THE 'NAC' CHALLENGE PANEL OF ISOLATES FOR VERIFICATION OF ANTIBIOTIC SUSCEPTIBILITY TESTING METHODS

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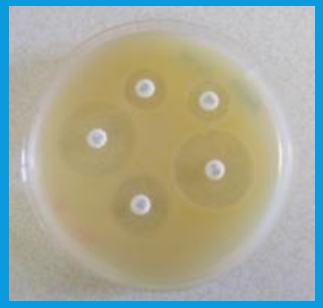
INTRODUCTION

Antimicrobial susceptibility testing (AST) in clinical laboratories

Minimal inhibitory concentration
Zone diameter



Breakpoints

60,5% EUCAST
34,5% CLSI
5% other

EKE WIV-ISP (2016/3, oktober 2016)

Susceptible
Intermediate
Resistant

INTRODUCTION

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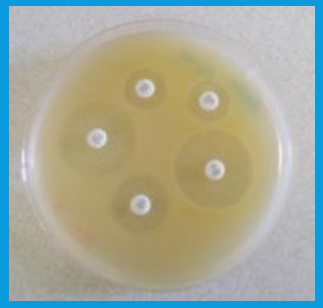


Implementation of (commercial AST) systems

Verification study
Quality control

INTRODUCTION

VERIFICATION STUDY

= process and study performed when

a system is first introduced into a laboratory

or

system is updated by the introduction of new antimicrobial agents, updated software or hardware, use of new breakpoints,…

Purpose

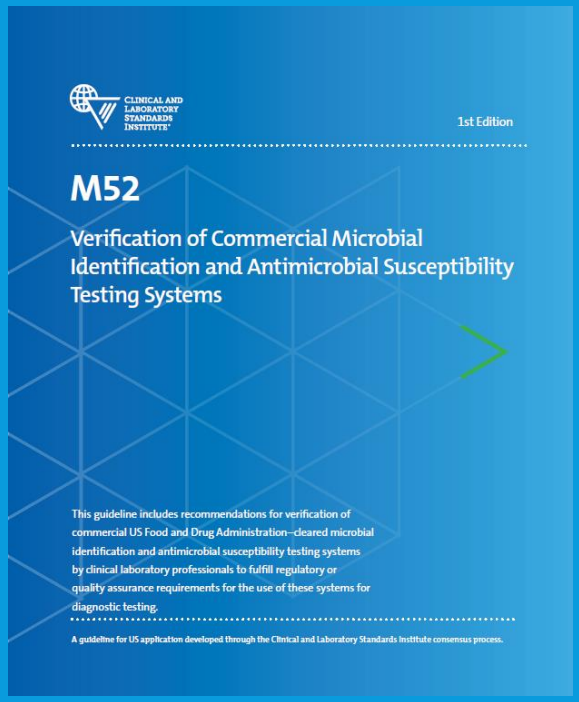
- Verify the system is performing within manufacturer's specifications
- Verify the ability of the laboratory staff to produce accurate and reproducible results
- Fulfill regulatory requirements

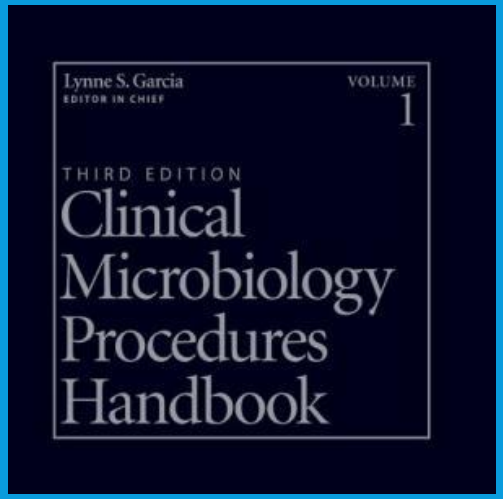
Objective

to detect a major flaw in the laboratory test process

CLSI. Verification of commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems. 1st ed. CLSI guideline M52.

VERIFICATION OF AST





VERIFICATION STUDY

- Precision (reproducibility)

  Closeness of agreement between results of successive measurements of the same analyte

- Accuracy

  Closeness of agreement between test result and comparator value

  reference method

  well-characterized specimens/ strains

at least 30 bacterial isolates per panel/card should be tested

-various species

-various antimicrobial susceptibility profiles

CHALLENGE PANEL

- In 2012 Belgian National Antimicrobial Susceptibility Testing Committee (NAC) decided to develop a Belgian **challenge panel**

useful for the implementation of EUCAST breakpoints or EUCAST methods in the clinical laboratories.

- Covering most important resistance mechanisms
- Including both Gram-negatives and Gram-positives
- Showing stable susceptibility results with both
  - Micro-dilution methods
    - Including the 3 most used automated commercial AST systems in Belgium
      - Phoenix, Vitek and Microscan
  - Disk diffusion methods
- Each strain covers preferentially all relevant antibiotics

DEVELOPMENT OF THE PANEL

Desmet et al. Development of a national EUCAST challenge panel for antimicrobial susceptibility testing, Clinical Microbiology and Infection (2016), http://dx.doi.org/10,2016/j.cmi.2016.05.011

117 strains

AST in 7 validation laboratories

Selection of 28 strains

AST in 20 Belgian clinical laboratories

STRAINS

Starting panel of
117 isolates

57 strains with known resistance mechanisms

selected by the National Reference Centers

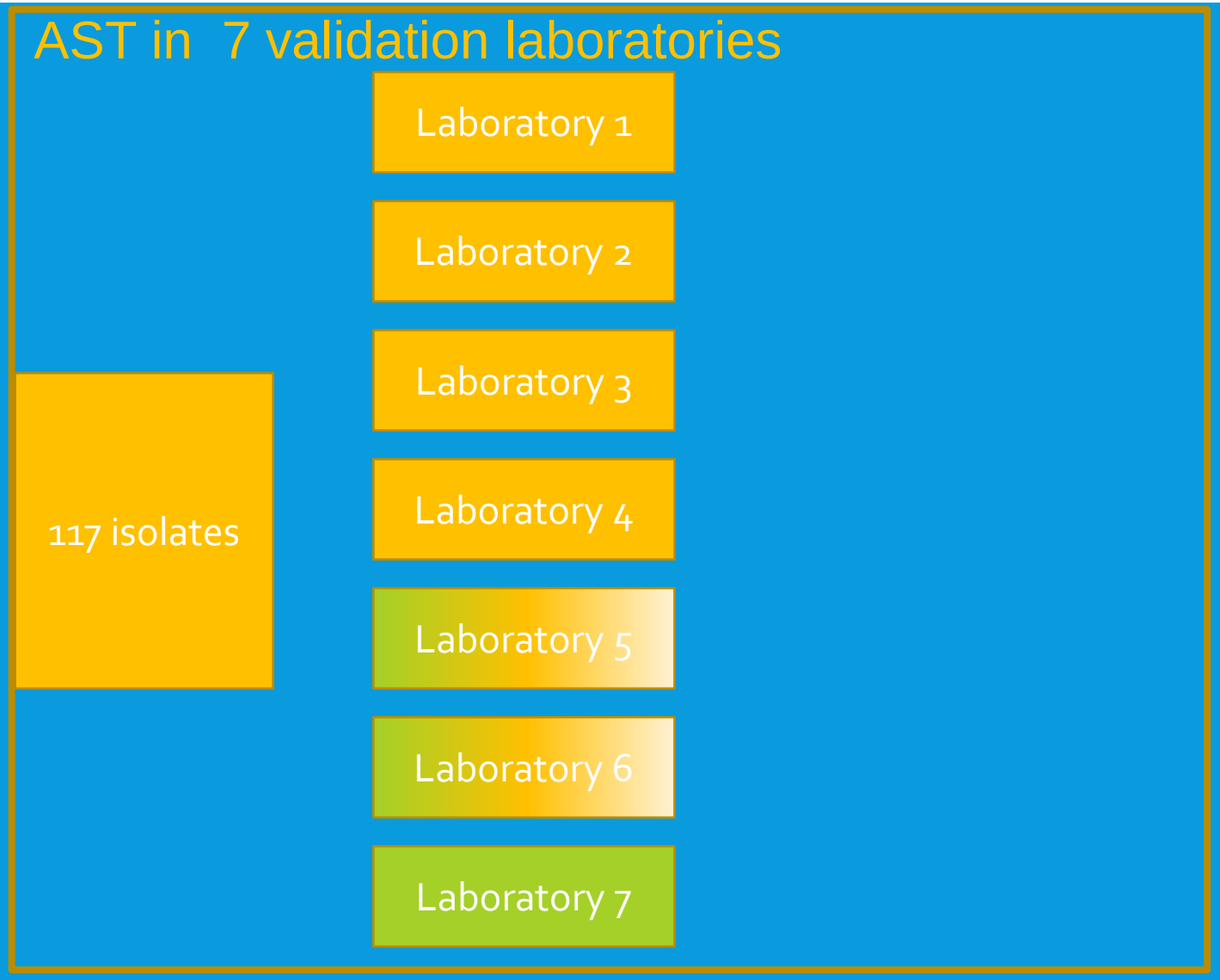
60 clinical isolates

10 per validation laboratory

STARTING PANEL (117 STRAINS)

Species	*n*	Resistance mechanism/profile	*n*
Aeromonas hydrophila	1	extended-spectrum β-lactamase-producing	8
Citrobacter braakii	1	AmpC-producing	3
Citrobacter freundii	2	carbapenemase-producing	8
Citrobacter koseri	2	OXA-48	4
Enterobacter aerogenes	5	VIM	2
Enterobacter cloacae complex	6	KPC	1
Escherichia coli	13	NDM	1
Klebsiella oxytoca	3	AmpC-producing with carbapenem porine deficiency	1
Klebsiella pneumoniae	9	wild-type	3
Morganella morganii	6	colistin-resistant (non-intrinsic)	1
Proteus mirabilis	6	combined or not genetically determined resistance	37
Proteus penneri	1		
Proteus vulgaris	1		
Providencia rettgeri	1		
Serratia marcescens	4		
Acinetobacter baumannii	1	AmpC-producing	1
Acinetobacter haemolyticus	1	carbapenemase-producing: OXA-58	1
Pseudomonas aeruginosa	9	wild-type	2
		carbapenemase-producing: VIM-2	2
		carbapenem-impermeability	2
		AmpC-producing	1
		combined or not genetically determined resistance	2
Enterococcus faecium	4	VanA	2
Enterococcus faecalis	4	VanB	2
		other	4
Coagulase-negative *Staphylococcus*	7	methicillin-resistant *Staphylococcus aureus*	4
Staphylococcus saprophyticus	1	wild-type	2
Staphylococcus aureus	11	other	14
Staphylococcus lugdunensis	1		
Streptococcus agalactiae	5	macrolide-resistant (4 MLSB, 2 macrolide-efflux pump, 1 phenotype L)	7
Streptococcus dysgalactiae	1	fluoroquinole-resistant	1
Streptococcus pyogenes	3	wild-type	1
		only fluoroquinole-resistant	1
		macrolide-resistant	1
		different combined resistance	4
Streptococcus anginosus group	1	wild-type	2
Streptococcus mitis group	1		

ANALYSIS


AST in 7 validation laboratories
117 isolates
Laboratory 1
Laboratory 2
Laboratory 3
Laboratory 4
Laboratory 5
Laboratory 6
Laboratory 7

AST SYSTEMS

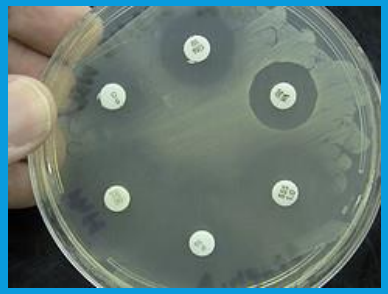
6 Automated AST – EUCAST panels/cards 2013

n=2 AZ Sint-Lucas Brugge
Sint-Jozefskliniek Izegem

n=2 Jessa Hasselt
OLV Aalst

n=2 UZ Leuven
CHU Liège

7 Disk diffusion



UZ Leuven

BD
Rosco

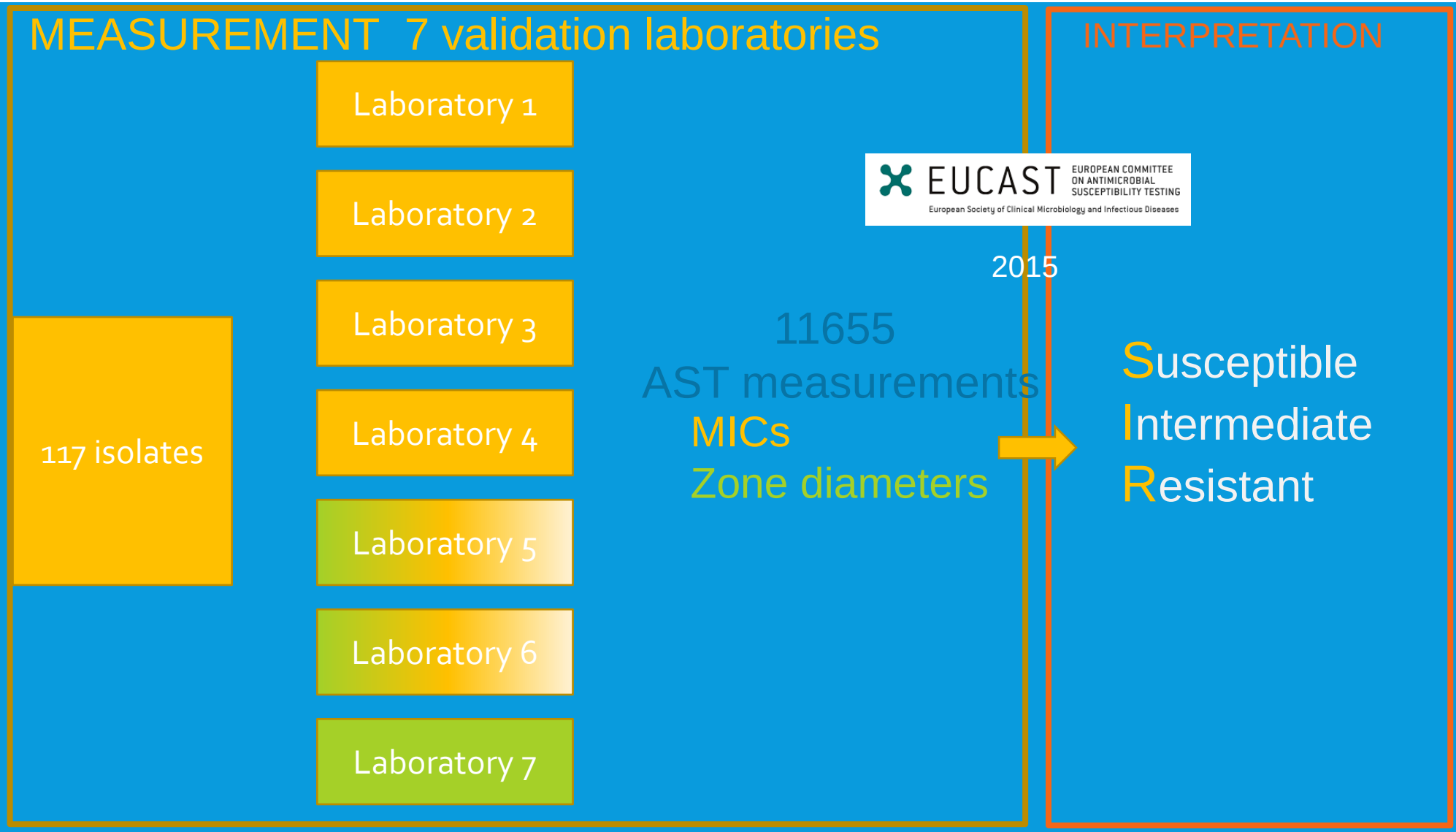
CHU Liège

BD
Rosco
Bio-rad
Biomérieux

UZ Brussel

i2a

ANALYSIS


MEASUREMENT 7 validation laboratories
INTERPRETATION
Laboratory 1
Laboratory 2
Laboratory 3
Laboratory 4
Laboratory 5
Laboratory 6
Laboratory 7
117 isolates
EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases
2015
11655
AST measurements
MICs
Zone diameters
Susceptible
Intermediate
Resistant

SELECTION OF STRAINS

- Based on
  - List of strains needed for verification studies
  - Results of panel validation study
    - Categorical agreement between all methods (MICs en diameters) per antibiotic (%)
    - Number of (very) major errors
    - Number of minor errors

SELECTION OF STRAINS

- List of susceptibility profiles/resistance mechanisms to be included in the panel

  - **Garcia**, L.S, Isenberg, H. D. Clinical microbiology procedures handbook. 3rd edition. ASM Press, Washington, DC.
  - Clark, R. B., M. A. Lewinski, M. J. Loeffelholz, and R. J. Tibbetts, 2009. **Cumitech** 31A, Verification and Validation of Procedures in the Clinical Microbiology Laboratory. Coordinating ed., S. E. Sharp. ASM Press, Washington, DC.
  - CLSI. Verification of Microbial Identification and Antimicrobial Susceptibility testing systems: Draft Guideline. **CLSI document M52** (proposed Draft). Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
  - Own expert group

SELECTION OF STRAINS

- Based on
  - List of strains needed for verification studies
  - Results of panel validation study
    - Categorical agreement between all methods per antibiotic (%)
    - Number of (very) major errors
    - Number of minor errors

Panel of 14 gram-negative and 14 gram-positive bacteria

No candidate strain for 6 susceptibility profiles

Some selected strains serve for different susceptibility profiles

NOT INCLUDED IN PANEL

- multidrug-resistant *Acinetobacter* spp.[2]
- extended-spectrum cephalosporine-resistant *Citrobacter freundii* and *Serratia marcescens*[1,2]
- Tobramycin-resistant *P. aeruginosa* [1]
- High-level aminoglycoside-resistant *Enterococcus*[1]
- Penicillin-resistant and penicillin-intermediate *Streptococcus viridans*[1]

[1] Garcia, L.S, Isenberg, H. D. Clinical microbiology procedures handbook. 3rd edition. ASM Press, Washington, DC.
[2] Clark, R. B., M. A. Lewinski, M. J. Loeffelholz, and R. J. Tibbetts, 2009. Cumitech 31A, Verification and Validation of Procedures in the Clinical Microbiology Laboratory. Coordinating ed., S. E. Sharp. ASM Press, Washington, DC.

PILOT STUDY

Panel of 14 gram-negatives and 14 gram-positives

Pilot study in 20 clinical laboratories (May-June 2015)

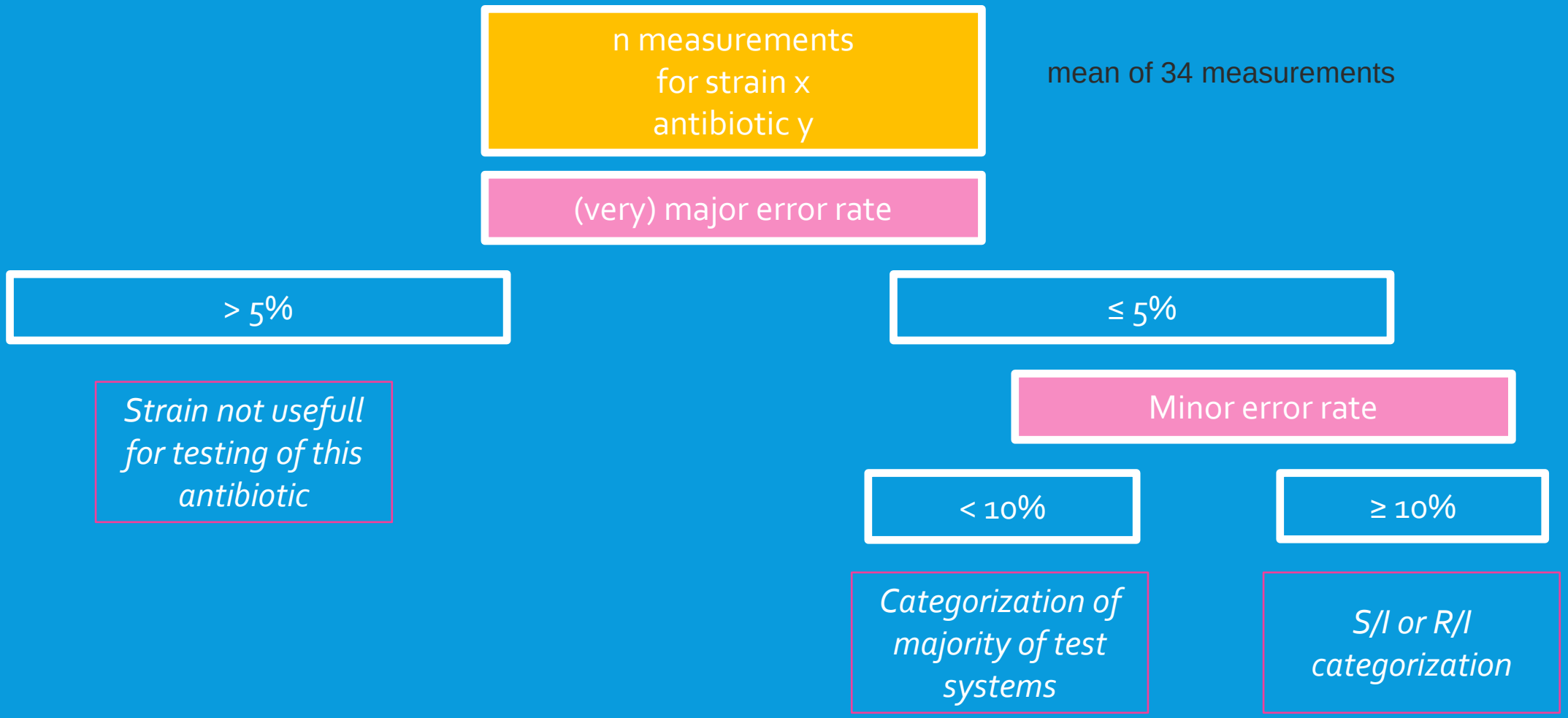
Vitek (n=8); Phoenix (n=7); Microscan (n=2)

Bio-rad discs (n=7), Rosco Neo-Sensitab discs (n=3)

- to explore stability of strains and expression of resistance mechanisms (+/-2 years between validation and pilot study)
- to define categorisation results

PILOT STUDY

To define susceptibility categorization (S or I or R)



GRAM-NEGATIVE BACTERIA

Number	Species	Resistance mechanism/susceptibility profile	Amikacin	Amoxicillin/ Ampicillin	Amoxicillin-clavulanate	Cefepime	Cefotaxime/ Ceftriaxone	Ceftazidime	Cefuroxime	Ciprofloxacin	Colistin	Gentamicin	Levofloxacin	Meropenem	Piperacillin-tazobactam	Tigecycline	Tobramycin	Trimethoprim-sulfamethoxazole
NAC14 §	*Klebsiella pneumoniae*	carbapenemase: OXA-48; ESBL: CTX-M-15	S	R	R	R/I	R	R	R	S	S	R	S	S/I	R	S	-	R
NAC20	*Klebsiella pneumoniae*	carbapenemase: KPC; ESBL: SHV-12, SHV-1, TEM-1	R	R	R	R	R	R	R	R	S	I/S	R	R/I	R	I/S	R	R
NAC15	*Klebsiella pneumoniae*	carbapenemase: OXA-48; ESBL: CTX-M-15, OXA-1	S	R	R	R/I	R	R	R	R/I	S	R	S/I	S/I	R	S/I	R	R
NACL2	*Escherichia coli*	WT	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NACA7	*Escherichia coli*	fluoroquinolone R	S	S	S	S	S	S	S	R	S	S	R	S	S	S	S	S
NACI4	*Escherichia coli*	ESBL	S/I	R	R	R	R	R	R	R	S	S	R	S	S/I	S	R	S
NACA9	*Morganella morganii*	colistin R	S	R	R	S	S	S	R	S	R	S	S	S	S	**	S	R
NAC29 #	*Morganella morganii*	AmpC hyper	S	R	R	-	-	R	R	-	R	R	-	S	S	**	-	-
NACI10	*Enterobacter cloacae complex*	resistant to extended-spectrum cephalosporins	S	R	R	S	R	R	R	S	S	S	S	S	R/I	S	S	S
NAC11 §	*Enterobacter aerogenes*	AmpC + carbapenem porine deficiency	S	R	R	-	R	R	R	S	S	S	S	I	R	S	S	S
NAC24	*Citrobacter koseri*	WT	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NAC4	*Pseudomonas aeruginosa*	carbapenemase: VIM-2	R	-	-	R	-	R	-	R	S	R	R	R/I	R	-	R	-
NAC3	*Pseudomonas aeruginosa*	AmpC	S/I	-	-	S	-	S	-	S	S	-	S	S	S	-	S	-
NAC5 §	*Pseudomonas aeruginosa*	carbapenemase: VIM-2	-	-	-	-	-	R	-	R	S	S	R	R	R	-	R	-

ESBL: extended-spectrum beta-lactamase; WT: wild type; '-': no categorisation; **: intrinsic resistance but tested as susceptible ; §: strain not useful for one antibiotic after pilot-testing; #: strain not useful for two antibiotics after pilot-testing

GRAM-NEGATIVE BACTERIA

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NAC14 §	*Klebsiella pneumoniae*	carbapenemase: OXA-48; ESBL: CTX-M-15	S	R	R	R/I	R	R	R	S	S	R	S	S/I	R	S	-	R
NAC20	*Klebsiella pneumoniae*	carbapenemase: KPC; ESBL: SHV-12, SHV-1, TEM-1	R	R	R	R	R	R	R	R	S	I/S	R	R/I	R	I/S	R	R
NAC15	*Klebsiella pneumoniae*	carbapenemase: OXA-48; ESBL: CTX-M-15, OXA-1	S	R	R	R/I	R	R	R	R/I	S	R	S/I	S/I	R	S/I	R	R
NACL2	*Escherichia coli*	WT	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NACA7	*Escherichia coli*	fluoroquinolone R	S	S	S	S	S	S	S	R	S	S	R	S	S	S	S	S
NACI4	*Escherichia coli*	ESBL	S/I	R	R	R	R	R	R	R	S	S	R	S	S/I	S	R	S
NACA9	*Morganella morganii*	colistin R	S	R	R	S	S	S	R	S	R	S	S	S	S	**	S	R
NAC29 #	*Morganella morganii*	AmpC hyper	S	R	R	-	-	R	R	-	R	R	-	S	S	**	-	-
NACI10	*Enterobacter cloacae complex*	resistant to extended-spectrum cephalosporins	S	R	R	S	R	R	R	S	S	S	S	S	R/I	S	S	S
NAC11 §	*Enterobacter aerogenes*	AmpC + carbapenem porine deficiency	S	R	R	-	R	R	R	S	S	S	S	I	R	S	S	S
NAC24	*Citrobacter koseri*	WT	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NAC4	*Pseudomonas aeruginosa*	carbapenemase: VIM-2	R	-	-	R	-	R	-	R	S	R	R	R/I	R	-	R	-
NAC3	*Pseudomonas aeruginosa*	AmpC	S/I	-	-	S	-	S	-	S	S	-	S	S	S	-	S	-
NAC5 §	*Pseudomonas aeruginosa*	carbapenemase: VIM-2	-	-	-	-	-	R	-	R	S	S	R	R	R	-	R	-

ESBL: extended-spectrum beta-lactamase; WT: wild type; '-': no categorisation; **: intrinsic resistance but tested as susceptible ; §: strain not useful for one antibiotic after pilot-testing; #: strain not useful for two antibiotics after pilot-testing

GRAM-POSITIVE BACTERIA

Number	Species	Resistance mechanism/susceptibility profile	Ampicillin	Benzylpenicillin	Cefotaxime	Cefoxitin	Ciprofloxacin	Clindamycin	Erythromycin	Gentamicin	Levofloxacin	Linezolid	Moxifloxacin	Oxacillin	Rifampicin	Teicoplanin	Tetracycline	Tigecycline	Trimethoprim-sulfamethoxazole	Vancomycin
NAC53 §	*Staphylococcus aureus*	MRSA, constitutive MLSb, fluoroquinolone R, rifampicin R, tetracycline R		R		R	R	R	R	R	R	S	R	R	R	S	-	S	S	S
NACB1	*Staphylococcus aureus*	MRSA, erythromycin en clindamycin R		R		R	R	R	R	-	R	S	R	R	S	S	S	S	S	S
NACB10	*Staphylococcus aureus*	MSSA peni R		R		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NACB7	*Staphylococcus warneri/Staphylococcus pasteuri*	WT				S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NAC44	*Enterococcus faecium*	VanA	R									S				R		S		R
NACL9	*Enterococcus faecalis*	WT	S									S				S		S		S
NAC45	*Enterococcus faecium*	VanB	R/I									S				S		S		R
NAC46	*Enterococcus faecium*	VanA, ampicillin-susceptible	S									S				R		S		R
NAC50	*Streptococcus pneumoniae*	fluoroquinolone R	S	S	S			S	S		R	S	R				S		S	S
NAC52	*Streptococcus pneumoniae*	penicillin R, cefotaxime R, macrolide R, tetracycline R	R	R/I	R/I			R	R		S	S	S				R		R	S
NAC48 §	*Streptococcus pneumoniae*	penicillin intermediate	S	I	S			S	S		-	S	S				S		-	S
NAC38	*Streptococcus agalactiae*	macrolide effluxpump: phenotype M		S				S	R		S	S	S				R		S	S
NAC42	*Streptococcus pyogenes*	MLSb ermB		S				R	R		S/I	S	S/I				R		S	S
NAC35	*Streptococcus agalactiae*	fluoroquinolone R		S				S	S		-	S	R				R		S	S

'-': no categorisation result; NT: not-tested; MRSA: methicillin-resistant Staphylococcus aureus; WT: wild type; §: strain not useful for one antibiotic after pilot-testing).

GRAM-POSITIVE BACTERIA

Number	Species	Resistance mechanism/susceptibility profile	Ampicillin	Benzylpenicillin	Cefotaxime	Cefoxitin	Ciprofloxacin	Clindamycin	Erythromycin	Gentamicin	Levofloxacin	Linezolid	Moxifloxacin	Oxacillin	Rifampicin	Teicoplanin	Tetracycline	Tigecycline	Trimethoprim-sulfamethoxazole	Vancomycin
NAC53 §	*Staphylococcus aureus*	MRSA, constitutive MLSb, fluoroquinolone R, rifampicin R, tetracycline R		R		R	R	R	R	R	R	S	R	R	R	S	-	S	S	S
NACB1	*Staphylococcus aureus*	MRSA, erythromycin en clindamycin R		R		R	R	R	R	-	R	S	R	R	S	S	S	S	S	S
NACB10	*Staphylococcus aureus*	MSSA peni R		R		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NACB7	*Staphylococcus warneri/Staphylococcus pasteuri*	WT				S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NAC44	*Enterococcus faecium*	VanA	R									S			R		S			R
NACL9	*Enterococcus faecalis*	WT	S									S			S		S			S
NAC45	*Enterococcus faecium*	VanB	R/I									S			S		S			R
NAC46	*Enterococcus faecium*	VanA, ampicillin-susceptible	S									S			R		S			R
NAC50	*Streptococcus pneumoniae*	fluoroquinolone R	S	S	S			S	S		R	S	R			S		S	S	
NAC52	*Streptococcus pneumoniae*	penicillin R, cefotaxime R, macrolide R, tetracycline R	R	R/I	R/I			R	R		S	S	S			R		R	S	
NAC48 §	*Streptococcus pneumoniae*	penicillin intermediate	S	I	S			S	S		-	S	S			S		-	S	
NAC38	*Streptococcus agalactiae*	macrolide effluxpump: phenotype M		S				S	R		S	S	S			R		S	S	
NAC42	*Streptococcus pyogenes*	MLSb ermB		S				R	R		S/I	S	S/I			R		S	S	
NAC35	*Streptococcus agalactiae*	fluoroquinolone R		S				S	S		-	S	R			R		S	S	

'-': no categorisation result; NT: not-tested; MRSA: methicillin-resistant Staphylococcus aureus; WT: wild type; §: strain not useful for one antibiotic after pilot-testing.

HOW TO USE THE PANEL?

NAC panel strains

- have defined susceptibility categorisation
- cover different important and stable resistance mechanisms
- show reproducible results with different AST systems

Developed for use in clinical microbiology laboratories
for **verification studies**
Accuracy
Reproducibility

- have no defined MIC ranges
- are not validated as (internal) quality control strains (CLSI M23A3)

PRACTICAL INFORMATION

- Distribution of strains by WIV-ISP
  - October 2016: distributed to 46 laboratories
  - December 2016: new distribution
  - Contact: **EQAtoolkit@wiv-isp.be**

  - Distributed on transport media (TSA-like)

- Strains should be stored at -20°C to -70°C
- Frozen isolates should be subcultured twice before testing
- Subcultures should be no older than 24 hours before testing

CONCLUSION

NAC panel of 14 gram-negative and 14 gram-positive bacteria

- Available for all Belgian clinical laboratories
- Usefull for verification studies of AST methods

SPECIAL THANKS TO

- Belgian National Antimicrobial susceptibility testing Committee
- WIV-ISP
- Prof. Dr. Jan Verhaegen and Prof. Dr. Veroniek Saegeman

Validation laboratories

- Centre Hospitalier Universitaire de Liège
- OLV Hospital Aalst
- AZ Sint-Lucas Brugge
- University Hospital Brussels
- Jessa Hasselt
- AZ Sint-Lucas Brugge
- Sint-Jozefskliniek Izegem

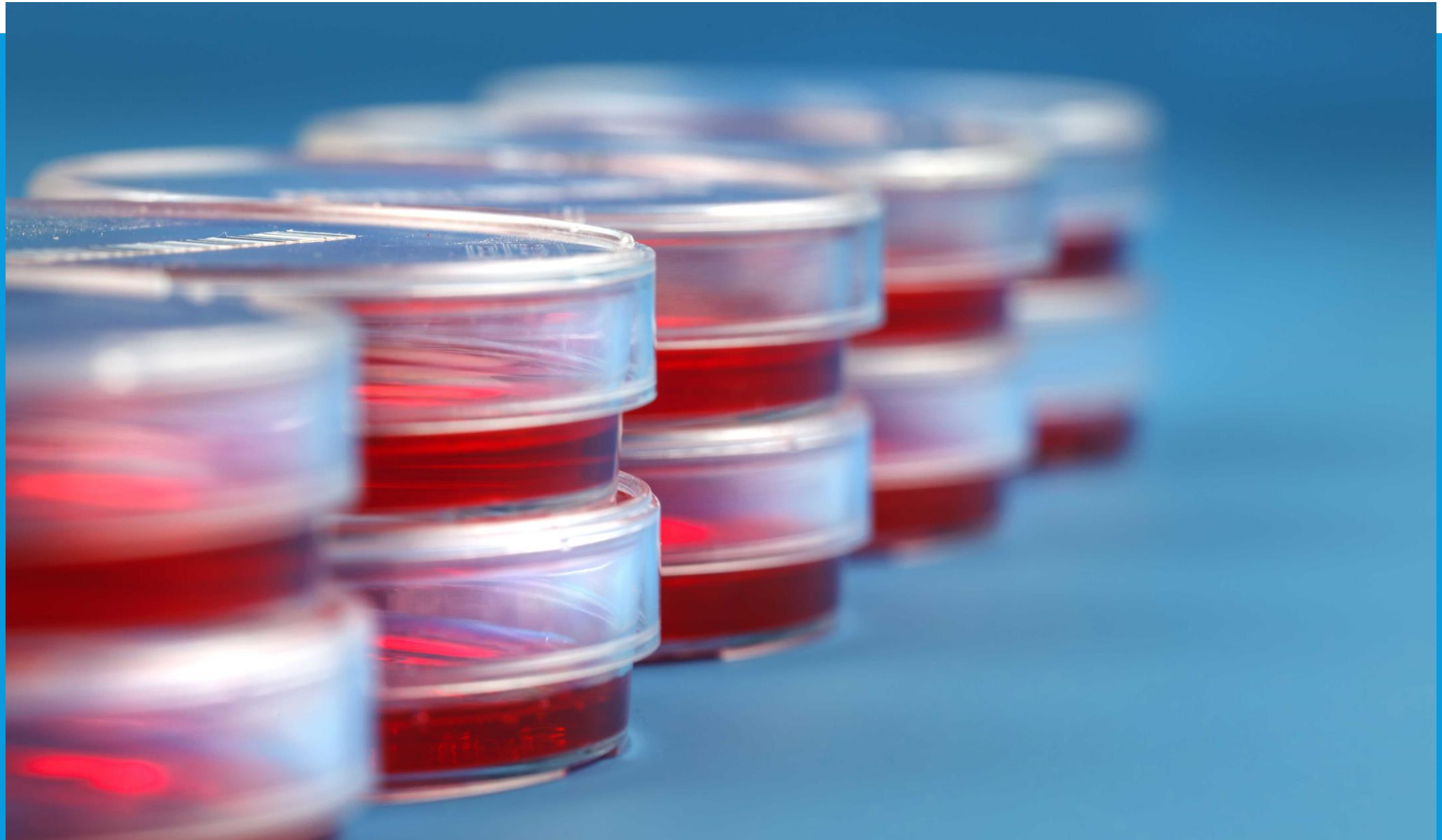
Pilot testing laboratories

- AZ Sint-Lucas, Ghent Annelies De Bel
- MCH Leuven
- GZA campus Sint-Augustinus Antwerp
- Hopital Erasme, Brussels
- AZ Turnhout, Turnhout
- AZ Delta,Roeselare
- Ghent University Hospital, Ghent
- AZ Nikolaas, Sint-Niklaas
- Cliniques Universitaires Saint Luc, Brussels
- Mariaziekenhuis, Overpelt
- AML,Antwerp
- Jan-Yperman, Ieper
- Heilig-Hart Hospital, Lier

National Reference Centers

- Belgian Reference Laboratory of multi-resistant Enterobacteriaceae and multi-resistent Pseudomonas and Acinetobacter ,CHU Mont-Godinne, Belgium
- Belgian Reference Laboratory of *Enterococcus* spp, *Streptococcus pyogenes* and other beta-hemolytic Streptococci non-group B, University hospital Antwerp, Antwerp, Belgium
- Belgian Reference Laboratory of *Staphylococcus aureus*, Hopital Erasme, Brussels, Belgium
- Belgian Reference Laboratory of Group B Streptococci, University hospital of Liège, Liège, Belgium

DISCUSSION TIME



ADDITIONAL SLIDES FOR QUESTIONS

VERIFICATION NEW AST CARDS (ON PREVIOUSLY VERIFIED AST SYSTEM)

Example of use of NAC strains in verification study

UZ LEUVEN | LABORATORIUMGENEESKUNDE

- Precision (reproducibility)
  - Testing: minimum of 5 isolates 3 times (separate inoculum)
  - → e.g. 3 quality control strains and 2 NAC panel strains
  - Criteria CLSI M52
    - At least 95% of results within EA (+/- 1 dilution)
    - At least 95% of the QC strain results within QC specifications
- Accuracy
  - Testing of minimum 30 isolates per card
    - Selection of NAC panel strains
      - comparator value = defined categorisation result
    - Selection of quality control strains
      - comparator value = defined categorisation result
    - Selection of strains tested with reference method
      - comparator value = value reference method
  - Criteria CLSI M52 based on categorical agreement (>=90%), major errors (not more than one) and very major errors (not more than one)
  - In case not fulfilling criteria: retesting of discordant isolates together with same number of concordant isolates to control if the result is indeed a random error

"THE EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING. ROUTINE AND EXTENDED INTERNAL QUALITY CONTROL FOR MIC DETERMINATION AND DISK DIFFUSION AS RECOMMENDED BY EUCAST. VERSION 6.1, 2016. HTTP://WWW.EUCAST.ORG."

Routine quality control
Escherichia coli ATCC 25922
Pseudomonas aeruginosa ATCC 27853
Staphylococcus aureus ATCC 29213
Enterococcus faecalis ATCC 29212
Streptococcus pneumoniae ATCC 49619
Haemophilus influenzae ATCC 49766
Campylobacter jejuni ATCC 33560
Control of the inhibitor component of β-lactam-β-lactamase inhibitor combination disks

Extended quality control for detection of resistance mechanisms with disk diffusion
ESBL production in Enterobacteriaceae
Methicillin resistance in *Staphylococcus aureus*
VanB-mediated glycopeptide resistance in enterococci
High-level aminoglycoside resistance in enterococci
Reduced susceptibility to β-lactam agents due to PBP mutations in *Haemophilus influenzae*

M23A3

Strains under consideration for use as new QC strains are first evaluated in a Tier 1 study to establish initial feasibility. Considerations for a Tier 1 study to evaluate a new QC strain include:

- reproducibility of test results;
- performance for the objective(s) of using the QC strain;
- stability of the strain and relevant resistance mechanisms through:
  - multiple passages; and
  - multiple freeze/thaw cycles;
- effect of culture age on test results; and
- any known issues with lyophilization of similar strains.

Subsequently, candidate strains are qualified in a Tier 2 study to obtain formal CLSI approval for use. To qualify a new QC strain and establish the expected range for the antimicrobial agent, follow Tier 2 study guidelines as described in the following section. Include at least one current QC strain if QC expected ranges exist for the antimicrobial agent.

TIER 1

Tier 1 Testing Guidelines:

	Broth MIC	Agar Dilution MIC
Organisms*	100	100
Total QC Replicates	20	20
Days (minimum)	2	2
Media lots	1	1
Laboratories	1	1

*Within the clinical spectrum for the antimicrobial agent.

Tier 2 Study: Requirements to Establish Quality Control Expected Range

Category	Number required for:	
	MIC Study	Disk Diffusion Study
Laboratories*	7	7
Media lots (different manufacturers)†	3	3
Replicates (individual inoculum, max 4 per day)‡	10	10
Disk lots (different manufacturers)	NA	2
Total data points	210 (7 × 3 × 10)	420 (7 × 3 × 10 × 2)

SELECTION OF STRAINS

117 isolates

- Group 1: 100% categorical agreement for all antibiotics
- Group 2: Only minor errors
- Group 3: Only (very) major error(s) for one antibiotic
- Group 4: (Very) major error(s) for more than one antibiotic

SELECTION OF STRAINS

Eucast interpretation group	number of strains per categorisation group				TOTAL
	group 1 100% CA	group 2 no (V)ME	group 3 1 (V)ME	group 4 >1 (V)ME	
Enterobacteriacea	4	9	16	32	61
Acinetobacter spp.	0	0	0	2	2
Pseudomonas spp.	0	1	4	4	9
Enterococcus spp.	1	3	3	1	8
Staphylococcus spp.	2	3	7	8	20
Streptococcus pneumoniae	1	2	2	1	6
Streptococcus groups A, B, C and G	3	3	2	1	9
Viridans group streptococci	0	0	1	1	2
TOTAL	12 (10%)	20 (17%)	35 (30%)	50 (43%)	117

SELECTION OF STRAINS

species	resistance mechanism/ susceptibility profile
Enterobacter cloacae	resistant to extended-spectrum cephalosporins
Enterobacter aerogenes	resistant to extended-spectrum cephalosporins
Citrobacter freundii	resistant to extended-spectrum cephalosporins
Serratia marcescens	resistant to extended-spectrum cephalosporins
Enterobacteriacea	gentamicin resistant
Enterobacteriacea	amikacin resistant
Enterobacteriacea	fluoroquinolones resistant
Morganella morganii	resistant to extended-spectrum cephalosporins
Klebsiella spp.	extended-spectrum beta-lactamase (ESBL)
Escherichia coli	extended-spectrum beta-lactamase (ESBL)
Escherichia coli - Enterobacteriaceae	ampicillin resistant
Klebsiella spp.	piperacillin resistant
Enterobacteriacea	meropenem low level resistance
Enterobacteriacea	carbapenemase producing
Enterobacteriacea	ampicillin MIC around breakpoint
Enterobacteriacea	cefepime resistant
Enterobacteriacea	colistin resistant
Enterobacteriacea	AmpC producing
Pseudomonas aeruginosa	amikacin resistant
Pseudomonas aeruginosa	gentamicin resistant
Pseudomonas aeruginosa	resistant to extended-spectrum cephalosporins
Pseudomonas aeruginosa	fluoroquinolones R
Pseudomonas aeruginosa	meropenem resistant
Pseudomonas aeruginosa	ceftazidim MIC around breakpoint
Pseudomonas aeruginosa	piperacillin-tazobactam MIC around breakpoint
Pseudomonas aeruginosa	meropenem MIC around breakpoint
Acinetobacter spp.	multidrug resistant

- Group 1> Group 2> Group 3
- Group 4 excluded
- Preferentially strains with the highest number of MICs in the measurable range of the testing system (no < and > results) and showing MICs close to the susceptibility breakpoints.

Citrobacter freundii	resistant to extended-spectrum cephalosporins	[1,2]	*NA*		
Serratia marcescens	resistant to extended-spectrum cephalosporins	[1,2]	*NA*		
Enterobacteriacea	gentamicin resistant	[1]	NAC14	NAC15	NAC29
Enterobacteriacea	amikacin resistant	[1]	NAC20		
Enterobacteriacea	fluoroquinolones resistant	[1]	NACA7	NACI4	NAC20
Morganella morganii	resistant to extended-spectrum cephalosporins	[1]	NAC29		
***Klebsiella* spp.**	extended-spectrum beta-lactamase (ESBL)	[1,2]	NAC15	NAC14	
Escherichia coli	extended-spectrum beta-lactamase (ESBL)	[1,2]	NACI4		
***Escherichia coli* - Enterobacteriaceae**	ampicillin resistant	[1]	NAC24	NACI4	
***Klebsiella* spp.**	piperacillin resistant	[1]	NAC14	NAC15	NAC20
Enterobacteriaceae	meropenem low level resistance	[3]	NAC11	NAC14	NAC15
Enterobacteriaceae	carbapenemase-producing	[2]	NAC14	NAC15	
Enterobacteriaceae	ampicillin MIC around susceptibility breakpoint	[3]	NACL2		
Enterobacteriaceae	cefepime resistant	[3]	NACI4		
Enterobacteriaceae	colistin resistant	[3]	NACA9	NAC29	
Enterobacteriaceae	AmpC-producing	[2]	NAC11	NAC29	
Pseudomonas aeruginosa	amikacin resistant	[1]	NAC4		
Pseudomonas aeruginosa	gentamicin resistant	[1]	NAC4		
Pseudomonas aeruginosa	resistant to extended-spectrum cephalosporins	[1]	NAC4	NAC5	
Pseudomonas aeruginosa	fluoroquinolones resistant	[1]	NAC4	NAC5	
Pseudomonas aeruginosa	meropenem resistant	[1,2]	NAC5		
Pseudomonas aeruginosa	ceftazidim MIC around susceptibility breakpoint	[3]	NAC3		
Pseudomonas aeruginosa	piperacillin-tazobactam MIC around susceptibility breakpoint	[3]	NAC3		
Pseudomonas aeruginosa	meropenem MIC around susceptibility breakpoint	[3]	NAC4		
***Acinetobacter* spp.**	multidrug resistant	[2]	*NA*		
Staphylococcus aureus	MRSA	[1,2]	NAC53	NACB1	
Coagulase-negative staphylococcus		[1,2]	NACB7		
***Staphylococcus* spp.**	gentamicin resistant	[1]	NAC53		
***Staphylococcus* spp.**	fluoroquinolones resistant	[1]	NAC53	NACB1	
***Staphylococcus* spp.**	clindamycin resistant	[1,2]	NAC53	NACB1	
***Staphylococcus* spp.**	erythromycin resistant	[1]	NAC53	NACB1	
***Staphylococcus* spp.**	oxacillin MIC around susceptibility breakpoint	[3]	NACB10		
Enterococcus faecium	ampicillin resistant	[1]	NAC44		
***Enterococcus* spp.**	high level aminoglycoside resistant	[1]	*NA*		
***Enterococcus* spp.**	vancomycin resistant	[1.2]	NAC44	NAC45	NAC46
***Enterococcus* spp.**	vancomycin MIC 2-4 mg/l	[3]	NACL9		
***Enterococcus* spp.**	vancomycin resistant teicoplanin susceptible	[3]	NAC46		
Streptococcus pneumoniae	penicillin resistant	[1]	NAC52		
Streptococcus pneumoniae	penicillin intermediate	[1]	NAC48		
Streptococcus pneumoniae	extended-spectrum cephalosporin resistant	[1]	NAC52		
Streptococcus pneumoniae	fluoroquinolones resistant	[1]	NAC50		
***Streptococcus* viridans**	penicillin resistant	[1]	*NA*		
***Streptococcus* viridans**	penicillin intermediate	[1]	*NA*		
***Streptococcus* spp.**	macrolide resistant	[1]	NAC42		
***Streptococcus* spp.**	clindamycin resistant	[1]	NAC42		
***Streptococcus* spp.**	only erythromycin resistant	[3]	NAC38		
***Streptococcus* spp.**	fluoroquinolones resistant	[3]	NAC35		

[1] Garcia, L.S, Isenberg, H. D. Clinical microbiology procedures handbook. 3rd edition. ASM Press, Washington, DC.

[2] Clark, R. B., M. A. Lewinski, M. J. Loeffelholz, and R. J. Tibbetts, 2009. Cumitech 31A, Verification and Validation of Procedures in the Clinical Microbiology Laboratory. Coordinating ed., S. E. Sharp. ASM Press, Washington, DC.

[3] own expert group

- To exclude interference of a mall functioning test system in a laboratory, not more than two (very) major errors of one system were accepted for the same strain.