

EUCAST in Belgium: a difficult road

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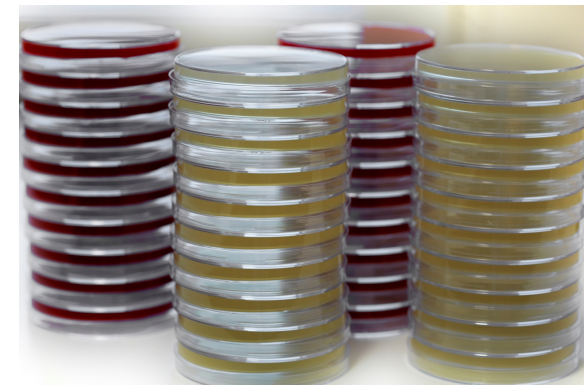
ANTIMICROBIAL SUSCEPTIBILITY METHODS

Methods for antimicrobial susceptibility testing

- **Phenotypic test methods**
 - Based on antimicrobial activity and breakpoints
 - Disk diffusion
 - Manual, Semi-automation (Adagio, SIRscan)
 - MIC Determination
 - Broth, agar, gradient diffusion (Etest, MICE)
 - (Semi-) automated systems
 - Vitek, Phoenix, Microscan, Sensititre
- **Genotypic test methods**
 - Based on the detection of a resistance gene or its product
 - *mecA*, *vanA*, ...
 - PBP2a, β -lactamase detection
- **By deduction**
 - Based on expert rules

Methods for antimicrobial susceptibility testing

- « EUCAST » Disk diffusion testing
 - Susceptibility testing media
 - MH
 - MH-Fastidious
 - MH + 5% horse blood + 20 mg/L β - nicotinamide adenine dinucleotide (NAD)
 - Inoculum
 - 0.5 McFarland std



Methods for antimicrobial susceptibility testing

« EUCAST » Disk diffusion testing

- The 15-15-15 minute rule

- Preparation of plates

- Use the inoculum within **15 minutes** of preparation (and never beyond 60 min)
 - Apply disks within **15 minutes** of inoculating plates
 - Start incubation within **15 minutes** of application of disks

Methods for antimicrobial susceptibility testing

« EUCAST » Disk diffusion testing

■ Incubation

Organism	Incubation conditions
Enterobacteriaceae	35±1 °C in air for 16-20h
<i>Pseudomonas</i> spp.	35±1 °C in air for 16-20h
<i>Stenotrophomonas maltophilia</i>	35±1 °C in air for 16-20h
<i>Acinetobacter</i> spp.	35±1 °C in air for 16-20h
<i>Staphylococcus</i> spp.	35±1 °C in air for 16-20h
<i>Enterococcus</i> spp.	35±1 °C in air for 16-20h
Streptococcus groups A, B, C and G	35±1 °C in air with 4-6% CO ₂ for 16-20h
Viridans group streptococci	35±1 °C in air with 4-6% CO ₂ for 16-20h
<i>Streptococcus pneumoniae</i>	35±1 °C in air with 4-6% CO ₂ for 16-20h
<i>Haemophilus</i> spp.	35±1 °C in air with 4-6% CO ₂ for 16-20h
<i>Moraxella catarrhalis</i>	35±1 °C in air with 4-6% CO ₂ for 16-20h
<i>Listeria monocytogenes</i>	35±1 °C in air with 4-6% CO ₂ for 16-20h
Other fastidious organisms	Pending

Methods for antimicrobial susceptibility testing

- Some practical issues with EUCAST breakpoints implementation
 - Lower ranges of concentrations are needed
 - EUCAST breakpoints often lower than CLSI
 - New testing panels for MIC determination
 - Desirable specification
 - To include drug concentration equal to ECOFFs
 - Allowing detection of wild type organisms
 - A technical change
 - Expression of breakpoints interpretation

	S	R
EUCAST	\leq	$>$
CLSI	\leq	\geq

Practical implementation of the EUCAST breakpoints and methods

VERIFICATION & VALIDATION

Recommandations for laboratories for verification of new test methods for AST

For (semi-) automated systems

- **Cumitech 31A. ASM Press 2009**
 - Verification and validation of procedures in the clinical microbiology laboratory
- **Clinical Microbiology Procedures Handbook, 3rd edition, Garcia. ASM Press 2010**
- **ISO 20776-2, 2007**
- **Guidance for Industry and FDA, 2009**
- **CLSI M50-A, 2008**

Recommandations for laboratories for verification of new test methods for AST

Clinical Microbiology Procedures Handbook, 3rd edition, Garcia. ASM Press 2010

- **Performed by « NAC », not by every lab ! (>Sept.2012)**
 - Lab could rely on these data + minimum implementation
 - > 50 isolates of
 - various species
 - various antimicrobial susceptibility profiles
 - ATCC strains
 - **To assess performance**
 - Precision (reproducibility)
 - Agreement

Recommandations for laboratories for verification of new test methods for AST

Clinical Microbiology Procedures Handbook, 3rd edition, Garcia. ASM Press 2010

For agreement with automated systems

- **Selected « validation » laboratories**
 - KUL & CHU Lg for Vitek
 - Alost & Hasselt for Phoenix
 - 2 others for Microscan
- **Selected strains**
 - **A challenge panel with varied resistance**
 - 50 strains provided by the Belgian NRC
 - GNB, *S.aureus*, Enterococci, GAS, GBS, *S.pneumoniae*
 - **60 clinical isolates**
 - 10 isolates /validation lab

Recommendations for laboratories for verification of new test methods for AST

Clinical Microbiology Procedures Handbook, 3rd edition, Garcia. ASM Press 2010

For agreement with automated systems

- **Use of latest panels available in Be**
- **Acceptation**
 - **< 1.5 % very major error**
 - **<= 3% major error**
 - **Essential agreement > 90%**

Recommandations for laboratories for verification of new test methods for AST

Clinical Microbiology Procedures Handbook, 3rd edition, Garcia. ASM Press 2010

For precision (reproducibility) with automated systems

- **Strains**
 - **Minimum 5 strains**
 - **Variety of species and resistance**
- **Test**
 - **Minimum 5 strains in triplicate for 3 days**
- **Acceptation**
 - **Results for each antimicrobial agent within +/- doubling dilution 95% of time**

DOCUMENTS & MATERIAL

DOCUMENTS & MATERIAL

NAC page from the SBIMC-BVIKM website

- **Link to EUCAST website and all available documents**
 - **Step by step document on implementation of AST with EUCAST breakpoints**
 - **Based on EUCAST documents**
 - **Disk diffusion test procedure and guide for reading and interpretation**
 - **Checklist to facilitate implementation**
 - **Results of the performed verification of the 3 mainly used (semi-)automated systems in Belgium**
- **Distribution of sets of « challenge » strains and instructions**
 - **During a NAC meeting (*end of 2012 or beginning 2013*)**

QUALITY CONTROL

QUALITY CONTROL

- **To monitor test performance**
 - **Use of the recommended routine quality control strains**
 - **EUCAST Quality control tables**
- **To confirm the ability to detect resistance**
 - **QC strains with defined resistance mechanisms may be used**

EUCAST routine quality control strains

Organism	Culture collection numbers	Characteristics
<i>E. coli</i>	ATCC 25922; NCTC 12241; CIP 7624 DSM 1103; CCUG 17620, CECT 434	Susceptible, wild-type
<i>P. aeruginosa</i>	ATCC 27853; NCTC 12903; CIP 76110 DSM 1117; CCUG 17619; CECT 108	Susceptible, wild-type
<i>S. aureus</i>	ATCC 29213; NCTC 12973; CIP 103429 DSM 2569; CCUG 15915; CECT 794	Weak β -lactamase producer
<i>E. faecalis</i>	ATCC 29212; NCTC 12697; CIP 103214 DSM 2570; CCUG 9997; CECT 795	Susceptible, wild-type
<i>S. pneumoniae</i>	ATCC 49619; NCTC 12977; CIP 104340 DSM 11967; CCUG 33638	Penicillin intermediate
<i>H. influenzae</i>	NCTC 8468; CIP5494, CCUG 23946	Susceptible, wild-type

ATCC, American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA.

NCTC, National Collection of Type Cultures, Health Protection Agency Centre for Infections, 61 Colindale Avenue, London NW9 5HT, UK.

CIP, Collection de Institut Pasteur, 25–28 Rue du Docteur Roux, 75724 Paris Cedex 15 France.

DSMZ, Deutsche Stammsammlung für Mikroorganismen und Zellkulturen, Mascheroder Weg 16, D-38124 Braunschweig, Germany.

CCUG, The Culture Collection University of Gothenburg <http://www.ccug.se/>

CECT. Colección Española de Cultivos Tipo. Universidad de Valencia. 46100. Burjassot. Valencia. Spain. <http://www.cect.org>

EUCAST strains for detection of specific resistance mechanisms *(under development)*

Organism	Culture collection numbers	Characteristics
<i>E. coli</i>	ATCC 35218; NCTC 11954; CIP 102181; DSM 5564; CCUG 30600; CECT 943	TEM-1 β -lactamase producer
<i>K. pneumoniae</i>	ATCC 700603; NCTC 13368; CCUG 45421; CECT 7787	ESBL producer (SHV-18)
<i>S. aureus</i>	NCTC 12493	Oxacillin hetero-resistant, <i>mecA</i> positive
<i>E. faecalis</i>	ATCC 51922; NCTC 13379; CIP 104676;	High-level aminoglycoside resistant (HLAR) and vancomycin resistant (<i>vanB</i> positive)
<i>H. influenzae</i>	ATCC 49247; NCTC 12699; CIP 104604; DSM 9999; CCUG 26214	β -lactamase negative, ampicillin-resistant (BLNAR)