



MINISTERIO  
DE SANIDAD



agencia española de  
medicamentos y  
productos sanitarios



Plan Nacional  
Resistencia  
Antibióticos



# I Jornada del Comité Español del Antibiograma (COESANT)

Madrid 24 de noviembre de 2022

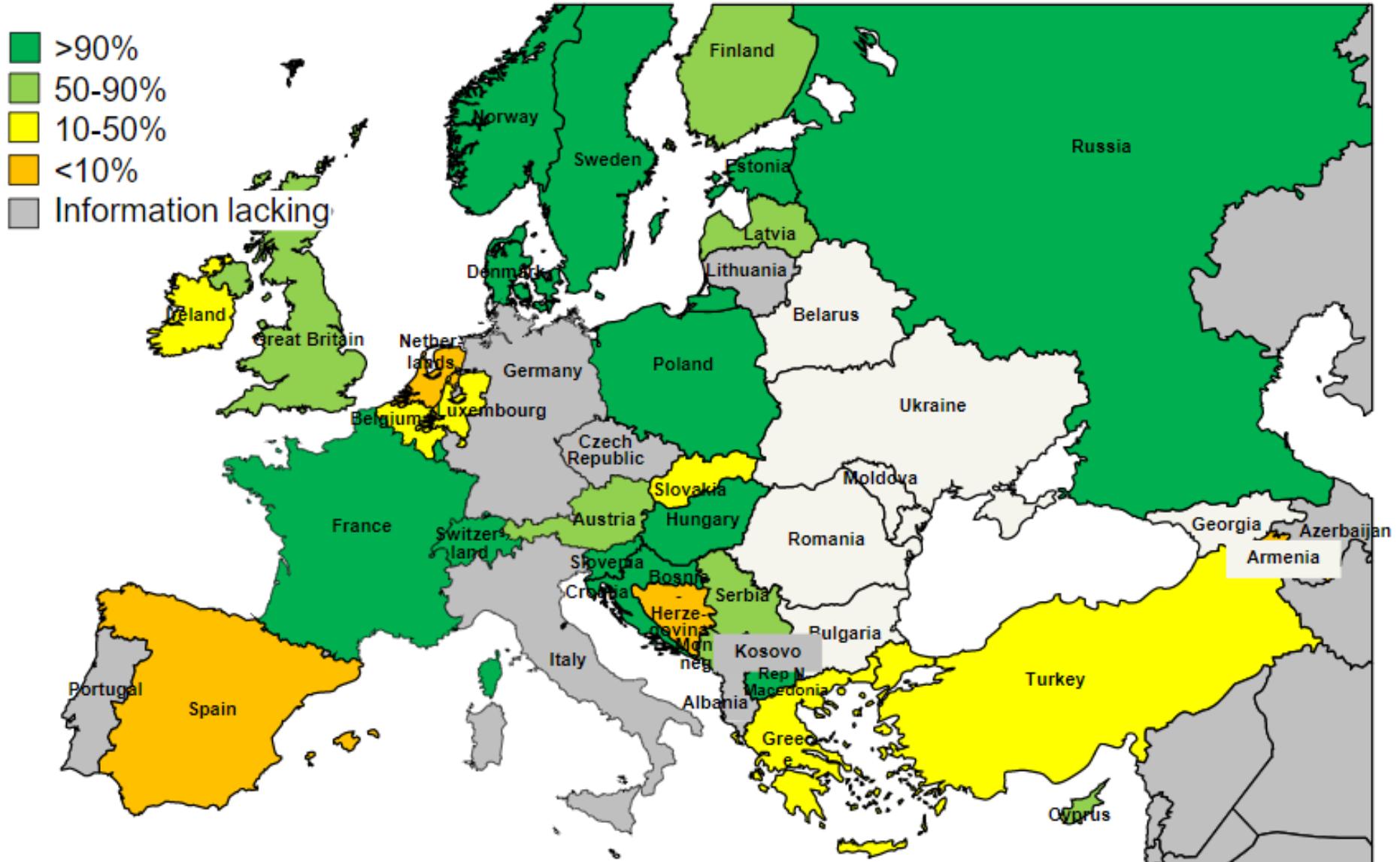


# Documento CoEsAnt de paneles disco difusión

Alba Rivera  
Servicio de Microbiología  
Hospital de la Santa Creu i Sant Pau

# Disk diffusion as main AST method, April 2019

## % Laboratories on disk diffusion as main method



Other countries:

Australia

Brazil

China

Canada

Iceland

Israel

Malta

Morocco

New Zealand

South Africa

USA

<https://www.eucast.org>

# Metodología

# Kirby–Bauer disk diffusion method

## ANTIBIOTIC SUSCEPTIBILITY TESTING BY A STANDARDIZED SINGLE DISK METHOD

A. W. BAUER, M.D., W. M. M. KIRBY, M.D., J. C. SHERRIS, M.D., AND  
M. TURCK, M.D.

Am J Clin Pathol 1966; 45:493-496.

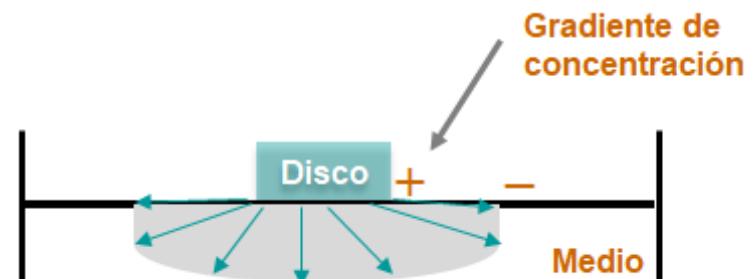
## ICS (International Collaborative Study) disk diffusion standard

## Antibiotic Sensitivity Testing

# Report of an International Collaborative Study

BY

HANS M. ERICSSON and JOHN C. SHERRIS



# Metodología



European Society of Clinical Microbiology and Infectious Diseases

## AST of bacteria

Organization

Consultations

EUCAST News

New definitions of S, I and R

Clinical breakpoints and dosing

Rapid AST in blood cultures

Expert rules and expected phenotypes

Resistance mechanisms

Guidance documents

SOP

MIC and zone distributions and ECOFFs

AST of bacteria

Media preparation

MIC determination

Disk diffusion methodology

Disk diffusion implementation



## EUCAST Disk Diffusion Test Methodology

The EUCAST disk diffusion test is based on MH media and disks of a good quality. It is calibrated to EUCAST clinical breakpoints using broth microdilution for MIC determination. Updates are published regularly.

See also EUCAST instruction videos.

- Disk diffusion - Manual v 10.0 (1 January, 2022)
- Disk diffusion - Slide show v 10.0 (1 January, 2022)
- Disk diffusion - Reading guide v 9.0 (1 January 2022)
- Anaerobic bacteria - disk diffusion methodology v 1.0 (1 January 2022) including QC recommendations (the difficulties related to ordering the QC strain *C. perfringens* DSM 25589 from Germany have been solved - it can now be ordered from DSM and CCUG). Disk diffusion breakpoints for anaerobic bacteria are valid for FAA with 5% mechanically defibrinated horse blood as the only additive.
- Anaerobic bacteria - disk diffusion reading guide v 1.0 (1 January 2022) Disk diffusion breakpoints for anaerobic bacteria are valid for FAA with 5% mechanically defibrinated horse blood as the only additive.



## Antimicrobial susceptibility testing

### EUCAST disk diffusion method

Version 10.0  
January 2022

EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing  
Version 10.0 (January 2022)  
[www.eucast.org](http://www.eucast.org)

## Variables

- Antimicrobiano
- Agar
- Incubación
- Microorganismo

Carga, tasa difusión, actividad

Profundidad, composición

Temperatura, duración, atmósfera

Tasa crecimiento, densidad inóculo

Contents	Page
<a href="#">Changes from previous version</a>	
<a href="#">Abbreviations and Terminology</a>	
1 <a href="#">Introduction</a>	5
2 <a href="#">Preparation and storage of media</a>	6
3 <a href="#">Preparation of inoculum</a>	8
4 <a href="#">Inoculation of agar plates</a>	10
5 <a href="#">Application of antimicrobial disks</a>	11
6 <a href="#">Incubation of plates</a>	12
7 <a href="#">Examination of plates after incubation</a>	14
8 <a href="#">Measurement of zones and interpretation of susceptibility</a>	15
9 <a href="#">Quality control</a>	17
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## AST of bacteria

- Organization
- Consultations
- EUCAST News
- New definitions of S, I and R
- Clinical breakpoints and dosing
- Rapid AST in blood cultures
- Expert rules and expected phenotypes
- Resistance mechanisms
- Guidance documents
- SOP
- MIC and zone distributions and ECOFFs

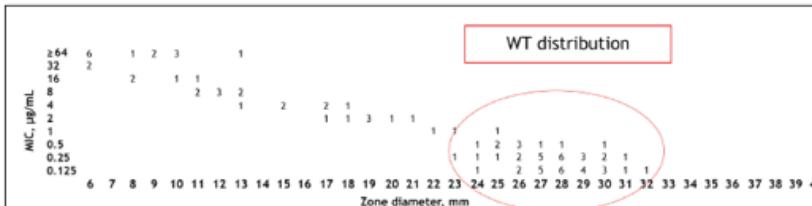


### Development and validation of EUCAST Disk Diffusion breakpoints

The EUCAST Disk Diffusion test was developed by EUCAST under the auspices of ESCMID and with the help of many laboratories. The help of these laboratories is gratefully acknowledged. Most are listed under the EUCAST laboratory network. The work started in 2009 and is ongoing - new species, new strains, revised or new breakpoints and new resistance mechanisms necessitates constant development and recalibration.

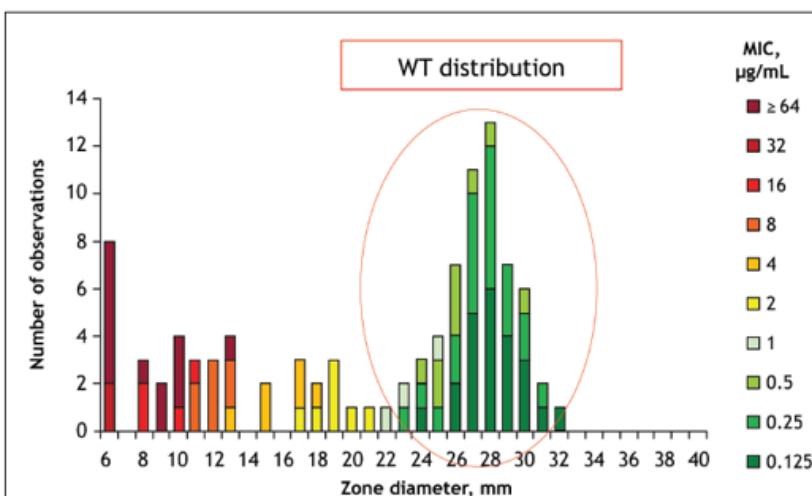
The files below list material and graphs used for determining zone diameter breakpoints to match MIC breakpoints (Exam)

- Media preparation
- MIC determination
- Disk diffusion methodology
- Disk diffusion implementation
- Breakpoint tables
- Quality Control
- Strains with defined susceptibility
- Calibration and validation
- Warnings!
- MIC testing services from EUCAST
- Previous versions of documents



Abbreviations: MIC, minimal inhibitory concentration; WT, wild-type.

**Figure 1A. Zone Diameter Scattergram With Zone Diameters Plotted Against Minimal Inhibitory Concentration Values.** Figures 1A and 1B represent the same dataset.



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

#### Enterobacterales

#### Pseudomonas spp.

#### Stenotrophomonas maltophilia

#### Acinetobacter spp.

#### Staphylococcus spp.

#### Enterococcus spp.

#### Streptococcus groups A, B, C and G

#### Streptococcus pneumoniae

#### Viridans group streptococci

#### Haemophilus influenzae

#### Moraxella catarrhalis

#### Listeria monocytogenes

#### Pasteurella multocida

#### Campylobacter jejuni and coli

#### Corynebacterium spp.

#### Aerococcus sanguinicola and urinae

#### Kingella kingae

#### Aeromonas spp.

#### Achromobacter xylosoxidans

#### Vibrio spp.

#### Bacillus spp.

#### Burkholderia pseudomallei

#### Bacteroides spp.

#### Prevotella spp.

#### Fusobacterium necrophorum

#### Clostridium perfringens

#### Cutibacterium acnes

# Puntos de corte disco difusión añadidos en los últimos años

## European Committee on Antimicrobial Susceptibility Testing

Breakpoint tables for interpretation of MICs and zone diameters

Bacteria	Año
<i>Aerococcus sanguinicola</i> and <i>urinae</i>	2017
<i>Kingella kingae</i>	2017
<i>Aeromonas</i> spp.	2018
<i>Burkholderia pseudomallei</i>	2020
<i>Achromobacter xylosoxidans</i>	2021
<i>Bacillus</i> spp.	2021
<i>Vibrio</i> spp.	2022
Anaerobios	2022
<i>Corynebacterium diphtheriae</i> and <i>ulcerans</i>	Prepublicación v. 13.0 2023

CLINICAL  
AND  
LABORATORY  
STANDARDS  
INSTITUTE®

13th Edition

**M02**

Performance Standards for Antimicrobial Disk Susceptibility Tests



This standard covers the current recommended methods for disk susceptibility testing and criteria for quality control testing.

A standard for global application developed through the Clinical and Laboratory Standards Institute consensus process.

## Discrepancias metodológicas

### EUCAST

### CLSI

#### Temperatura incubación

 $35 \pm 1^\circ\text{C}$  $35 \pm 2^\circ\text{C}$ 

#### Duración incubación

16-20 h  
40-44 h<sup>1</sup>16-18 h  
20-24h<sup>2</sup>  
24h<sup>3</sup>

#### Medio

Mueller Hinton+5% sangre caballo +20 mg/Lβ-NAD  
(MH-F)  
Fastidious anaerobe agar+5% sangre caballo (FAA)Mueller Hinton +5% sangre carnero (MHA)  
*Haemophilus* Test Medium (HTM)  
GC + 1% suplemento

#### Carga discos antimicrobiano

Benzylpenicillin  
Ampicillin  
Amoxicillin-clavulanate  
Piperacillin  
Piperacillin-tazobactam  
Cefotaxime  
Ceftaroline  
Ceftazidime  
Gentamicin (test for HLAR)  
Vancomycin  
Linezolid  
Nitrofurantoin

10 units  
10 µg  
20-10 µg  
100 µg  
100-10 µg  
30 µg  
30 µg  
30 µg  
120 µg  
30 µg  
30 µg  
300 µg

1 unit  
2 and 10 µg<sup>b</sup>  
2-1 and 20-10 µg<sup>c</sup>  
30 µg  
30-6 µg  
5 µg  
5 µg  
10 µg  
30 µg  
5 µg  
10 µg  
100 µg

#### Puntos de corte

Ausencia para *Neisseria gonorrhoeae*,  
*Neisseria meningitidis*, *Burkholderia cepacia*Ausencia para *Aerococcus* spp., *Kingella kingae*, *Listeria monocytogenes*,  
*Corynebacterium* spp., *Bacillus* spp.,  
anaerobios

<sup>1</sup>*Corynebacterium*, *Aerococcus*, *Kingella*, *Campylobacter* si crecimiento insuficiente en 16-20h ; <sup>2</sup>*Acinetobacter* spp., *Burkholderia*, *Stenotrophomonas maltophilia*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Streptococcus* spp., <sup>3</sup>cefoxitina/*Staphylococcus* spp.

# Disco difusión

## Ventajas e inconvenientes

### Flexibilidad

Elección antimicrobianos

### Visualización crecimiento:

- detección cultivos mixtos
- heteroresistencia
- interacciones entre antibióticos

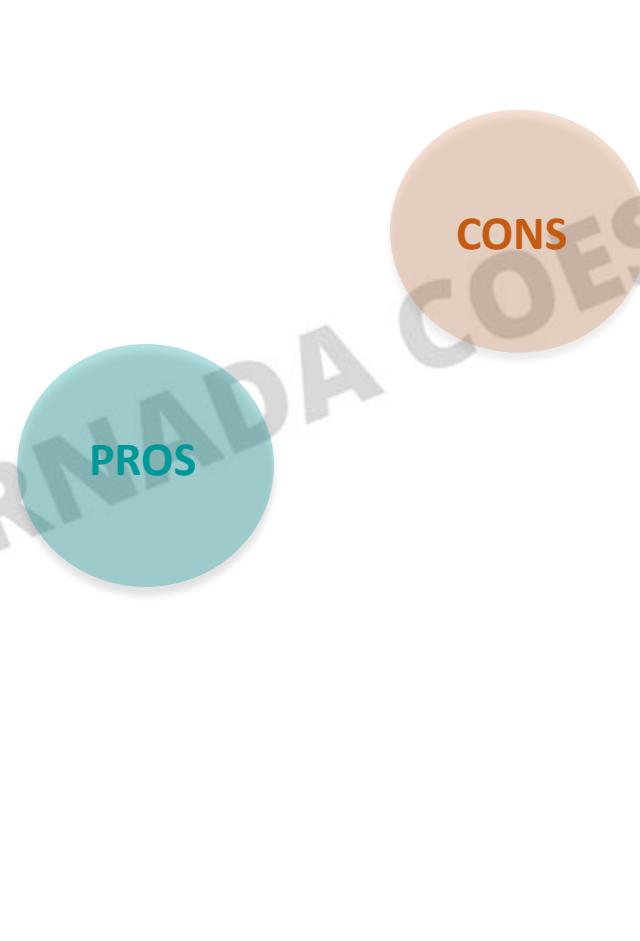
### Posibilidad de automatización

Lectura de halos de inhibición

### Método rápido

Lectura 4, 6, 8 horas

### Coste



### No determina CIM

### No estandarizado para algunos microorganismos

*Nocardia, Actinomyces, Streptomyces, Helicobacter*

### No adecuado para algunos antibióticos

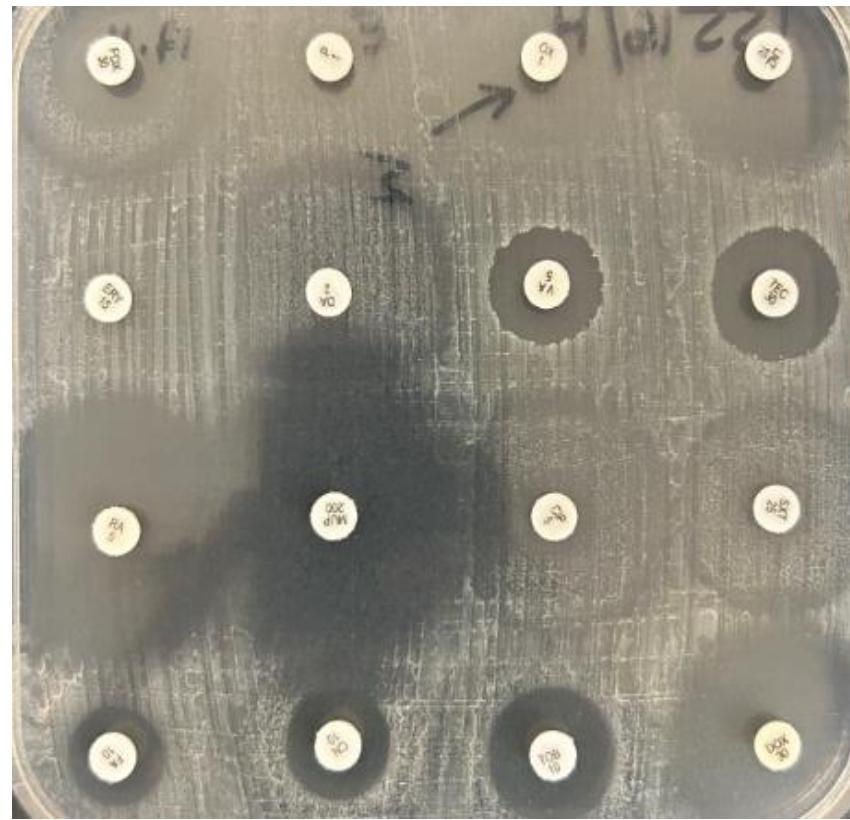
Glucopéptidos, lipoglucopéptidos, polimixinas

### Laborioso

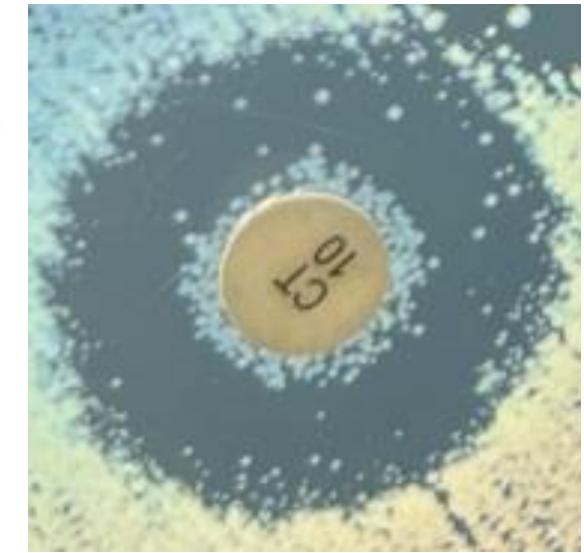
### Variabilidad interobservador en resultados

# Visualización del crecimiento

Detección cultivos mixtos

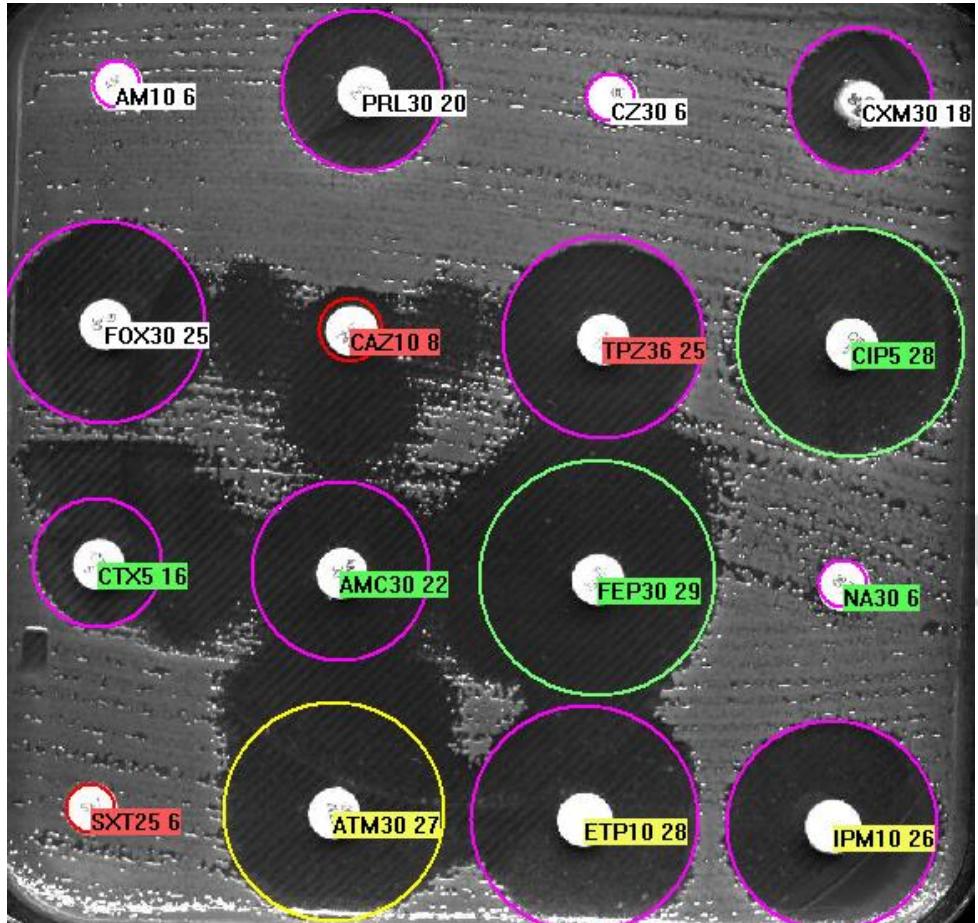


Heteroresistencia

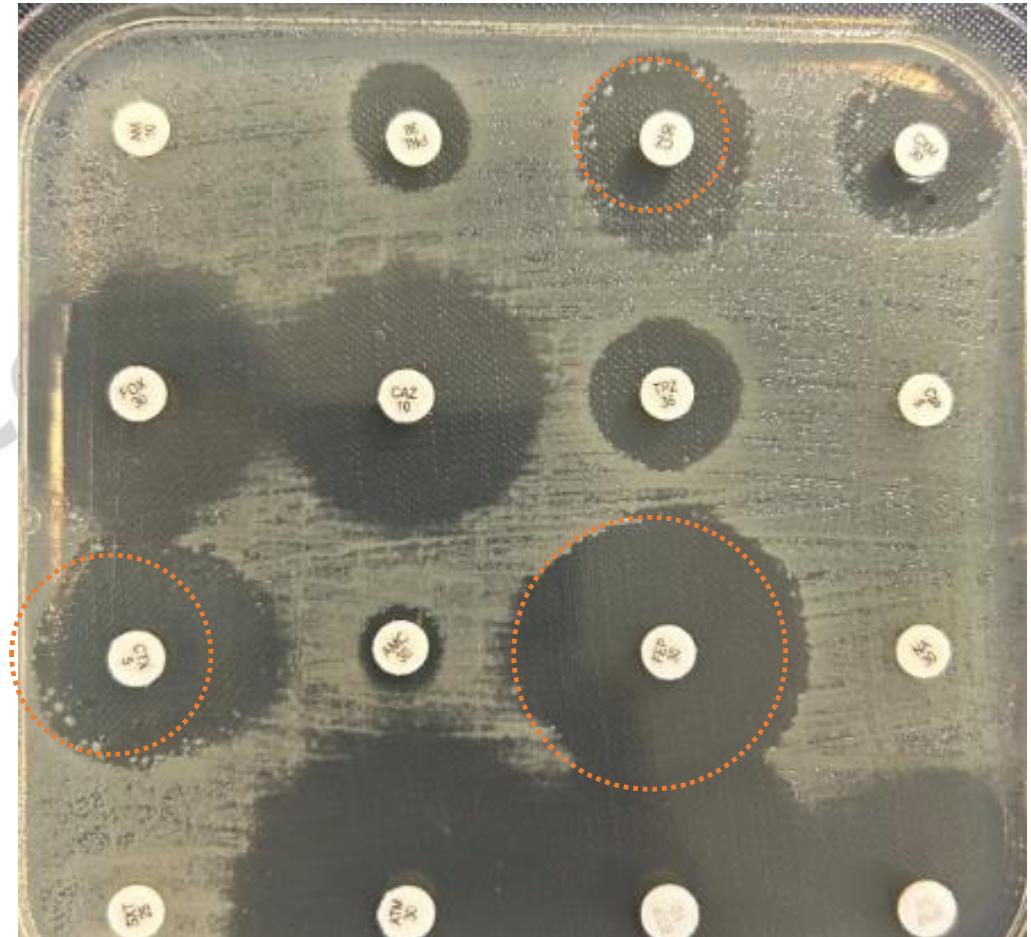


# Visualización del crecimiento

## Interacciones entre antibióticos



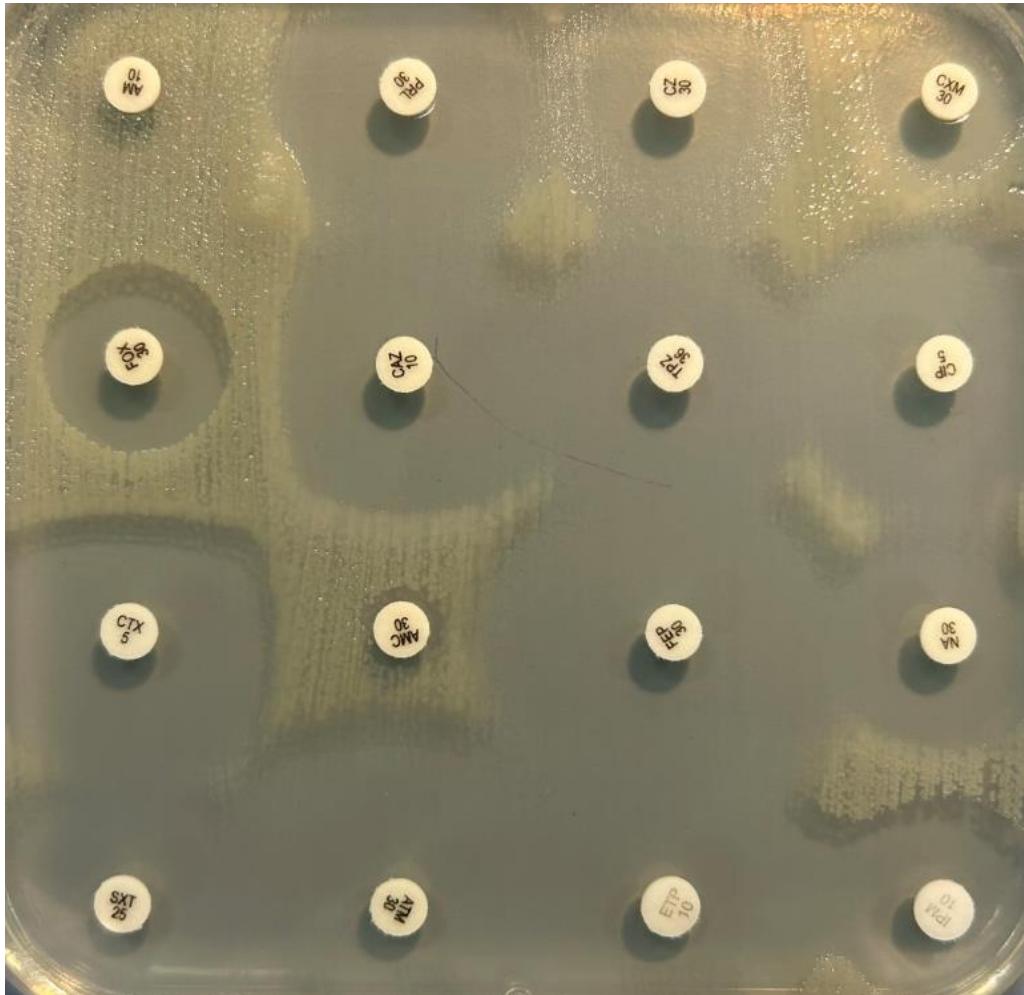
*Aeromonas hydrophila*  
VEB-1



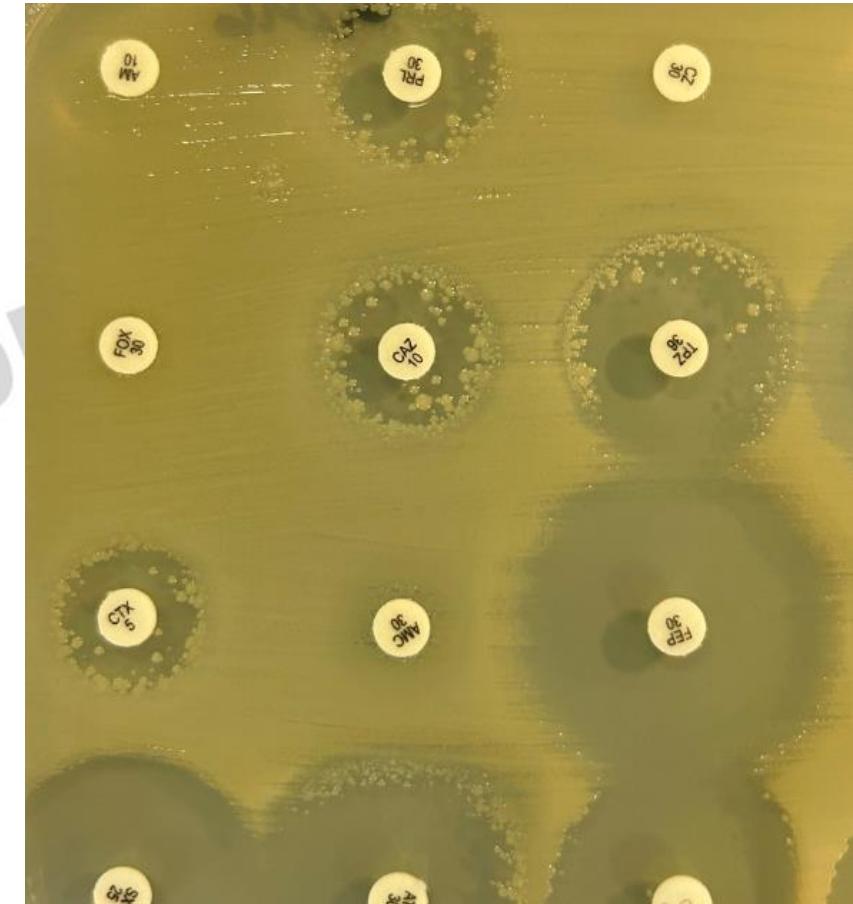
*Escherichia coli*  
CTX-M-15,OXA-1

# Visualización del crecimiento

## Interacciones entre antibióticos



*Morganella morganii*



*Escherichia coli*  
CMY-2

# Automatización



SIRScan (i2a, Francia)



BIOMIC V3 (Giles Scientific Inc, USA)



ADAGIO (Bio-Rad, Francia)



Copan WASP Srl (Copan Diagnostics, Italia)

Colibri, Radian in-Line Carousel, Radian Expert System

BD Kiestra (Becton Dickinson, USA)

Kiestra Inoqua, Kiestra Read A

- Lectura automática e interpretación resultados disco difusión
- Sistema experto
- Almacenamiento y gestión de datos para su explotación en estudios epidemiológicos

Automatización completa:

- Preparación inóculo
- Siembra de placas
- Colocación discos
- Lectura e interpretación

# Método rápido



**Methodology - EUCAST rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles.**

Version 3.0

April 2022

**Screening for ESBL and carbapenemases in *E. coli* and *K. pneumoniae* for epidemiological purposes as part of the RAST procedure.**

**EUCAST Guidelines for detection of resistance mechanisms and specific resistance of clinical and/or epidemiological importance using EUCAST rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles.**

Version 2.0

April 2022

## Lectura en 4, 6, 8, 16-20h

**The proportion of zone diameters (%) which are possible to read\* after 4 – 20 h of incubation.**

Organism	4 hours	6 hours	8 hours	16-20 hours
<i>Escherichia coli</i>	90	99	99	100
<i>Klebsiella pneumoniae</i>	96	98	98	100
<i>Pseudomonas aeruginosa</i>	-	88	97	100
<i>Acinetobacter baumannii</i>	99	100	100	ND
<i>Staphylococcus aureus</i>	55**	91	95	100
<i>Enterococcus faecalis</i>	93	99	100	ND
<i>Enterococcus faecium</i>	44	93	99	ND
<i>Streptococcus pneumoniae</i>	68	83	95	100

## Incubation conditions for antimicrobial susceptibility test plates

Organism	Incubation time	Medium	Incubation
<i>Escherichia coli</i>	4, 6 and 8 hours	MH	35±1°C in air
<i>Klebsiella pneumoniae</i>	16-20 hours		
<i>Staphylococcus aureus</i>			
<i>Pseudomonas aeruginosa</i>	6 and 8 hours 16-20 hours	MH	35±1°C in air
<i>Acinetobacter baumannii</i>	4, 6 and 8 hours	MH	35±1°C in air
<i>Enterococcus faecalis</i>			
<i>Enterococcus faecium</i>			
<i>Streptococcus pneumoniae</i>	4, 6 and 8 hours 16-20 hours	MH-F	35±1°C in 4-6% CO <sub>2</sub> in air

EUCAST RAST Breakpoint Tables version 5.1 (2022-05-02)

European Committee on Antimicrobial Susceptibility Testing

## Zone diameter breakpoint tables for rapid antimicrobial susceptibility testing (RAST) directly from blood culture bottles

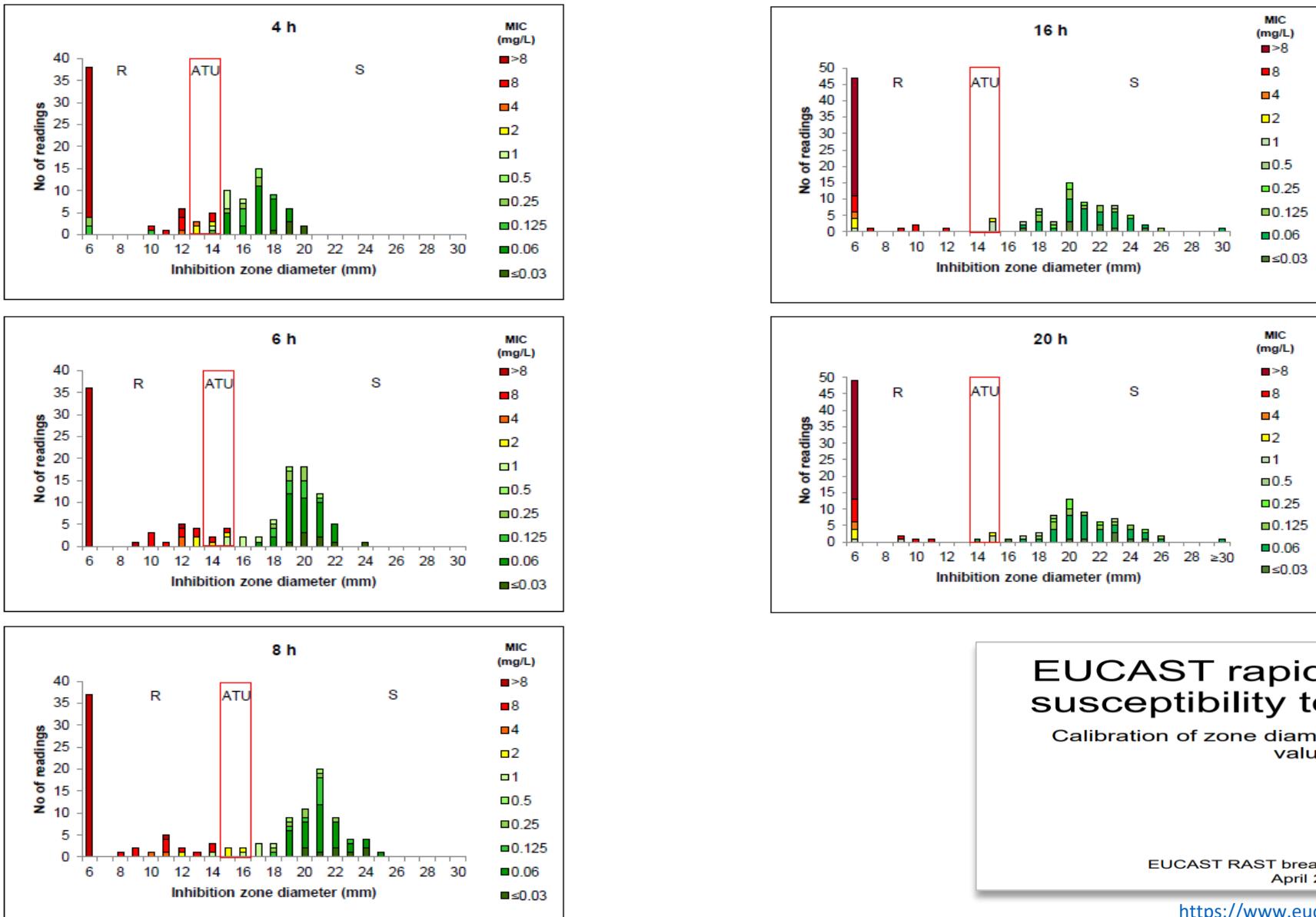
Version 5.1, valid from 2022-05-02

This document should be cited as "The European Committee on Antimicrobial Susceptibility Testing. Zone diameter Breakpoint Tables for rapid antimicrobial susceptibility testing (RAST) directly from blood culture bottles. Version 5.1, 2022. <http://www.eucast.org>."

Content	Page	Additional information
Changes	1	
Notes	3	
Guidance on reading EUCAST RAST Breakpoint Tables	4	
Information on technical uncertainty	5	
<i>Escherichia coli</i>	6	Breakpoints for 4, 6, 8 and 16-20 h
<i>Klebsiella pneumoniae</i>	7	Breakpoints for 4, 6, 8 and 16-20 h
<i>Pseudomonas aeruginosa</i>	8	Breakpoints for 6, 8 and 16-20 h
<i>Acinetobacter baumannii</i>	9	Breakpoints for 4, 6 and 8 h
<i>Staphylococcus aureus</i>	10	Breakpoints for 4, 6, 8 and 16-20 h
<i>Enterococcus faecalis</i>	11	Breakpoints for 4, 6 and 8 h
<i>Enterococcus faecium</i>	12	Breakpoints for 4, 6 and 8 h
<i>Streptococcus pneumoniae</i>	13	Breakpoints for 4, 6 and 16-20 h

# Método rápido

*E. coli* and cefotaxime 5 µg, spiked blood culture bottles  
RAST vs. broth microdilution 16-20h



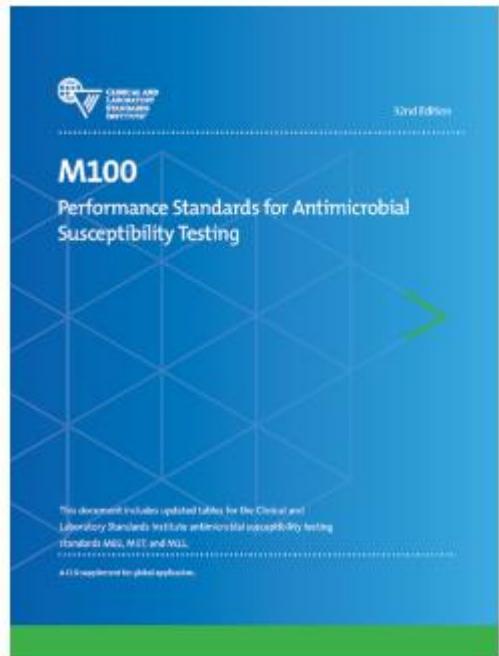
**EUCAST rapid antimicrobial susceptibility testing (RAST)**

Calibration of zone diameter breakpoints to MIC values.

EUCAST RAST breakpoints version 5.0  
April 2022

[https://www.eucast.org/rapid\\_ast\\_in\\_bloodcultures](https://www.eucast.org/rapid_ast_in_bloodcultures)

# Método rápido



- Table 3E-1. Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth
- Table 3E-2. Zone Diameter Disk Diffusion Breakpoints for Enterobacteriales Direct From Blood Culture
- Table 3E-3. Zone Diameter Disk Diffusion Breakpoints for *Pseudomonas aeruginosa* Direct From Blood Culture

**Table 3E-2. Zone Diameter Disk Diffusion Breakpoints for Enterobacteriales Direct From Blood Culture**

General Comments

(1) The dosage regimens shown in the Comments column below are needed to achieve plasma drug exposure (in adults with normal renal and hepatic function) on which breakpoints were based. When new breakpoints are implemented, it is strongly recommended that laboratories share this information with infectious diseases practitioners, pharmacists, pharmacy and therapeutics committees, infection prevention committees, and the antimicrobial stewardship team.

(2) For additional testing and reporting recommendations, refer to Table 2A.

NOTE: Information in boldface type is new or modified since the previous edition.

Test/Report Group	Antimicrobial Agent	Disk Content	Read Times, hours	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Comments
				S	SDD	I	R	
<b>PENICILLINS</b>								
A	Ampicillin	10 µg	8-10 16-18	- ≥ 17	-	14-16	≤ 13	(3) Results of ampicillin testing can be used to predict results for amoxicillin.  (4) Breakpoints are based on an ampicillin dosage regimen of 2 g parenterally administered every 4-6 h or an amoxicillin dosage regimen of 1-2 g parenterally administered every 6 h.
<b>CEPHEMS (PARENTERAL) (including cephalosporins I, II, III, and IV. Please refer to Glossary I.)</b>								
B	Ceftriaxone	30 µg	8-10 16-18	≥ 23 ≥ 23	-	20-22	≤ 19	(5) Breakpoints are based on a dosage regimen of 1 g administered every 24 h.
C	Ceftazidime	30 µg	8-10 16-18	≥ 21 ≥ 21	-	18-20	≤ 17	(6) Breakpoints are based on a dosage regimen of 1 g administered every 8 h.

Test	Direct Disk Diffusion
Test method	Disk diffusion using positive blood culture broth
Organism group	Enterobacteriales and <i>Pseudomonas aeruginosa</i>
Medium	MHA
Antimicrobial concentration	Standard disk contents for the antimicrobials are detailed in Table 3E-2 (Enterobacteriales) and Table 3E-3 ( <i>P. aeruginosa</i> )
Inoculum	Positive blood culture broth with gram-negative bacilli, used within 8 hours of flagging positive by the blood culture system
Test procedure	<ol style="list-style-type: none"> <li>Invert blood culture bottle 5-10 times to thoroughly mix.</li> <li>Sterilize the top of the bottle with an alcohol wipe (allow to dry) and insert 20-gauge venting needle into the blood culture bottle.</li> <li>Dispense 4 drops of blood culture broth onto an MHA plate. As a purity check, use an inoculated blood agar plate streaked for isolation.</li> <li>Spread blood culture broth across the entire surface of the MHA plate using a sterile cotton swab.</li> <li>Repeat this procedure by streaking twice more, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum.</li> <li>Leave the lid ajar for 3-5 minutes (ideally) but no more than 15 minutes.</li> <li>Dispense antimicrobial disks onto the surface of the inoculated MHA plate.</li> <li>Press each disk down to ensure complete contact with the agar surface.</li> <li>Invert the plate and place in the incubator within 15 minutes of disks being applied.</li> </ol>
Incubation conditions	35°C ± 2°C; ambient air
Incubation length	8-10 hours or 16-18 hours
Results	<ol style="list-style-type: none"> <li>Examine the blood agar purity plate to ensure pure growth.</li> <li>Examine the test plate to ensure confluent lawn of growth appropriate to read disk zone tests per M02.<sup>1</sup></li> <li>Measure the zone diameters according to routine disk diffusion recommendations in M02.<sup>1</sup></li> <li>Report results using the interpretive categories and zone diameter breakpoints in Table 3E-2 or Table 3E-3 if the gram-negative bacillus tested is confirmed to be an Enterobacteriales or <i>P. aeruginosa</i>, respectively. If species is identified as another organism, do not interpret or report results.</li> </ol>
Additional testing and reporting	<ul style="list-style-type: none"> <li>If there is an inconsistent growth pattern on the plate (eg, mixed inoculum, nonconfluent growth, growth is too faint to read), do not interpret or report results from the direct disk diffusion test, and perform standard susceptibility testing from pure colony growth.</li> <li>Antimicrobial agents to which the organism is intrinsically resistant (see Appendix B) should be reported as resistant, regardless of measured zone size.</li> <li>If two zones of growth inhibition are observed, measure the inner zone diameter. In case of colonies present within zones, or presence of both inner and outer zones, check the purity plate and, if pure, record the inner zone diameter.</li> </ul>
QC recommendations	<ul style="list-style-type: none"> <li>Perform QC according to the standard disk diffusion QC procedures per M021 (eg, daily or weekly).</li> </ul>

Article

# Multicentre Evaluation of the EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) Extending Analysis to 16–20 Hours Reading Time

Gabriele Bianco <sup>1,\*</sup>, Donatella Lombardo <sup>2</sup> , Guido Ricciardelli <sup>1,3</sup>, Matteo Boattini <sup>1,3</sup> , Sara Comini <sup>1,3</sup>, Rossana Cavallo <sup>1,3</sup>, Cristina Costa <sup>1,3</sup> and Simone Ambretti <sup>2</sup>

*Antibiotics* **2022**, *11*, 1404. <https://doi.org/10.3390/antibiotics11101404>

n  
641

Multicéntrico (Italia)  
Comparación con Microscan  
Especies validadas y no validadas (*Enterobacteriales* n=61, ConNS n=72)

**Lectura 4h** >90% excepto: 0% *P. aeruginosa*, 51,7% *Enterococcus* spp., 46,1% *Staphylococcus* spp.

**ATU:** Piperacilina-tazobactam/*Enterobacteriales*, *P. aeruginosa* (4, 6, 8h)  
clindamicina, gentamicina/*Staphylococcus* spp., linezolid, vancomicina/*Enterococcus* spp.

**VME:** Aminoglucósidos, cefalosporinas/*Enterobacteriales*, gentamicina, clindamicina/*Staphylococcus* spp.

	4 h %	6 h %	8 h %	16-20 h %
<b>Readable zones</b>	75,7	96,6	100	100
<b>CA</b>	98,9	-	-	99,4
<b>ME</b>	0,2	0,4	0,3	0,3
<b>VME</b>	3,3	3,7	3,4	1
<b>ATU</b>	9,9	5,9	5	5,2



AMERICAN  
SOCIETY FOR  
MICROBIOLOGY

Journal of  
Clinical Microbiology®

# Fully Automated EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) from Positive Blood Cultures: Diagnostic Accuracy and Implementation

October 2022 Volume 60 Issue 10

✉ Abdessalam Cherkoui,<sup>a,b</sup> Didier Schorderet,<sup>a</sup> Nouria Azam,<sup>a</sup> Luigi Crudeli,<sup>a</sup> José Fernandez,<sup>a</sup> Gesuele Renzi,<sup>a</sup> Adrien Fischer,<sup>a</sup> Jacques Schrenzel<sup>a,c</sup>

Fase 1

n  
779

Bacteriology Laboratory Geneva University Hospitals  
Copan System / disco difusión estàndard EUCAST

Fase 2

n  
534

Fase 1: hemocultivos inoculados 100-200 UFC  
Fase 2: estudio clínico prospectivo

Lectura 4h >95% excepto: 0% *P. aeruginosa*, 93,9% *Enteroccus* spp., 87,3% *S. aureus*

ATU: Piperacilina-tazobactam, amikacina, ciprofloxacino, cotrimoxazol/Gram neg (4h), gentamicina, norfloxacino/*S. aureus*, vancomicina, linezolid/*Enterococcus* spp.

CA:>95%

VME: imipenem, ciprofloxacino, tobramicina/*P. aeruginosa*

MRSA n=20, identificados 100% a las 4h

ESBL n=122, detectados por sinergia doble disco 67% 4h, 100% 6h

VRE n=30, detectados 97% 4h, 100% a las 6h

Carbapenemasas 100% correlación con cribado por disco difusión estàndar

Resistencia inducible a clindamicina n=49, detectados 8,2% 4h, 75,5% 6h, 83,7% 8h

## Automatización del procesamiento

- óptimas condiciones de crecimiento con temperatura estable
- lectura programable a diferentes tiempos
- observación aumentada de las imágenes
- sistema experto

# Impact of EUCAST rapid antimicrobial susceptibility testing (RAST) on management of Gram-negative bloodstream infection

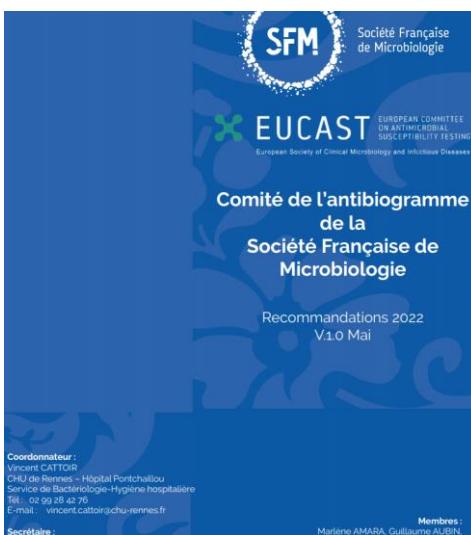
Emilie Cardot Martin <sup>a,\*</sup>, Marie Alice Colombier <sup>b</sup>, Lucie Limousin <sup>a</sup>, Orianne Daude <sup>a</sup>, Oscar Izarn <sup>a</sup>, Pierre Cahen <sup>a</sup>, Eric Farfour <sup>a</sup>, Philippe Lesprit <sup>c</sup>, Marc Vasse <sup>a</sup>

**n RAST**  
61  
Unicéntrico (France)  
Episodios bacteriemia por gramnegativos (*E. coli*, *K. pneumoniae*, *P. aeruginosa*)  
Comparación grupo RAST con grupo control (antibiograma directo según SFM 16h incubación)  
Evaluación actitud terapéutica y evolución pacientes

**n Control**  
49

**Terapia efectiva el día de hemocultivo positivo:**  
100% grupo RAST vs 88% grupo control ( $p=0,007$ )  
No diferencia en mortalidad ni estancia hospitalaria

	4 h %	6 h %	8 h %
No Readable zones	7,4	2,3	0,5
CA	99,3	99,6	99,6
ME	0,3	0	0
VME	0,2	0	0
ATU	9,4	5,6	4,4



**ANNEXE 4**  
Antibiogramme direct par dilution à partir de flacons d'hémocultures positives.

Dilution	BGN	Staphylocoques	Streptocoques
Dilution	1/50 <sup>e</sup>	1/50 <sup>e</sup>	1/5 <sup>e</sup>
Equivalent en gouttes*	15 gouttes / 9 mL NaCl 0,9 %	15 gouttes / 9 mL NaCl 0,9 %	15 gouttes / 1 mL NaCl 0,9 %



EUROPEAN COMMITTEE  
ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

## Combinaciones antibiótico-microorganismo sin puntos de corte para disco difusión

Antibiotic	Group of bacteria
<b>Fosfomycin</b>	<i>Enterobacteriales</i> except <i>E. coli</i> , <i>Staphylococcus</i> spp.
<b>Ciprofloxacin</b>	<i>Salmonella</i> spp.
<b>Colistin</b>	All Gram-negative bacilli
<b>Tigecycline</b>	<i>Enterobacteriales</i> except <i>E. coli</i>
<b>Beta-lactams</b>	Penicillin non-susceptible <i>Streptococcus pneumoniae</i>
<b>Glycopeptides</b>	<i>Staphylococcus</i> spp.
<b>Daptomycin</b>	All Gram-positive
<b>Lipoglycopeptides</b>	All Gram-positive
<b>All antibiotics</b>	Some anaerobes, <i>Neisseria</i> spp., <i>Helicobacter pylori</i>

# Recomendaciones CoEsAnt paneles disco difusión

Categories	Definitions
A	Antimicrobials that must be routinely studied and reported. They are relevant for both clinical purpose and for the process of interpretive reading of the antibiogram.
B	Antimicrobials that must be routinely studied but selectively reported. They are useful for the process of interpretive reading of the antibiogram and should be selectively reported according to the type of patient, type of infection or the inferred resistance mechanism.
C	Antimicrobials that should be selectively studied and reported according to the type of patient, type of infection or to the inferred resistance mechanism.
D	Antimicrobials that are recommended to be routinely studied and reported in urine isolates.
E	Antimicrobials that should be studied but not reported. They are useful for the detection of antimicrobial resistance mechanisms, application of an expert rule or as surrogate markers of the susceptibility testing result of other antimicrobials.

# Paneles

## *Enterobacteriales*

<i>Enterobacteriales</i>				<i>Enterobacteriales ITU</i>			
AMP	PIT	AMI	GEN	AMP	PIT	AMI	GEN
CXI	CTA	CUR	ERT	FOS	CTA	CUR	ERT
CTZ	AMC	AZT	CIP	CTZ	AMC	AZT	CIP
CTV	CEP	MER	TRS	NIT	CEP	MER	TRS

Categoría	Antibiótico
A	Ampicilina Amoxicilina-ác clavulánico Piperacilina-tazobactam Cefuroxima Ceftazidima Cefotaxima Cefepime Ertapenem Ciprofloxacino Amikacina Gentamicina Cotrimoxazol
B	Aztreonam Meropenem Ceftazidima-avibactam
D	Fosfomicina Nitrofurantoina
E	Cefoxitina

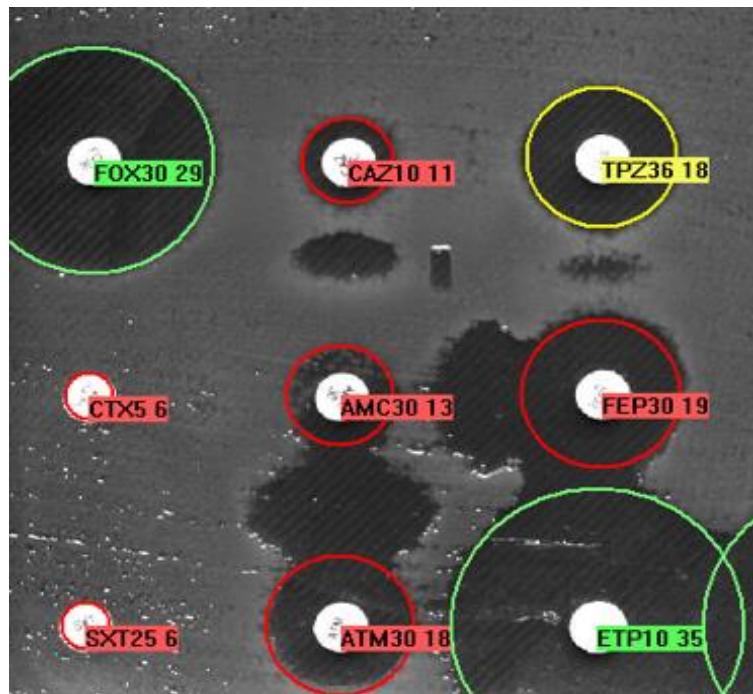
AMC Amoxicillin-clavulanate, AMI Amikacin, AMP Ampicillin, AZT Aztreonam, CEP Cefepime, CIP Ciprofloxacin, CTA Cefotaxime, CTV Ceftazidime- avibactam, CTZ Ceftazidime, CUR Cefuroxime, CXI Cefoxitin, ERT Ertapenem, FOS Fosfomycin, GEN Gentamicin, MER Meropenem, NIT Nitrofurantoin, PIT Piperacillín-tazobactam, TRS Trimethoprim-sulfamethoxazole.

# Enterobacterales

## Detección de mecanismos de resistencia

- **BLEE: sinergia doble disco**

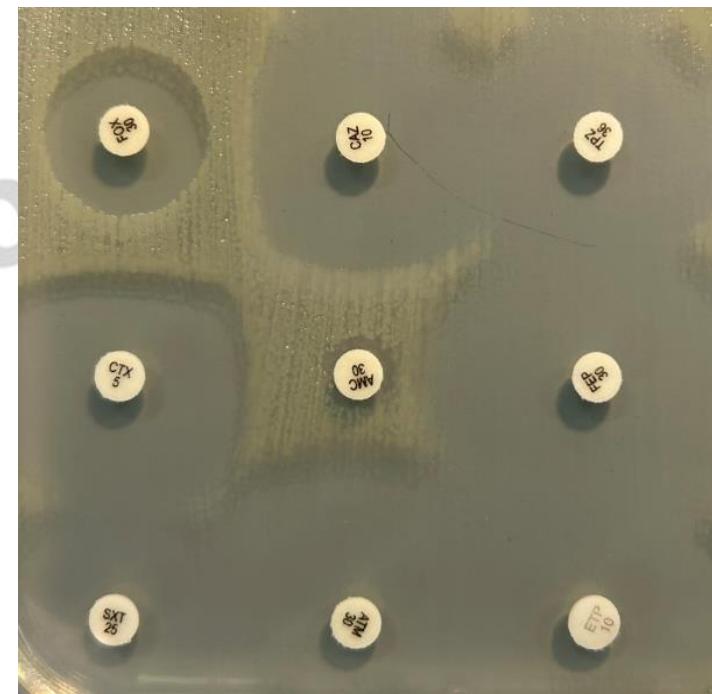
Colocación amoxicilina clavulánico cercano a C3G/C4G



- **AmpC inducible**

Achatamiento halo C3G , aztreonam

Colocación inductores débiles C3G, aztreonam cercanos a inductores fuertes AMC, FOX, MER



- **Resistencia a fluoroquinolonas en *Salmonella enterica*, *Vibrio* spp.**

Disco pefloxacino 5 µg permite excluir resistencia a fluoroquinolonas

# Paneles

## Bacilos gramnegativos no fermentadores

### BGNNF

PIP	CTZ	AMS	MER
CEP	PIT	AZT	IMI
GEN	TOB	AMI	CIP
CTT	MIN	FOS	TRS

### *Pseudomonas spp.*

Categoría	Antibiótico
A	Piperacilina-tazobactam Ceftazidima Cefepime Imipenem Meropenem Aztreonam Ciprofloxacino Amikacina Tobramicina
B	Ceftolozano-tazobactam
C,D	<b>Fosfomicina</b>
E	Piperacilina

### *Acinetobacter spp.*

Antibiótico
<b>Ampicilina-sulbactam</b>
<b>Piperacilina-tazobactam</b>
<b>Ceftazidima</b>
Imipenem
Meropenem
Ciprofloxacino
Amikacina
Gentamicina
Tobramicina

AMI Amikacin, AMS Ampicillin- sulbactam, AZT Aztreonam, CEP Cefepime, CIP Ciprofloxacin, CTT Ceftolozane-tazobactam, CTZ Ceftazidime, FOS Fosfomycin, GEN Gentamicin, IMI Imipenem, MER Meropenem, MIN Minocycline, PIP Piperacillin, PIT Piperacillin-tazobactam, TOB Tobramycin, TRS Trimethoprim-sulfamethoxazole.

# Paneles

## *Sataphylococcus spp.*

<i>Staphylococcus spp.</i>			
BEN	OXA	VAN	TEI
ERY	CLI	LIN	TET
RIF	GEN	LEV	TRS
FUS	MUP	TOB	CXI

Categoría	Antibiótico
A	Penicilina Oxacilina Eritromicina Clindamicina Levofloxacino Gentamicina Tobramicina <b>Vancomicina</b> <b>Teicoplanina</b> Cotrimoxazol
B	Tetraciclina Linezolid Ácido fusídico Mupirocina Rifampicina
E	Cefoxitina

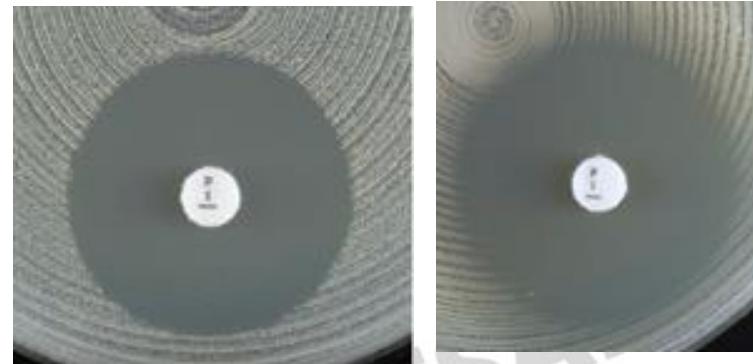
BEN Benzylpenicillin, CLI Clindamycin, CXI Cefoxitin, ERY Erythromycin, FUS Fusidic acid, GEN Gentamicin, LEV Levofloxacin, LIN Linezolid, MUP Mupirocin, OXA Oxacillin, RIF Rifampicin, TEI Teicoplanin, TET Tetracycline, TOB Tobramycin, TRS Trimethoprim-sulfamethoxazole, VAN Vancomycin.

# *Staphylococcus spp.*

## Detección de mecanismos de resistencia

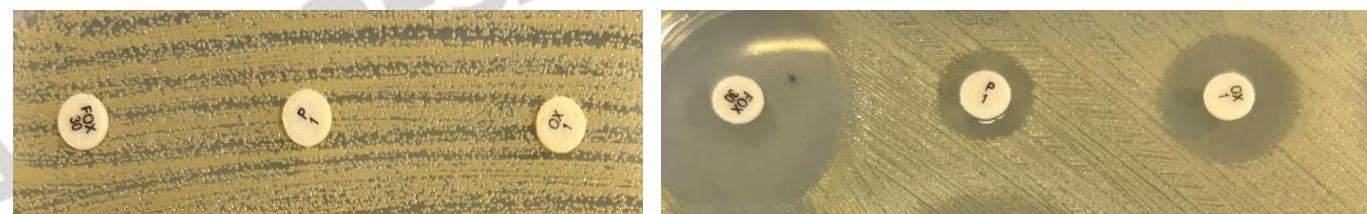
- **Producción de betalactamasa**

Si diámetro de **pencilina**  $\geq 26$  mm y borde de halo cortante interpretar resistente, si borde en bisel interpretar sensible



- **Resistencia oxacilina**

Disco **cefoxitina 30 µg** predice resistencia a meticilina en estafilococos excepto *Staphylococcus pseudintermedius*, *Staphylococcus schleiferi* y *Staphylococcus coagulans*



- **Resistencia inducible a clindamicina**

Achatamiento halo de inhibición de **clindamicina** en zona cercana a **eritromicina** (zona efecto-D)



- **Resistencia a fluoroquinolonas**

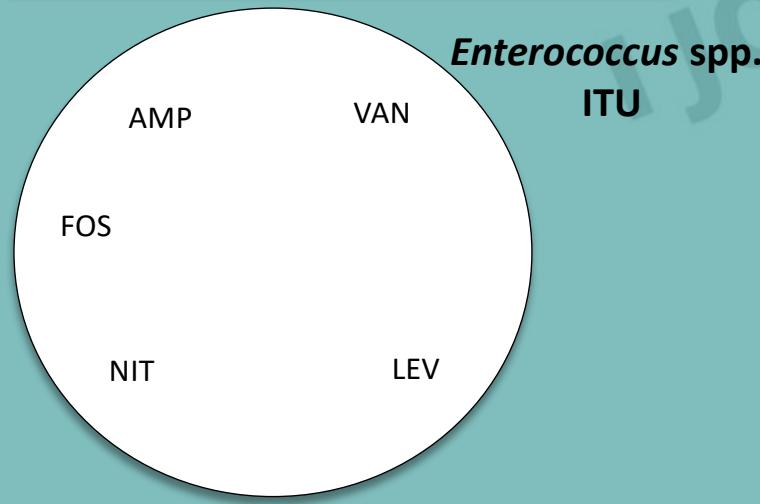
Disco **norfloxacino 10 µg** permite excluir resistencia a fluoroquinolonas

# Paneles

## *Streptococcus spp. / Enterococcus spp.*

### *Streptococcus spp./Enterococcus spp.*

BEN	AMP	OXA	LIN
ERY	CLI	TET	TEI
VAN	CTA	STR	GEN
LEV	TRS	RIF	



Estreptococos beta-hemolíticos		Estreptococos grupo viridans	<i>S. pneumoniae</i>	<i>Enterococcus spp.</i>
Categoría	Antibiótico	Antibiótico	Antibiótico	Antibiótico
A	Penicilina <b>Cefotaxima</b> Eritromicina Clindamicina Levofloxacino	Penicilina <b>Cefotaxima</b> Eritromicina Clindamicina <b>Levofloxacino</b>	Penicilina Ampicilina <b>Cefotaxima</b> Eritromicina Clindamicina Levofloxacino	Ampicilina Vancomicina Teicoplanina Levofloxacino
B	Rifampicina Cotrimoxazol	<b>Tetraciclina</b>	Tetraciclina Cotrimoxazol	Linezolid
C	Vancomicina Teicoplanina Tetraciclina Linezolid	Vancomicina Teicoplanina <b>Linezolid</b> <b>Rifampicina</b> Cotrimoxazol	Vancomicina Teicoplanina Linezolid Rifampicina	Gentamicina HL Estreptomicina HL <b>Rifampicina</b>
D				Levofloxacino <b>Fosfomicina</b> Nitrofurantoina
E			Oxacilina	<b>Eritromicina</b> <b>Tetraciclina</b> Cotrimoxazol

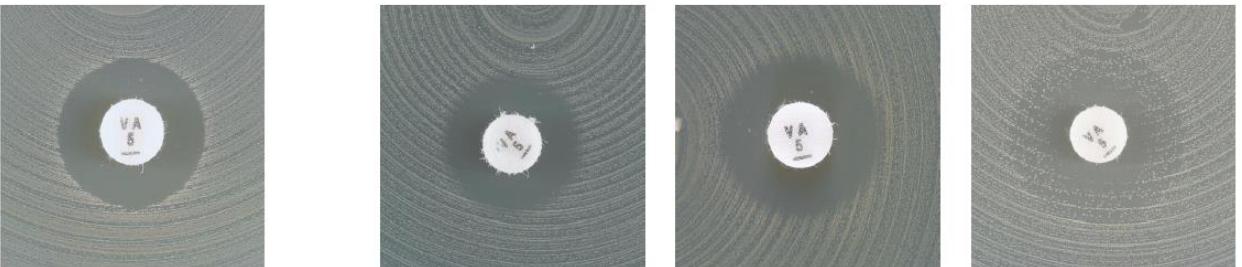
AMP Ampicillin, BEN Benzylpenicillin, CLI Clindamycin, CTA Cefotaxime, ERY Erythromycin, FOS Fosfomycin, GEN Gentamicin, LEV Levofloxacin, LIN Linezolid, NIT Nitrofurantoin, OXA Oxacillin, RIF Rifampicin, STR Streptomycin, TEI Teicoplanin, TET Tetracycline, TRS Trimethoprim-sulfamethoxazole, VAN Vancomycin.

# *Streptococcus spp./ Enterococcus spp.*

## Detección de mecanismos de resistencia

- **Resistencia a glucopéptidos en *Enterococcus* spp.**

Borde del halo de vancomicina en bisel o colonias en la zona de inhibición



non-VRE

VRE

- **Resistencia a betalactámicos en *S. pneumoniae***

Disco **oxacilina 1 $\mu$ g** permite excluir resistencia a betalactámicos



- **Resistencia a betalactámicos en estreptococos grupo viridans**

Disco **penicilina 1U** permite excluir resistencia a betalactámicos

- **Resistencia inducible a clindamicina**

Achatamiento halo de inhibición de **clindamicina** en zona cercana a **eritromicina** (zona efecto-D)

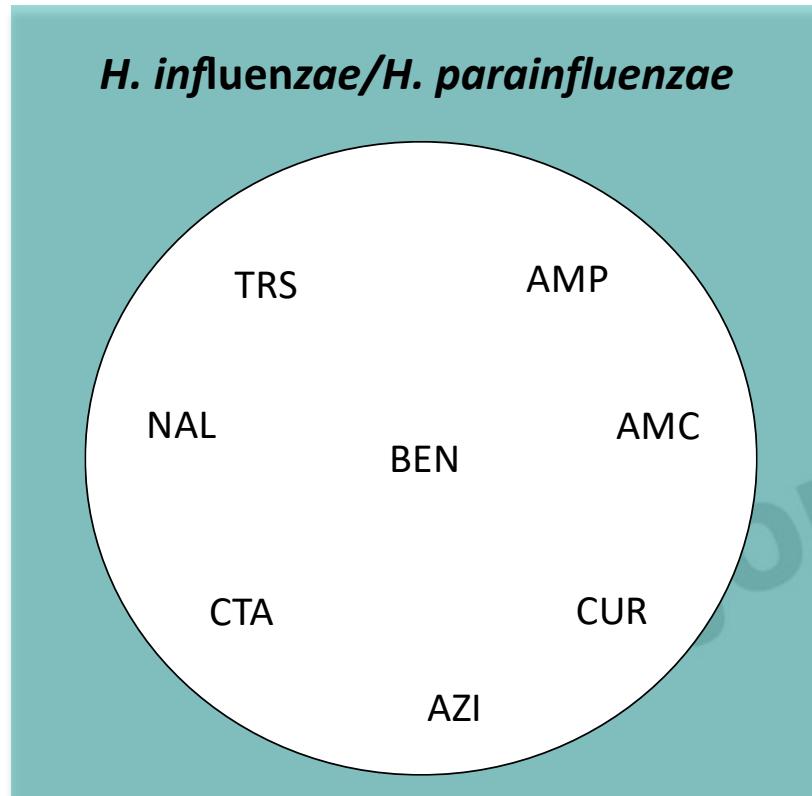


- **Resistencia a fluoroquinolonas**

Disco **norfloxacino 10  $\mu$ g** permite excluir resistencia a fluoroquinolonas

# Paneles

## *Haemophilus influenzae / Haemophilus parainfluenzae*



Categoría	Antibiótico
A	Ampicilina Amoxicilina-clavulánico Cefuroxima Cefotaxima Ciprofloxacino/Levofloxacino <b>Azitromicina</b>
B	Cotrimoxazol
E	Penicilina Ácido nalidíxico

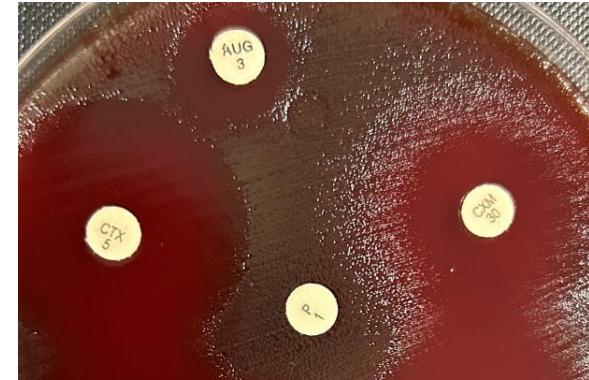
AMC Amoxicillin-clavulanate, AMP Ampicillin, AZI Azithromycin, BEN Benzylpenicillin, CTA Cefotaxime, CUR Cefuroxime, NAL Nalidixic acid, TRS Trimethoprim-sulfamethoxazole.

# *H. influenzae / H. parainfluenzae*

## Detección de mecanismos de resistencia

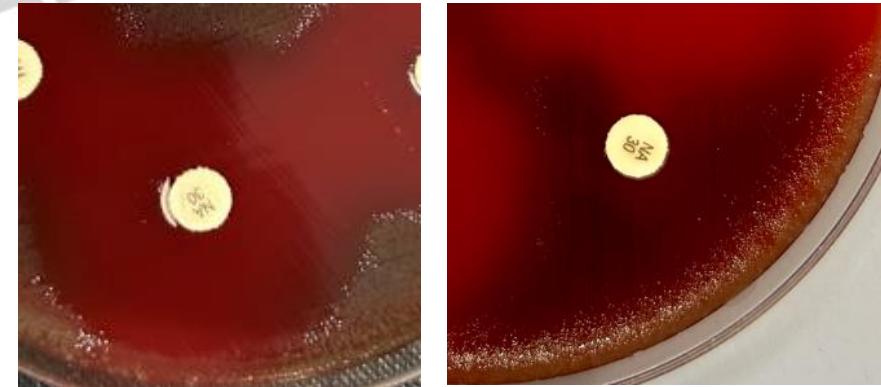
- **Resistencia a betalactámicos en *H. influenzae***

Disco penicilina 1U permite excluir resistencia a betalactámicos



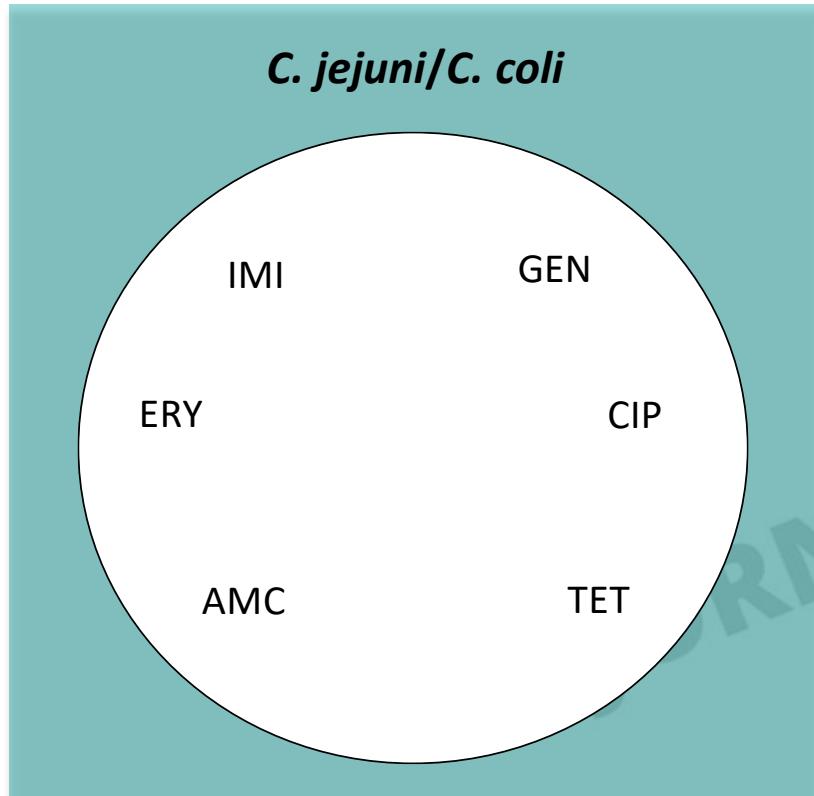
- **Resistencia a fluoroquinolonas en *H. influenzae***

Disco ácido nalidíxico 30 µg permite excluir resistencia a quinolonas



# Paneles

## *Campylobacter jejuni / Campylobacter coli*



Categoría	Antibiótico
A	<b>Amoxicilina-ác clavulánico</b> Eritromicina Ciprofloxacino
B	Imipenem <b>Gentamicina</b> Tetraciclina

AMC Amoxicillin-clavulanate, CIP Ciprofloxacin, ERY Erythromycin, GEN Gentamicin, IMI Imipenem, TET Tetracycline.

## Conclusiones

- La técnica de disco difusión constituye un método estandarizado con puntos de corte calibrados que combina flexibilidad en la elección de antimicrobianos y bajo coste.
- El método rápido validado por EUCAST para el estudio de sensibilidad por disco difusión a partir de hemocultivos positivos se presenta como una herramienta de fácil implementación para mejorar el tiempo de respuesta.
- Las recomendaciones sobre los antimicrobianos a incluir en los paneles se basan en las indicaciones y la utilidad para detección de mecanismos de resistencia y deben adaptarse las necesidades de cada centro.