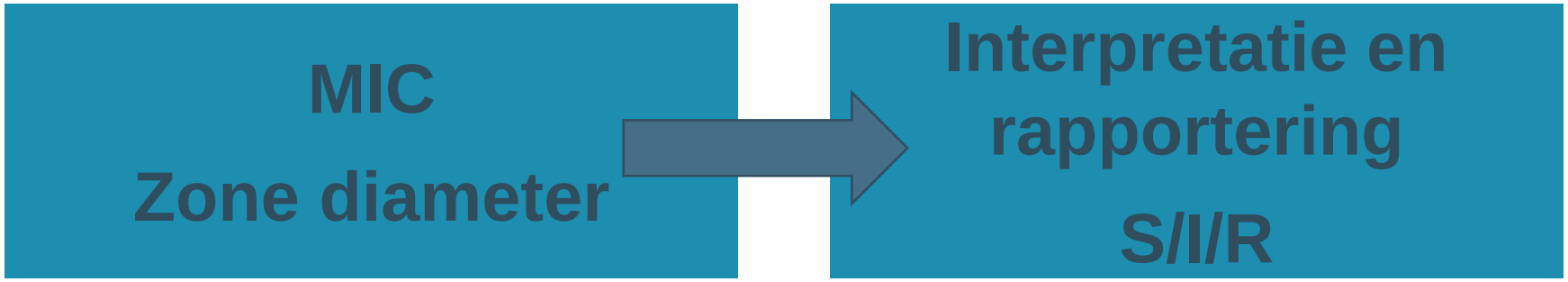
MRSA, ESBL, CPE, induceerbare clindamycine resistentie

Voorbeeld expertregel

Rule No.	Organisms	Indicator Agent*	Agents affected*	Rule	Remarks	Grade	References
5	*Enterobacter* spp., *K. aerogenes, Citrobacter freundii*, *Serratia* spp., *Morganella morganii, Hafnia alvei, Providencia* spp.	cefuroxime	cefuroxime other 2nd generation cephalosporins	IF susceptible to cefuroxime, THEN report cefuroxime and/or any other 2nd generation cephalosporin as resistant	Although the breakpoint table does not list cefuroxime breakpoints for species other than *E. coli*, *P. mirabilis, Klebsiella* spp. (except *K. aerogenes*) and *Raoultella* spp., isolates may appear susceptible in vitro but the MICs tend to be higher than with the mentioned species and therapy with cefuroxime is not recommended. In addition, de-repressed mutants may be selected as with a third-generation cephalosporin.	C	

EUCAST Expert Rules v 3.2 on Enterobacterales

Interpretatie gevoeligheidsbepaling



Klinische breekpunten

Expert regels

Intrinsieke resistenties

Resistentiemechanismen

MRSA, ESBL, CPE, induceerbare clindamycine resistentie

Intrinsieke resistentie/verwachte resistentie

- Micro-organismen kunnen intrinsiek/verwacht resistent zijn tegen een antibioticum

- Onafhankelijk van het bekomen test resultaat is het antibioticum waaraan de kiem intrinsiek resistant is geen goede therapeutische optie
- In praktijk wordt dit steeds als R gerapporteerd
- Er wordt bij voorkeur geen gevoeligheidbepaling uitgevoerd

Expected resistant phenotypes v 1.1 (25 March, 2022)

Table 1 Expected resistant phenotype (susceptibility not expected) in *Enterobacterales* and *Aeromonas* spp. *Enterobacterales* and *Aeromonas* spp. are also expected to be resistant to benzylpenicillin, glycopeptides, lipoglycopeptides, fusidic acid, macrolides (with some exceptions[1]), lincosamides, streptogramins, rifampicin, and oxazolidinones

Rule	Organisms	Ampicillin/Amoxicillin	Amoxicilin-clavulanic acid	Ampicillin-sulbactam	Ticarcillin	Cefazolin, Cephalothin Cefalexin, Cefadroxil	Cefoxitin[2]	Cefuroxime	Tetracyclines	Tigecycline	Polymyxin B, Colistin	Fosfomycin	Nitrofurantoin
1.1	*Citrobacter koseri, Citrobacter amalonaticus*[3]	R			R								
1.2	*Citrobacter freundii*[4]	R	R	R		R	R						
1.3	*Enterobacter cloacae* complex	R	R	R		R	R						
1.4	*Escherichia hermannii*	R			R								
1.5	*Hafnia alvei*	R	R								R		
1.6	*Klebsiella aerogenes*	R	R	R		R	R						
1.7	*Klebsiella pneumoniae* complex	R			R								
1.8	*Klebsiella oxytoca*	R			R								
1.9	*Leclercia adecarboxylata*											R	
1.10	*Morganella morganii*	R	R	R		R			R		R		R
1.11	*Plesiomonas shigelloides*	R	R	R									
1.12	*Proteus mirabilis*								R	R	R		R
1.13	*Proteus penneri*	R				R		R	R	R	R		R
1.14	*Proteus vulgaris*	R				R		R	R	R	R		R
1.15	*Providencia rettgeri*	R	R	R		R			R		R		R
1.16	*Providencia stuartii*	R	R	R		R			R		R		R
1.17	*Raoultella* spp.	R			R								
1.18	*Serratia marcescens*	R	R	R		R	R	R	R[5]		R		R

Table 2 Expected resistance in non-fermentative gram-negative bacteria. Non-fermentative gram-negative bacteria are also generally intrinsically resistant to benzylpenicillin, first- and second-generation cephalosporins, glycopeptides, lipoglycopeptides, fusidic acid, macrolides, lincosamides, streptogramins, rifampicin and oxazolidinones

Rule	Organisms	Ampicillin, Amoxicillin	Amoxicillin-clavulanic acid	Ampicillin-sulbactam	Ticarcillin	Ticarcillin-clavulanic acid	Piperacillin	Piperacillin-tazobactam	Ceftriaxone, Cefotaxime	Ceftazidime	Cefepime	Aztreonam	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Chloramphenicol	Aminoglycosides	Trimethoprim	Fosfomycin	Tetracyclines	Tigecycline	Polymyxin B/Colistin
2.1	*Acinetobacter baumannii*, *Acinetobacter pittii*, *Acinetobacter nosocomialis*	R	R	Note[1]					R			R	R						R	R	R[2]	Note[2]	
2.2	*Achromobacter xylosoxidans*	R							R				R										
2.3	*Burkholderia cepacia* complex[3]	R	R	R	R	R	R	R	R			R	R			R	R	R[4]	R	R			R
2.4	*Elizabethkingia meningoseptica*	R	R	R	R	R	R		R	R	R	R	R	R	R								R
2.5	*Ochrobactrum anthropi*	R	R	R	R	R	R	R	R	R	R	R	R										
2.6	*Pseudomonas aeruginosa*	R	R	R					R				R				R	Note[5]	R		R	R	
2.7	*Stenotrophomonas maltophilia*	R	R	R	R		R	R	R			R	R	R	R			R[4]	R[6]	R	R[7]		

Table 4 Expected resistance in gram-positive bacteria. Gram-positive bacteria are also intrinsically resistant to aztreonam, temocillin, polymyxin B/colistin and nalidixic acid

Rule	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Macrolides	Clindamycin	Quinupristin-dalfopristin	Vancomycin	Teicoplanin	Fosfomycin	Novobiocin	Sulfonamides
4.1	*Staphylococcus saprophyticus*	R	R								R	R	
4.2	*Staphylococcus cohnii*		R									R	
4.3	*Staphylococcus xylosus*		R									R	
4.4	*Staphylococcus capitis*		R								R		
4.5	Other coagulase-negative staphylococci and *S. aureus*		R										
4.6	*Streptococcus* spp.	R	R		R[1]								
4.7	*Enterococcus faecalis*	R	R	R	R[1]	R	R	R					R
4.8	*Enterococcus gallinarum, Enterococcus casseliflavus*	R	R	R	R[1]	R	R	R	R				R
4.9	*Enterococcus faecium*	R	R	R	R[1,2]	R							R
4.10	*Corynebacterium* spp.										R		
4.11	*Listeria monocytogenes*		R	R									
4.12	*Leuconostoc* spp., *Pediococcus* spp.								R	R			
4.13	*Lactobacillus* spp. (*L. casei, L. casei* var. *rhamnosus*)								R	R			

[1] Low-level resistance (LLR) to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against isolates that are susceptible to cell wall inhibitors and do not display high-level resistance to aminoglycosides

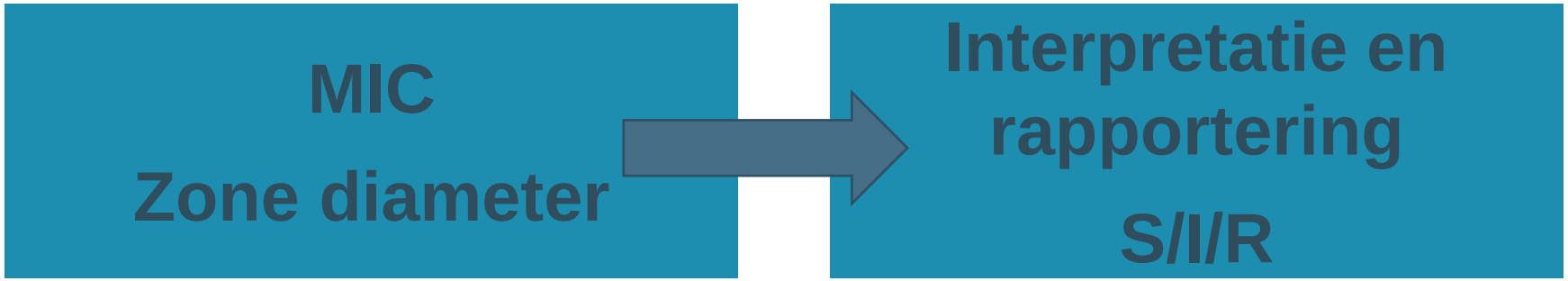
[2] In addition to LLR to aminoglycosides, *Enterococcus faecium* produces a chromosomal AAC(6')-I enzyme that is responsible for the loss of synergism between aminoglycosides (except gentamicin, amikacin and streptomycin) and penicillins or glycopeptides

Belangrijke resistentie mechanismen

- ESBL, CPE, MRSA, induceerbare clindamycine resistentie
  - Therapie
  - Infectiecontrole – ziekenhuishygiëne
  - Epidemiologie

  - Specifieke fenotypische of genotypische confirmatietesten

Interpretatie gevoeligheidsbepaling



Klinische breekpunten

Expert regels

Intrinsieke resistenties

Resistentiemechanismen

MRSA, ESBL, CPE, induceerbare clindamycine resistentie

Interpretatie gevoeligheidsbepaling

MIC

Zone diameter

→

Interpretatie en rapportering

S/I/R

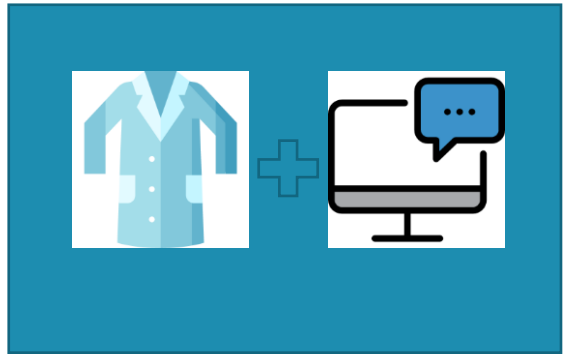
Klinische breekpunten

Expert regels

Intrinsieke resistenties

Resistentiemechanismen

MRSA, ESBL, CPE, induceerbare clindamycine resistentie



Selectief rapporteren
Voorbeeld *Escherichia coli* in UZL

- 18 antibiotica getest - 8 antibiotica gerapporteerd

Antibioticum	Diameter Sirscan	MIC Sirscan	MIC Vitek	Overrule	Resistentie (gemeten)	Resistentie (rapport)
temocilline			8.00		S	
amoxicilline			>= 32.00		R	R
amoxicilline-clavulaanzuur			8.00		S	S
piperacilline-tazobactam			<= 4.00		S	S
cefuroxime			4.00		S	S
cefuroxime-axetil			4.00		S	
cefotaxime			<= 0.25		S	
ceftazidime			0.25		S	
cefepime			<= 0.12		S	
meropenem			<= 0.25		S	
amikacine			<= 2.00		S	
gentamicine			<= 1.00		S	S
tobramycine			<= 1.00		S	S
levofloxacine			>= 8.00		R	R
tigecycline			<= 0.50		S	
nitrofurantoine			64.00		I	
colistine			<= 0.50		S	
co-trimoxazol			>= 320.00		R	R

Escherichia coli

S : amoxicilline-clavulaanzuur, cefuroxime, gentamicine, piperacilline-tazobactam, tobramycine

R : amoxicilline , co-trimoxazol, levofloxacine

ESBL: negatief

Selectief rapporteren

- Selectie van gerapporteerde antibiotica beïnvloedt keuze clinicus voor antibioticum opstart/switch
- Toename van aantal adequate behandelingen in UWI (Coupat et al 2013)
- Cascade rapportering (2016 guideline Implementing an **Antibiotic Stewardship Program**: Guidelines by IDSA and the Society for Healthcare Epidemiology of America)
  - Stapsgewijs rapporteren van initieel de meest aanbevolen antibiotica en pas in geval van resistentie, allergie, intolerantie of polybacteriële infecties gevoeligheden van andere antibiotica op het rapport
  - Niet rapporteren van breedspectrum antibiotica wanneer het isolaat gevoelig is aan antibiotica met een nauwer spectrum
- Aanbeveling om antibiotica die in vitro resistent gemeten → steeds op rapport
- Rapporteren mogelijks ook afhankelijk van staalsoort

Antibioticum	Diameter	MIC Vitek	Overrule	Resistentie (gemeten)	Resistentie (rapport)
piperacilline-tazobactam		<= 4.00		I	I
ceftazidime		2.00		I	I
cefepime		2.00		I	I
meropenem		1.00		S	
amikacine		<= 2.00		S	S
gentamicine		<= 1.00			
tobramycine		<= 1.00		S	S
levofloxacine		0.25		I	
co-trimoxazol					R

Pseudomonas aeruginosa +++

S : amikacine, tobramycine

I : piperacilline-tazobactam, ceftazidime, cefepime

R : co-trimoxazol

- *I = gevoelig, verhoogde expositie. Dit betekent dat de kiem behandeld kan worden met antibiotica die als I gecategoriseerd worden, mits hoge dosis zie doseringstabel antibioticagids (https://www.antibioticagids.be/pages/6).*
- *Antibiotica als I gerapporteerd zijn een volwaardige therapeutische optie mits hoge dosis zie doseringstabel antibioticagids (https://www.antibioticagids.be/pages/6).*

Interpretatie fenotypische gevoeligheidsbepalingen

MIC

Zone diameter

→

Interpretatie en rapportering

S/I/R

Klinische breekpunten

Expert regels

Intrinsieke resistenties

Exceptionele fenotypes

Resistentiemechanismen

MRSA, ESBL, CPE, induceerbare clindamycine resistentie

Cascade rapportering

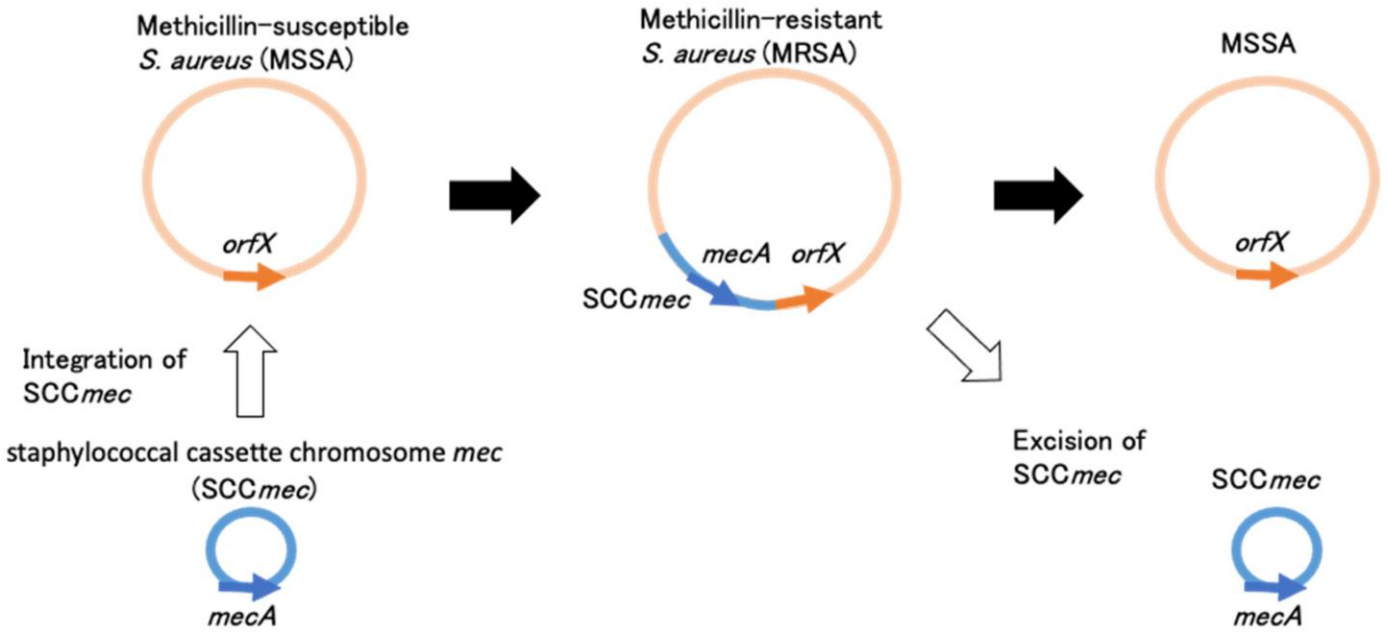
Gevoeligheidsbepalingen

- **Fenotypisch** – detectie van groei in aanwezigheid van het antibioticum (MIC, zone diameter, geautomatiseerde systemen)

- Detectie van resistentiegen of product
  - Antigentest
  - Moleculaire technieken PCR
  - Whole genome sequencing

Detectie resistentiegen of genproduct

- Voorbeeld:
  - MRSA screening aan de hand van PCR op staal/stam
  - Detectie van *mecA* gen (en SCCmec)



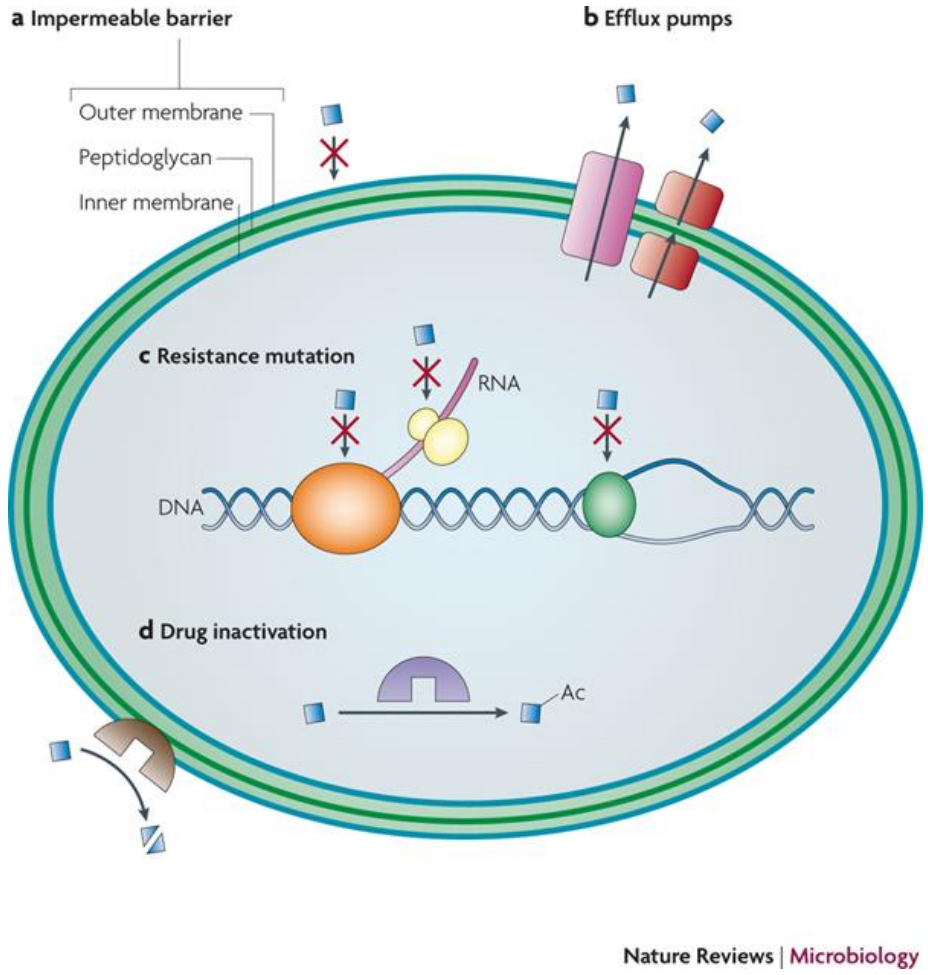
Genotypische gevoeligheidsbepaling

Genotypische gevoeligheidsbepalingen
Opsporen resistentie genen

↕

Fenotypische gevoeligheidsbepalingen
Reflecteren complexe en multiple interacties

- Cel permeabiliteit
- Influx/efflux
- Beschikbaarheid target
- Binding aan het target
- Gen expressie
- Enzymatische expressie
- Enzymatische activiteit



Predictie van gevoeligheidspatroon bacterie op basis van WGS

- Rule-based tools: detectie van resistentiegen, mutaties
  - Resfinder
  - ARIBA (direct from reads)

- Model-based tools
  - Predictie van categorisatie en/of MIC via modellen die vaak getraind werden door datasets met AST resultaten.

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Prediction of antimicrobial susceptibility of pneumococci based on whole-genome sequencing data: a direct comparison of two genomic tools to conventional antimicrobial susceptibility testing

Gerardo J. Sanchez [1], Lize Cuypers[1,2], Lies Laenen[1,2], Peter Májek[3,4], Katrien Lagrou[1,2], Stefanie Desmet [1,2]

TABLE 3 CA, ME, VME, and EA rates for AREScloud and PW WGS-AST compared to phenotypic AST[a] (Table view)

	ARESCloud				Pathogenwatch			
Antibiotic	CA (%)	ME (%)	VME (%)	EA (%)	CA (%)	ME (%)	VME (%)	EA (%)
(Benzyl)penicillin	**94.2**	**0.0**	**0.0**	88.4	**94.4**	**0.0**	**0.0**	**92.2**
Amoxicillin	**97.3**	**0.2**	**0.0**	**95.3**	**96.5**	**0.0**	**0.0**	**96.1**
Cefotaxime/ceftriaxone	**98.5**	**0.0**	**0.0**	**97.7**	**98.8**	**0.0**	**0.0**	**98.4**
Erythromycin	**95.9**	**0.5**	14.3	NA	88.2	**0.3**	53.6	NA
Trimethoprim-sulfamethoxazole	85.5	8.8	**2.5**	NA	81.2	**1.3**	**1.3**	NA
Tetracycline	**93.8**	**2.0**	19.1	NA	88.4	**1.5**	47.0	NA

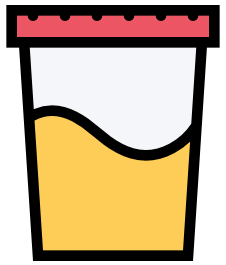
[a] CA was calculated for antibiotics with complete data for phenotypical methods and predictive tools. EA was calculated only for beta-lactam antibiotics with MIC results from broth microdilution and the predictive tool. Values in bold passed the CLSI (M52 guideline) (28) acceptance criteria for AST systems: CA and EA ≥90%, ME and VME <3%. NA, not available.

Uitvoering antibiogram in routine klinisch laboratorium

Rapportering van antibiogram

Labo

Clinicus



Bacteriologie culturen

Urine

09-06-2017 08:00 - urinestaal (geloosd, midstream)

Aantal bacterien	> 100	10**3/mL
Cultuur		
groei van:		
Escherichia coli +++		
S : amikacine, cefuroxime, co-trimoxazol, levofloxacine, nitrofurantoine, piperacilline-tazobactam, temocilline		
I : amoxicilline-clavulaanzuur		
R : amoxicilline , gentamicine, tobramycine		
ESBL:	negatief	

BVIKM/SBIMC
Belgische Vereniging voor Infectiologie en Klinische Microbiologie
Société Belge d'Infectiologie et de Microbiologie Clinique

WHO WE ARE WORKING GROUPS EVENTS PAS

NATIONAL ANTIBIOGRAM COMMITTEE

- MISSION STATEMENT
- NAC GUIDELINES
- ORDER MDR PANELS FOR CLINICAL TEST VALIDATION
- NAC STUDIES
- PRESENTATIONS AT SCIENTIFIC MEETINGS
- EUCAST GUIDELINES
- BOARD MEMBERS
- ARCHIVE - PRESENTATIONS AT SCIENTIFIC MEETINGS

https://sbimc-bvikm.be/en/news/national-antibiogram-committee

KU LEUVEN

Interpretatie fenotypische gevoeligheidsbepalingen

MIC

Zone diameter

→

Interpretatie en rapportering

S/I/R

Klinische breekpunten

Expert regels

Intrinsieke resistenties

Exceptionele fenotypes

Resistentiemechanismen

MRSA, ESBL, CPE, induceerbare clindamycine resistentie

Cascade rapportering