analyse

Was gibt es Neues in der Austestung?

Nationales Referenzzentrum für nosokomiale Infektionen und AMR am Krankenhaus der Elisabethinen Linz

Petra Apfalter

ESCMID
COLLABORATIVE CENTRE

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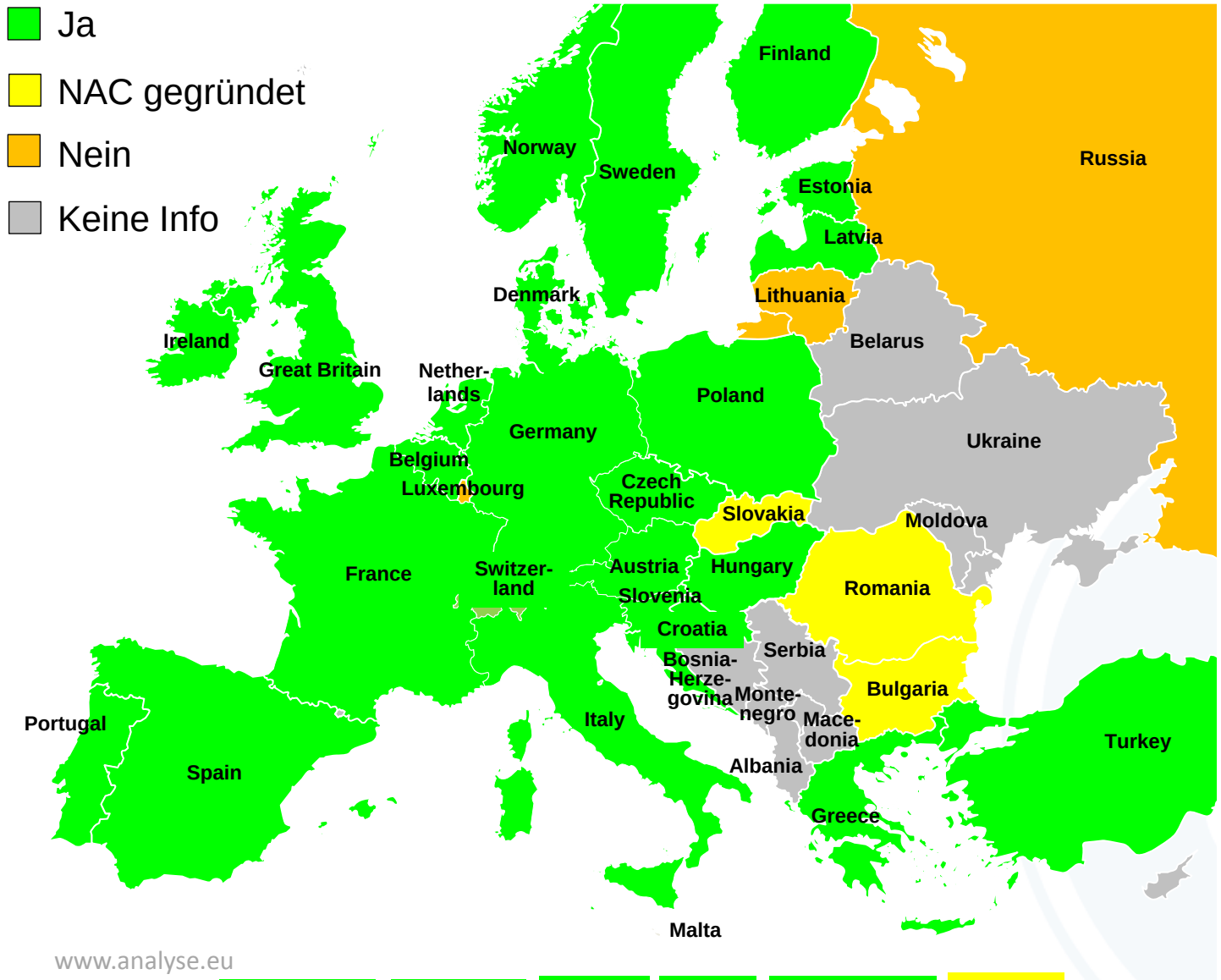
EUCAST 2014



www.analyse.eu

Nicht im Bild: Australia | Iceland | Israel | USA | Südafrika | China

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Wohin die Reise geht

- **EUCAST**
  - EARS-Net
  - AURES
  - EuSCAPE - europ. Surveillance von Carbapenemaseproduzierende Enterobakterien
    - **Geplant: Österreichweite Ausdehnung über EuSCAPE-AT Net**
    - **Nutzen**
      - **Labors: unentgeltliche NRZ Abklärung bei V. auf CPE**
      - **NRZ:** Aufbereitung von CPE-Daten als Basis für gesundheitspolitische Entscheidungen
      - **BMG:** Basis für Meldepflicht, „Agieren statt Reagieren“ in einer globalen Welt (Resistenzen kennen keine Grenzen)

Worum es geht

- **EUCAST 2014**
  - Methodik
  - Topische Antibiotika
  - Resistenzmechanismen

- **EARS-Net QC feed-back**

- **2015: Fokus auf Qualitätskontrolle**
  - EUCAST ist mehr als die Anwendung von breakpoints

Neues Make-up

European Committee on Antimicrobial Susceptibility Testing

Breakpoint tables for interpretation of MICs and zone diameters

Version 4.0, valid from 2014-01-01

This document should be cited as

"The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 4.0, 2014. http://www.eucast.org."

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Neues Make-up

Guidance on reading EUCAST Breakpoint Tables

EUCAST Clinical Breakpoint Table v. 4.0, valid from 2014-01-01

The intermediate category is not listed but is inferred as the values between the S and the R breakpoints. If the S and R breakpoints are the same value there is no intermediate category.

Agent A: No intemediate category
Agent B: Intermediate category: 4 mg/L, 23-25 mm
Agent G: Intermediate category: 1-2 mg/L, 24-29 mm

Disk diffusion (EUCAST standardised disk diffusion method)
Medium:
Inoculum:
Incubation:
Reading:
Quality control:

EUCAST method for antimicrobial susceptibility testing by disk diffusion and recommendations for quality control

Breakpoints with a species name apply only to that particular species (in this example *S. aureus*)

Antimicrobial agent	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Antimicrobial agent A	1[1]	1[1]	X	20[A]	20[A]	1. Comment on MIC breakpoints A. Comment on disk diffusion
Antimicrobial agent B, *S. aureus*	2	4	Y	26	23	
Antimicrobial agent C	IE	IE		IE	IE	
Antimicrobial agent D	-	-		-	-	
Antimicrobial agent E	IP	IP		IP	IP	
Antimicrobial agent F (screen)	NA	NA		25	25	
Antimicrobial agent G	0.5	2	Z	30	24	

Screening breakpoint to differentiate between isolates without and with resistance mechanisms

Not Applicable

Changes from previous version highlighted in yellow

In Preparation

No breakpoints. Susceptibility testing is not recommended

Link to MIC distribution if highlighted in blue

Link to zone diameter distribution if highlighted in blue

Link to rationale document if highlighted in blue

Insufficient evidence that the species in question are a good target for therapy with the drug

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Enterobakterien

- **Amoxicillin/Clavulansäure**
  - **Neuer BP für unkomplizierte HWI (S ≥ 16 statt 19mm)**
  - Achtung: BP gilt bei fixierter c von Clavulansäure (2mg/L; kein Gradient) … (derzeit) nicht möglich mit automatisierten Systemen

- **Doripenem neue BP**

- **Pefloxacin Screen-Blättchen (5µg; S ≥ 24) zum Nachweis einer Ciprofloxacinresistenz bei *Salmonella* spp.**

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Enterobakterien – Amoxi/Clav

Material : Blutkultur

ERGEBNIS

Kultur
Aerobe Kultur : **Wachstum**
Anaerobe Kultur : **kein Wachstum**

Kultur :
nachgewiesene Keime :
1. **Escherichia coli**

Antibiogramm	1
Ampicillin	-
Amoxicillin + Clavulansäure	-
Cefuroxim	+
Cefotaxim	+
Cefepim	+
Meropenem	+
Gentamicin	+
Ciprofloxacin	+
Trimethoprim + Sulfonamid	+

+ = sensibel, +/- = intermediär, - = resistent
[] minimale Hemmkonzentration in mg/L

2 Breakpoints!

unkomplizierter HWI: S ≥ 16mm
andere Infektionen: S ≥ 19mm

Material : Harn

ERGEBNIS

Keimzahl : **10 hoch 7 / ml**

Kultur :
nachgewiesene Keime :
1. **Escherichia coli** (10^7/ml)

Aminopenicillin/Betalaktamaseinhibitor-Kombinationen sind für unkomplizierte Harnwegsinfektionen als empfindlich, bei allen anderen Infektionen als resistent zu werten.

Antibiogramm	1
Ampicillin	-
Amoxicillin + Clavulansäure	+
Cefalexin	+
Cefuroxim	+
Cefotaxim	+
Cefepim	+
Meropenem	+
Gentamicin	+
Ciprofloxacin	+
Nitrofurantoin	+
Trimethoprim + Sulfonamid	+
Mecillinam	+

∅ 16, 17, oder 18mm

+ = sensibel, +/- = intermediär, - = resistent
[] minimale Hemmkonzentration in mg/L
Bei Fällen ohne Interpretation sind derzeit keine Grenzwerte festgelegt. Eine sichere Beurteilung des Therapieerfolges ist daher nach heutigem Wissensstand nicht möglich.

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Pseudomonas

- Piperacillin /Tazobactam

Piperacillin[1]	16	16	30	18	18
Piperacillin-tazobactam[1]	16[2]	16[2]	30-6	18	18
Ticarcillin[3]	16	16	75	18	18
Ticarcillin-clavulanate[3]	16[4]	16[4]	75-10	18	18

1. Breakpoints are based on high dose therapy (with or without tazobactam, 4 g x 4).
2. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
3. Breakpoints are based on high dose therapy (with or without clavulanate, 3 g x 4).
4. For susceptibility testing purposes, the concentration of clavulanate is fixed at 2 mg/L.

- Cefepim
- Doripenem

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Stenotrophomonas

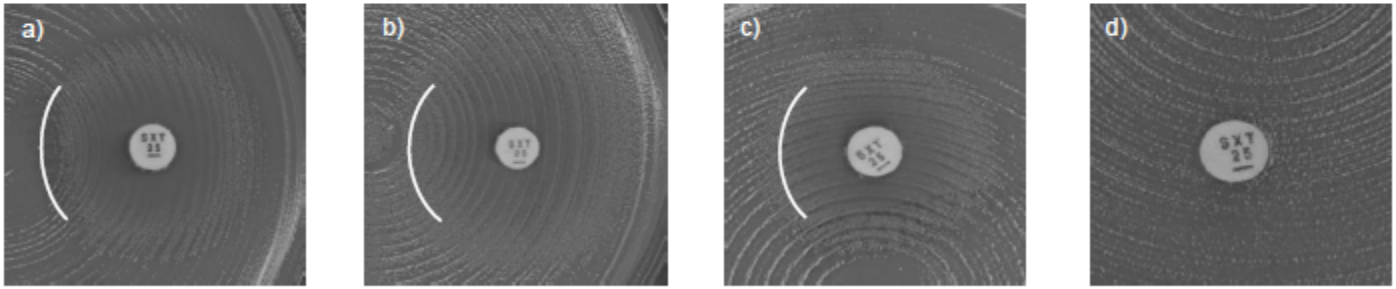
Stenotrophomonas maltophilia

EUCAST Clinical Breakpoint Table v. 4.0, valid from 2014-01-01

Trimethoprim-sulfamethoxazole is the only agent for which EUCAST breakpoints are currently available. For further information, see guidance document on www.eucast.org.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1°C, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.
Quality control: *Escherichia coli* ATCC 25922

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Trimethoprim-sulfamethoxazole[1]	4	4	1.25-23.75	16[A]	16[A]	1. Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration. A. Ignore haze or fine growth within the inhibition zone (see pictures below).



Examples of inhibition zones for *Stenotrophomonas maltophilia* with trimethoprim-sulfamethoxazole.
a-c) An outer zone can be seen. Report susceptible if the zone diameter ≥ 16 mm.
d) Growth up to the disk **and** no sign of inhibition zone. Report resistant.

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Induzierbare Clindamycin Resistenz

2. Inducible clindamycin resistance can be detected by antagonism of clindamycin activity by a macrolide agent. If not detected, then report as susceptible. If detected, then report as resistant and consider adding this comment to the report: "Clindamycin may still be used for short-term therapy of less serious skin and soft tissue infections as full resistance is unlikely to develop during such therapy".
B. Place the erythromycin and clindamycin disks 12-20 mm apart (edge to edge) and look for antagonism (the D phenomenon).

- **Staphylokokken**
- **Streptokokken Serogruppe A, B, C und G**
- **Pneumokokken**
- **Viridans Streptokokken**

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Pneumokokken

Screening for beta-lactam resistance in *S. pneumoniae*

Supplementary table

Oxacillin 1 µg disk Zone diameter	Antimicrobial agent	Further testing and/or interpretation
≥ 20 mm	All beta-lactam agents for which clinical breakpoints are listed (including those with "Note")	Report susceptible irrespective of clinical indication, except for cefaclor, which if reported, should be reported as intermediate.
< 20 mm*	Benzylpenicillin (meningitis) and phenoxymethylpenicillin (all indications)	Report resistant.
	Benzylpenicillin (for infections other than meningitis)	Determine the MIC and interpret according to the clinical breakpoints.
	Ampicillin, amoxicillin and piperacillin (without and with beta-lactamase inhibitor), cefepime, cefotaxime, ceftaroline and ceftriaxone	**Oxacillin zone diameter ≥ 8 mm:** Report susceptible. In meningitis confirm by determining the MIC for the agent considered for clinical use.
		Oxacillin zone diameter < 8 mm: Determine the MIC of the beta-lactam agent intended for clinical use but for ampicillin, amoxicillin and piperacillin (without and with beta-lactamase inhibitor) infer susceptibility from the MIC of ampicillin.
	Other beta-lactam agents	Determine the MIC of the agent considered for clinical use and interpret according to the clinical breakpoints.

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H. influenzae

Screening for beta-lactam resistance in *H. influenzae*

Supplementary table

Benzylpenicillin 1 unit disk Zone diameter	Beta-lactamase	Further testing and/or interpretation
≥ 12 mm	Do not test	Report susceptible to all beta-lactam agents for which clinical breakpoints are listed (including those with "Note"), and cefuroxime oral, which if reported, should be reported intermediate.
< 12 mm	Beta-lactamase negative	A resistance mechanism other than beta-lactamase production is present. As the effect on individual beta-lactam agents differs, test susceptibility to the beta-lactam agent intended for clinical use.
	Beta-lactamase positive	For ampicillin, amoxicillin and piperacillin, report resistant.
		For other beta-lactam agents, test susceptibility to the beta-lactam agent intended for clinical use as another resistance mechanism cannot be excluded by the screen test.

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Enterokokken

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Ciprofloxacin (uncomplicated UTI only)	4	4	5	IP[A]	IP[A]
Levofloxacin (uncomplicated UTI only)	4	4	5	IP[A]	IP[A]
Moxifloxacin	-	-		-	-
Nalidixic acid (screen)	NA	NA		NA	NA
Norfloxacin (screen)	NA	NA	10	12[B]	12[B]
Ofloxacin	-	-		-	-

Notes
Numbers for comments on MIC breakpoints
Letters for comments on disk diffusion

A. The norfloxacin disk diffusion test can be used to screen for fluoroquinolone resistance. See Note B.

B.Susceptibility of ciprofloxacin and levofloxacin can be inferred from the norfloxacin susceptibility.

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Corynebakterien

***Corynebacterium* spp.**
except *Corynebacterium diphtheriae*

EUCAST Clinical Breakpoint Table v. 4.0, valid from 2014-01-01

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
Incubation: 5% CO_2, 35±1°C, 18±2h. Isolates with insufficient growth after 16-20 h incubation are reincubated immediately and inhibition zones read after a total of 40-48 h incubation.
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Streptococcus pneumoniae* ATCC 49619

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Benzylpenicillin	0.12	0.12	1 unit	29	29	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Ciprofloxacin	1	1	5	25	25	
Moxifloxacin	0.5	0.5	5	25	25	

Aminoglycosides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Gentamicin	1	1	10	23	23	

Glycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Vancomycin	2	2	5	17	17	

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Topische Antibiotika

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

Breakpoints for topical use of antimicrobial agents
EUCAST Guidance Document

- **Schwierig da keine vernünftigen PK/PD Daten, keine outcome Daten, Mischungen …**
- **Vorschlag: PK/PD breakpoints verwenden**

In the absence of clinical data on outcome related to MIC of infecting organisms EUCAST does not find it possible to reach a consensus that resolves the conflicting opinions on these two alternative proposals:

1. Use ECOFFs for all agents when used topically.
2. Use clinical breakpoints when available and ECOFFs when there are no clinical breakpoints.

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Topische Antibiotika / Auge, Ohr, Haut*

ECOFFs and systemic clinical breakpoints for antimicrobial agents that are used topically

Organisms	ECOFF (mg/L) Systemic clinical breakpoint (S≤/R>, mg/L)	Gentamicin[1]	Ciprofloxacin[1]	Levofloxacin[1]	Ofloxacin[1]	Chloramphenicol[1]	Colistin[1] (for Polymyxin B)	Fusidic acid[1]	Neomycin (framycetin)	Bacitracin	Mupirocin	Retapamulin
Enterobacteriaceae	ECOFF	2	0.12	0.25	0.5	16	2	-	8	-	-	-
	Systemic clinical breakpoint	2/4	0.5/1	1/2	0.5/1	8/8	2/2	-	-	-	-	-
Acinetobacter spp.	ECOFF	4	1	0.5	1	-	2	-	ND	-	-	-
	Systemic clinical breakpoint	4/4	1/1	1/2	-	-	2/2	-	-	-	-	-
P. aeruginosa	ECOFF	8	0.5	2	2	-	4	-	ND	-	-	-
	Systemic clinical breakpoint	4/4	0.5/1	1/2	-	-	4/4	-	-	-	-	-
S. aureus	ECOFF	2	1	1	1	16	-	0.5	1	ND	1/1[2]	0.5
	Systemic clinical breakpoint	1/1	1/1	1/2	1/1	8/8	-	1/1	-	-	-	-
S. pneumoniae	ECOFF	-	2	2	4	8	-	32	ND	ND	-	-
	Systemic clinical breakpoint	-	0.12/2	2/2	0.12/4	8/8	-	-	-	-	-	-
β-haemolytic streptococci	ECOFF	-	2	2	4	8	-	32	ND	ND	0.5	0.12
	Systemic clinical breakpoint	-	-	1/2	-	8/8	-	IE	-	-	-	-
H. influenzae	ECOFF	4	0.06	0.06	0.12	1	-	ND	ND	-	-	-
	Systemic clinical breakpoint	IE	0.5/0.5	1/1	0.5/0.5	2/2	-	-	-	-	-	-
Moraxella spp.	ECOFF	0.25	0.12	0.12	0.25	2	-	ND	ND	-	-	-
	Systemic clinical breakpoint	IE	0.5/0.5	1/1	0.5/0.5	2/2	-	-	-	-	-	-

*nicht: Darm, Atemwege (inhalativ)

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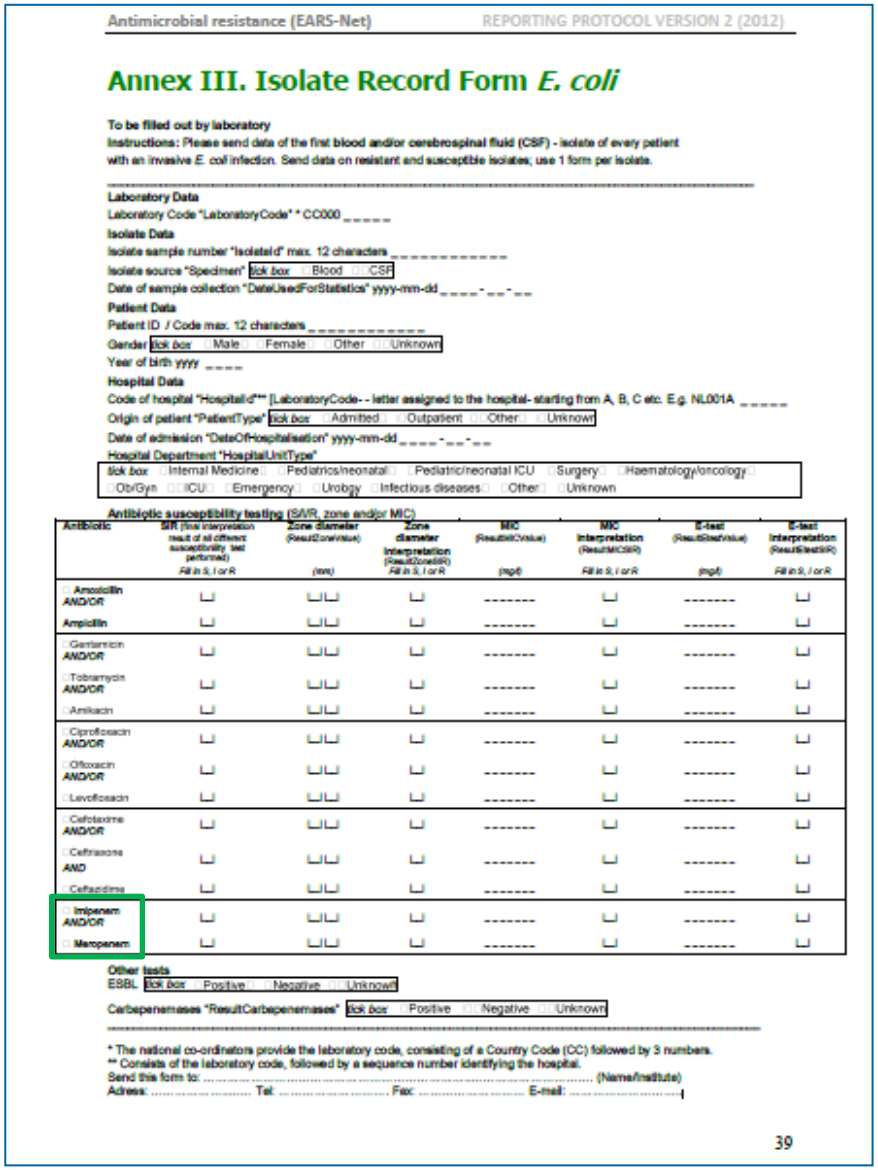
EARS-Net reporting form



Antimicrobial resistance (EARS-Net) REPORTING PROTOCOL VERSION 2 (2012)

Annex III. Isolate Record Form *E. coli*

To be filled out by laboratory
Instructions: Please send data of the first blood and/or cerebrospinal fluid (CSF) - isolate of every patient with an invasive *E. coli* infection. Send data on resistant and susceptible isolates; use 1 form per isolate.

Laboratory Data
Laboratory Code "LaboratoryCode" * CC000 _ _ _ _ _
Isolate Data
Isolate sample number "IsolateId" max. 12 characters _ _ _ _ _ _ _ _ _ _ _ _
Isolate source "Specimen" tick box ☐ Blood ☐ CSF
Date of sample collection "DateUsedForStatistics" yyyy-mm-dd _ _ _ _ - _ _ - _ _
Patient Data
Patient ID / Code max. 12 characters _ _ _ _ _ _ _ _ _ _ _ _
Gender tick box ☐ Male ☐ Female ☐ Other ☐ Unknown
Year of birth yyyy _ _ _ _
Hospital Data
Code of hospital "HospitalId"** [LaboratoryCode- - letter assigned to the hospital- starting from A, B, C etc. E.g. NL001A _ _ _ _ _
Origin of patient "PatientType" tick box ☐ Admitted ☐ Outpatient ☐ Other ☐ Unknown
Date of admission "DateOfHospitalisation" yyyy-mm-dd _ _ _ _ - _ _ - _ _
Hospital Department "HospitalUnitType"
tick box ☐ Internal Medicine ☐ Pediatrics/neonatal ☐ Pediatric/neonatal ICU ☐ Surgery ☐ Haematology/oncology ☐ Ob/Gyn ☐ ICU ☐ Emergency ☐ Urology ☐ Infectious diseases ☐ Other ☐ Unknown

Antibiotic susceptibility testing (S/I/R, zone and/or MIC)

Antibiotic	SIR (first interpretation result of all different susceptibility test performed) Fill in S, I or R	Zone diameter (ResultZoneValue) (mm)	Zone diameter Interpretation (ResultZoneSIR) Fill in S, I or R	MIC (ResultMICValue) (mg/l)	MIC Interpretation (ResultMICSIR) Fill in S, I or R	E-test (ResultEtestValue) (mg/l)	E-test Interpretation (ResultEtestSIR) Fill in S, I or R
☐ Amoxicillin AND/OR	☐	☐☐	☐	-------	☐	-------	☐
Ampicillin	☐	☐☐	☐	-------	☐	-------	☐
☐ Gentamicin AND/OR	☐	☐☐	☐	-------	☐	-------	☐
☐ Tobramycin AND/OR	☐	☐☐	☐	-------	☐	-------	☐
☐ Amikacin	☐	☐☐	☐	-------	☐	-------	☐
☐ Ciprofloxacin AND/OR	☐	☐☐	☐	-------	☐	-------	☐
☐ Ofloxacin AND/OR	☐	☐☐	☐	-------	☐	-------	☐
☐ Levofloxacin	☐	☐☐	☐	-------	☐	-------	☐
☐ Cefotaxime AND/OR	☐	☐☐	☐	-------	☐	-------	☐
☐ Ceftriaxone AND	☐	☐☐	☐	-------	☐	-------	☐
☐ Ceftazidime	☐	☐☐	☐	-------	☐	-------	☐
☐ Imipenem AND/OR	☐	☐☐	☐	-------	☐	-------	☐
☐ Meropenem	☐	☐☐	☐	-------	☐	-------	☐

Other tests
ESBL tick box ☐ Positive ☐ Negative ☐ Unknown
Carbepenemases "ResultCarbepenemases" tick box ☐ Positive ☐ Negative ☐ Unknown

* The national co-ordinators provide the laboratory code, consisting of a Country Code (CC) followed by 3 numbers.
** Consists of the laboratory code, followed by a sequence number identifying the hospital.
Send this form to: ..... (Name/Institute)
Adress: ..... Tel: ..... Fax: ..... E-mail: .....

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- **Letzte EARS-Net QC**
  - A. baumanii √
  - E. coli √
  - **K. pneumoniae (OXA 48)**
  - S. aureus (MRSA) √
  - Pneumokokken (MR) √
  - **P. aeruginosa**

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Fehlerquellen

Medium	Aufbewahrung
	Nicht ordnungsgemäss hergestellt
	Batch to batch Variation oder Herstellerwechsel
	Supplemente (batch to batch variations, c abgelaufen)
	pH
	Agar Schichtdicke/Agar Volumen
	Ablaufdatum
Test Konditionen	“15-15-15”-Regel nicht eingehalten (Suspension binnen 15 min verwendet, disks binnen 15 min gestempelt, Inkubation binnen 15 min)
	Inkubation (Temperatur, Atmosphäre und Zeit)
	Inkorrektes Inokulum
	Ablesekonditionen, Hemmhofränder
Blättchen	inkorrekt (falsches AB oder Beladung)
	Disk potency (incorrect storage, labile agent, expiry date)
	Blättchen nicht bei RT
	Zuviele Blättchen auf Platte
Kontroll Stämme	Falscher QC Stamm
	Mutation
	Kontamination
	Alter der Kultur

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Clin Microbiol Infect 2014; 20:O255-O266

ORIGINAL ARTICLE BACTERIOLOGY

Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories

E. Matuschek[1], D. F. J. Brown[2] and G. Kahlmeter[1]

1) *EUCAST Laboratory for Antimicrobial Susceptibility Testing, Växjö, Sweden and* 2) *EUCAST Scientific Secretary, Peterborough, UK*

Abstract

With the support of ESCMID and European countries, EUCAST has developed a disk diffusion test with zone diameter breakpoints correlated with the EUCAST clinical MIC breakpoints. The development of the EUCAST disk diffusion method and quality control criteria are described, together with guidance on quality control and implementation of the method in clinical microbiology laboratories. The method includes the use of Mueller–Hinton agar without supplements for non-fastidious organisms and with 5% mechanically defibrinated horse blood and 20 mg/L β-NAD for fastidious organisms, a standardized inoculum resulting in confluent growth, an incubation time of 16–20 h, a reading guide on how to read zone diameters on individual species-agent combinations and zone diameter breakpoints calibrated to the EUCAST clinical MIC breakpoints. EUCAST recommendations are described in detail and updated regularly on the EUCAST website (http://www.eucast.org).

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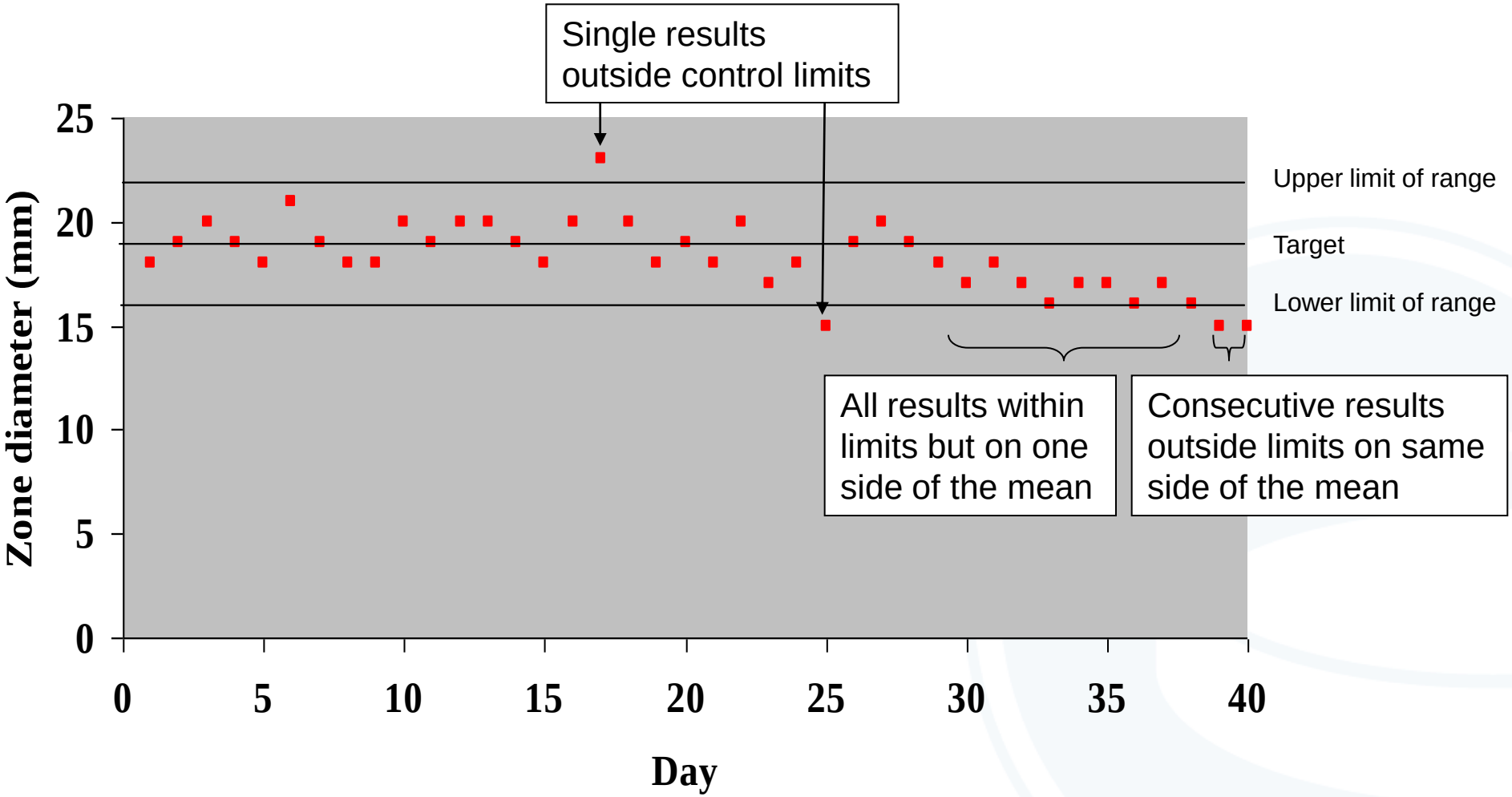
Tägliche Routine-QC



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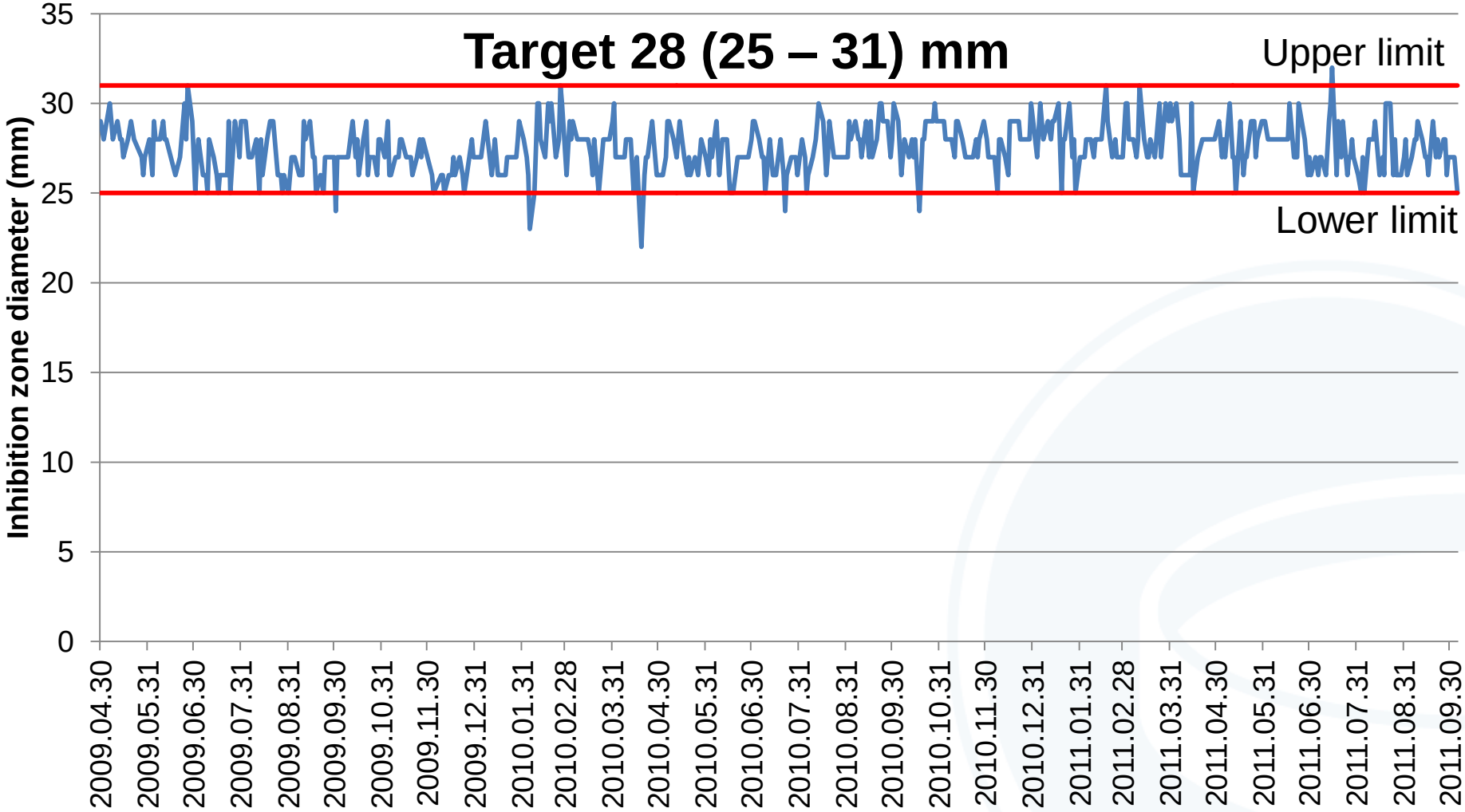
Tägliche Routine-QC



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