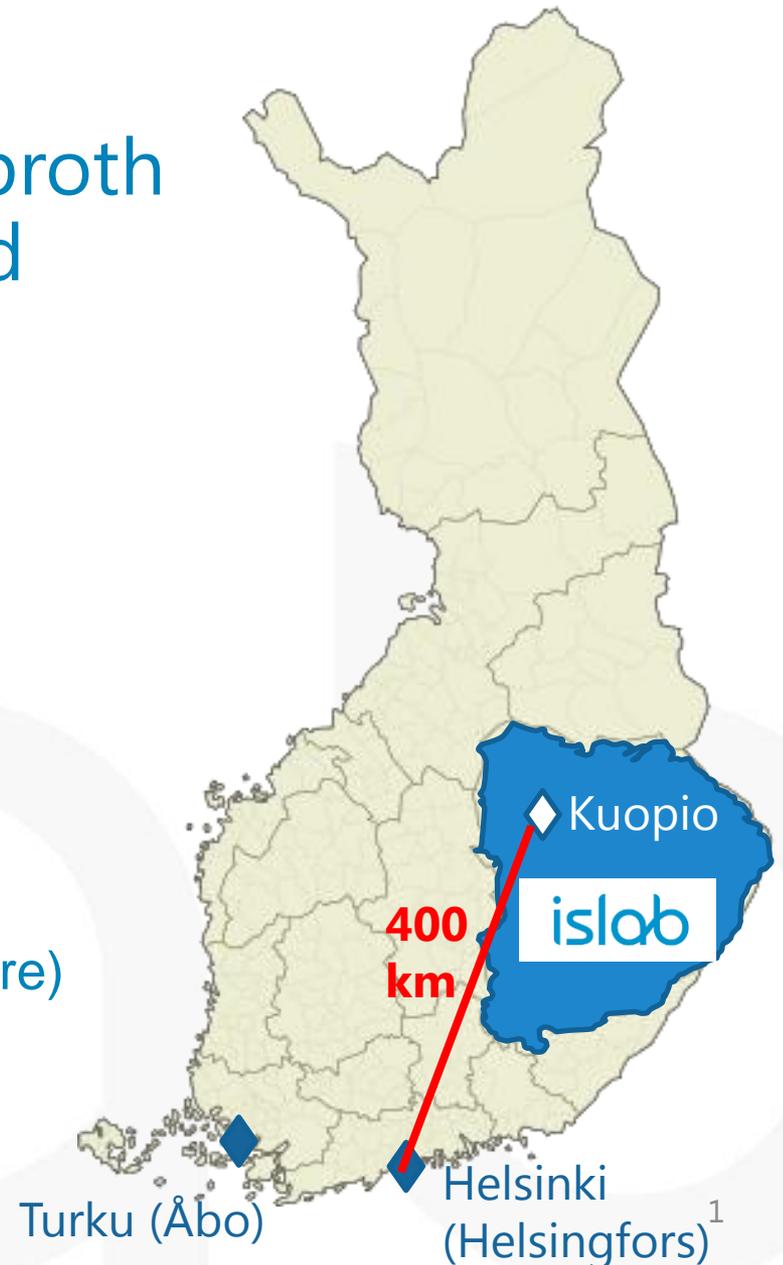


Implementation of manual broth microdilution (BMD) method

- experiences from a middle-size laboratory

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Manual Sensititre™ implemented in September 2016: why?

- EUCAST Warnings!
 - Problems with colistin gradient tests – the warning issued July 2016
- Methods routinely used:
 - EUCAST disk diffusion
 - Gradient tests if no DD method
 - Small scale limited use of VITEK2
- Colistin BMD service not available in Finland at that time
 - isolate transported from Kuopio to Helsinki or Turku generates an extra day delay (on weekends 2-4 days)

Why did we choose manual Sensititre™?

- Suitable for "very seldom random access"
 - occasionally for a selected isolate
 - colistin susceptibility needed for 10-20 isolates per year
 - MDR *Pseudomonas aeruginosa*, CPE, MDR *Acinetobacter*
- Low cost & quick implementation possible: equipment ready
 - turbidimeter (Densichek)
 - 8-channel or automated serial pipette
 - mirror table
- Technical support readily available by the manufacturer/distributor

Implementation – part one

Demonstration (2 days) by Thermo Scientific Application Specialist at our laboratory

- 3 technicians, 1 hospital microbiologist, 1 clinical microbiologist tutored to be the responsible persons
- other available staff briefly oriented to preparing (day 1) and reading (day 2) the plates
- 4 gram-negative isolates → 4 plates prepared and analyzed during demo



Shopping list for Sensititre demo

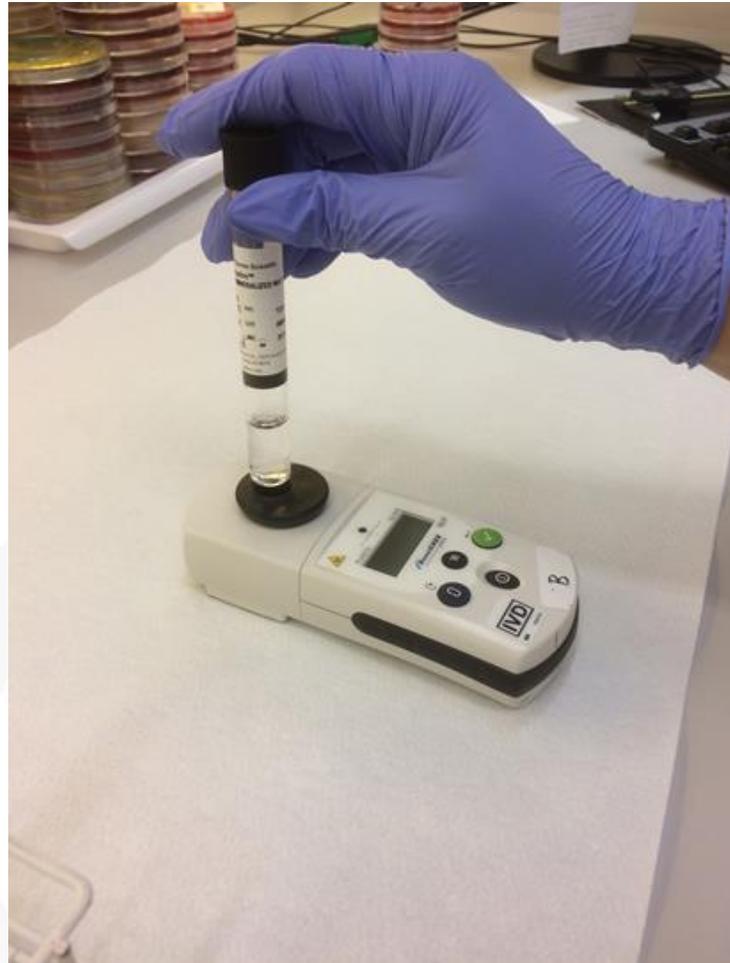
- ✓ T3339: Demineralized water, 5 ml
- ✓ T3463: Müller-Hinton broth, 11 ml
- ✓ GNX2F: Sensititre Gram Negative Xtra MIC Plate

Starting with OOPS..

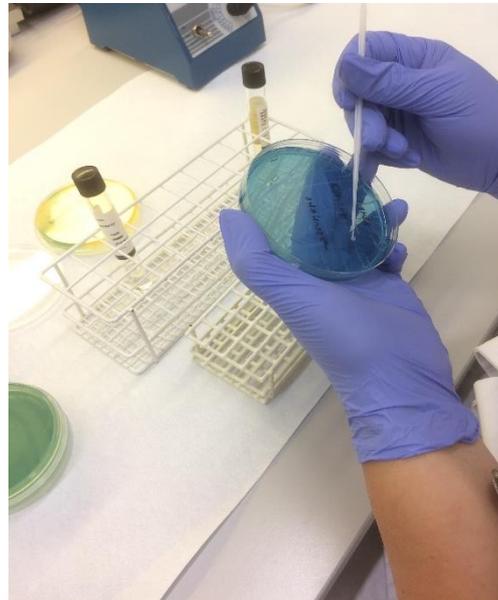
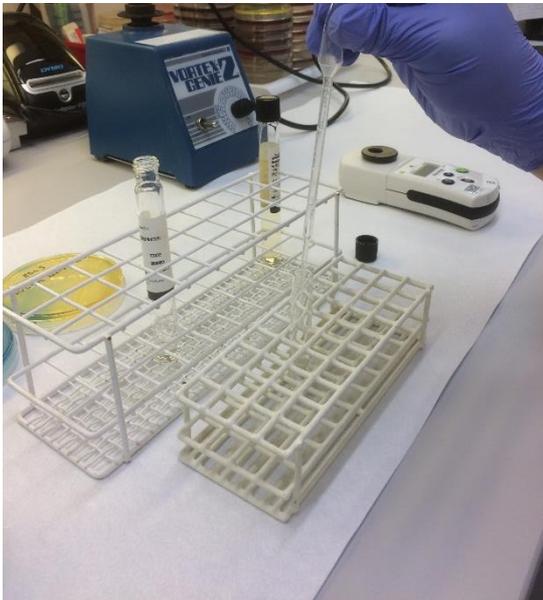
Thermo
demineralized water
tube is

too thick

for Densichek

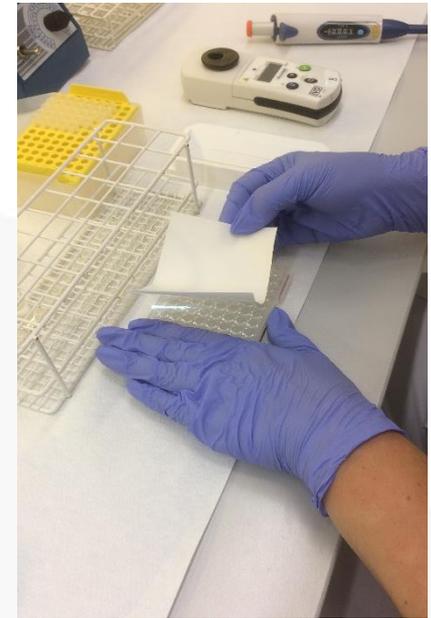
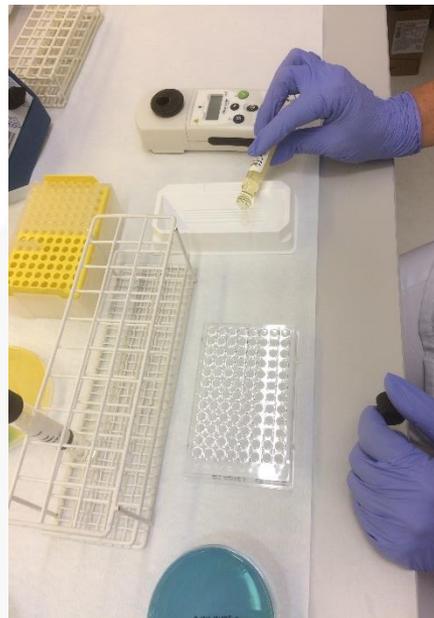
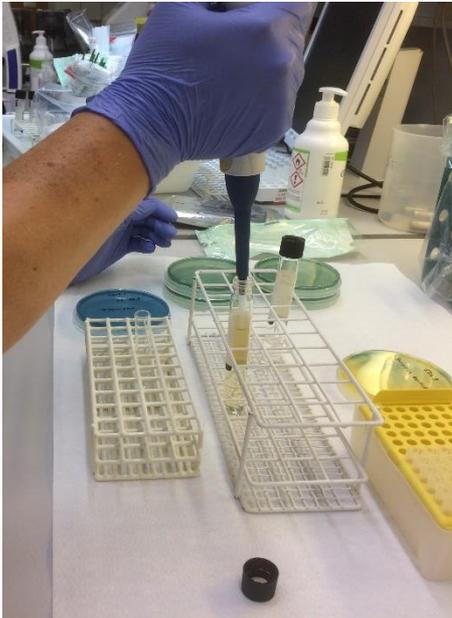


... so 2 ml of demineralized water is transferred into a smaller sterile tube to prepare the 0,5 McFarland suspension.



10 μ l of vortexed suspension to Müller-Hinton medium tube.

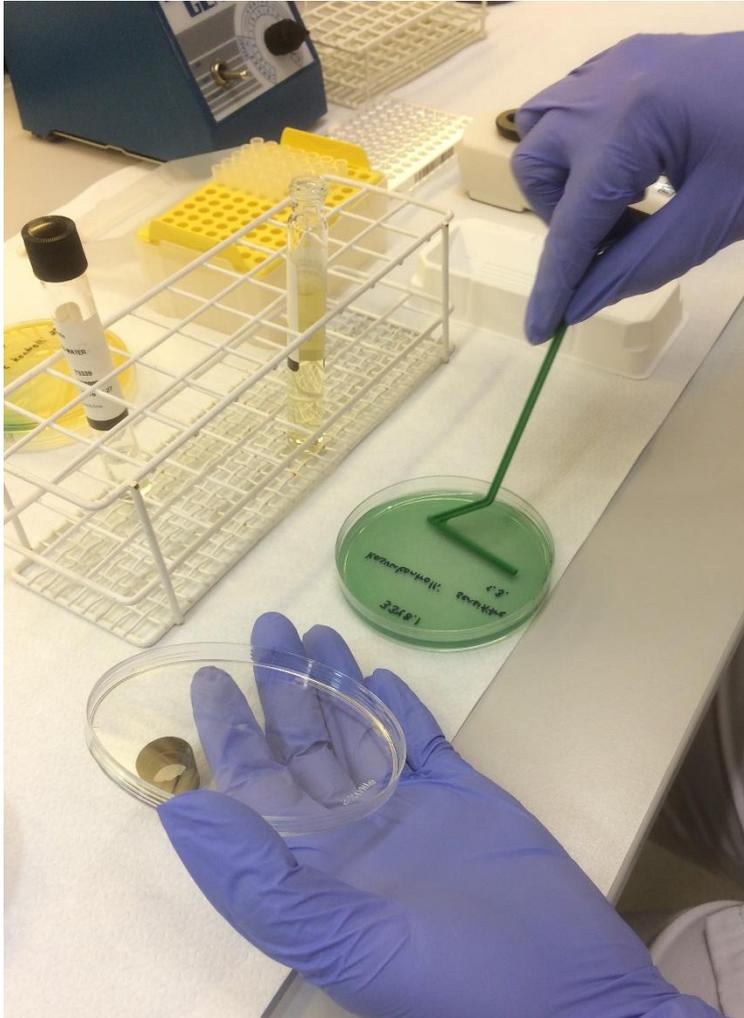
Vortexing again... then 50 μ l to each well.



Be careful not to touch the bottom of the wells or to produce air bubbles.

Cover with adherent plastic and incubate +35°C ambient air 18-24h.

Technical quality controlling



Purity check plate:

1 μ l from the positive control well on agar plate

Growth control plate:

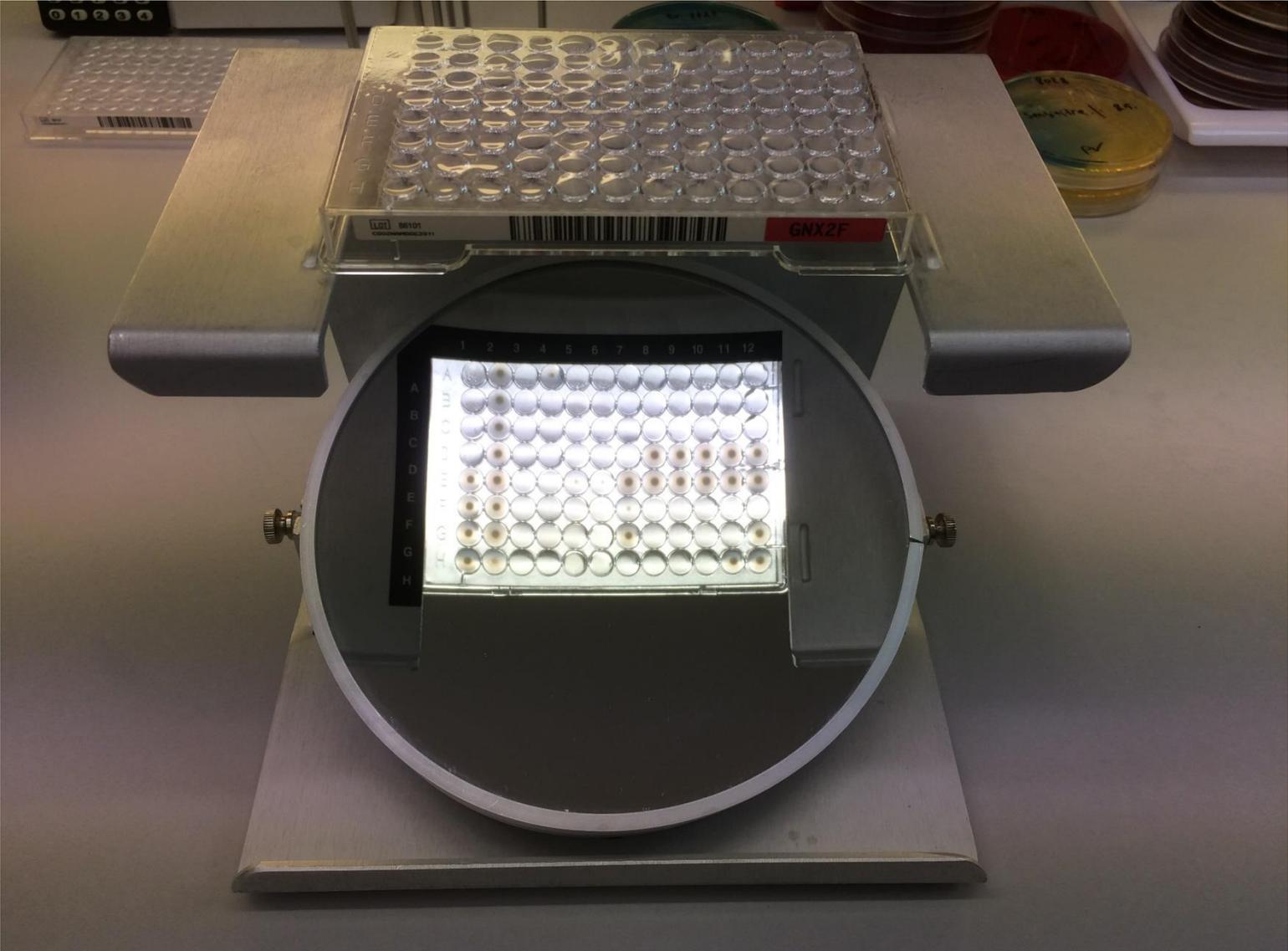
100 μ l of the left-over suspension into a new Müller-Hinton broth tube \rightarrow vortex \rightarrow 100 μ l on agar plate

Should yield around 100 cfu

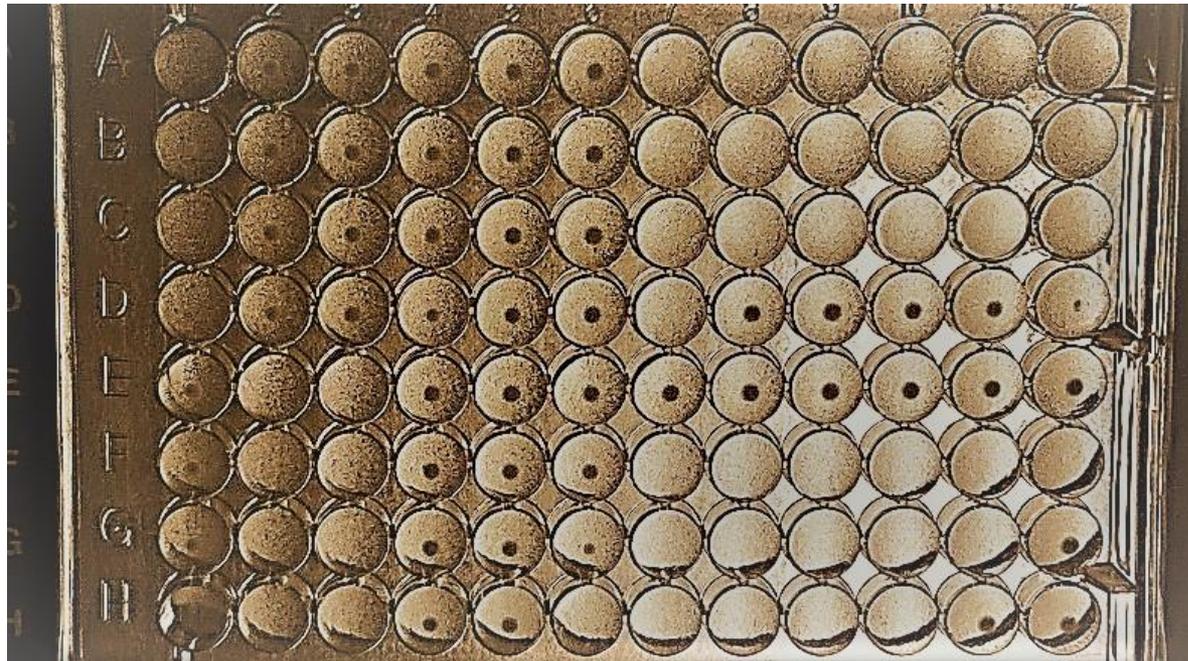
Some sweet dreams
recommended



Demo day 2



Manual reading on the mirror table

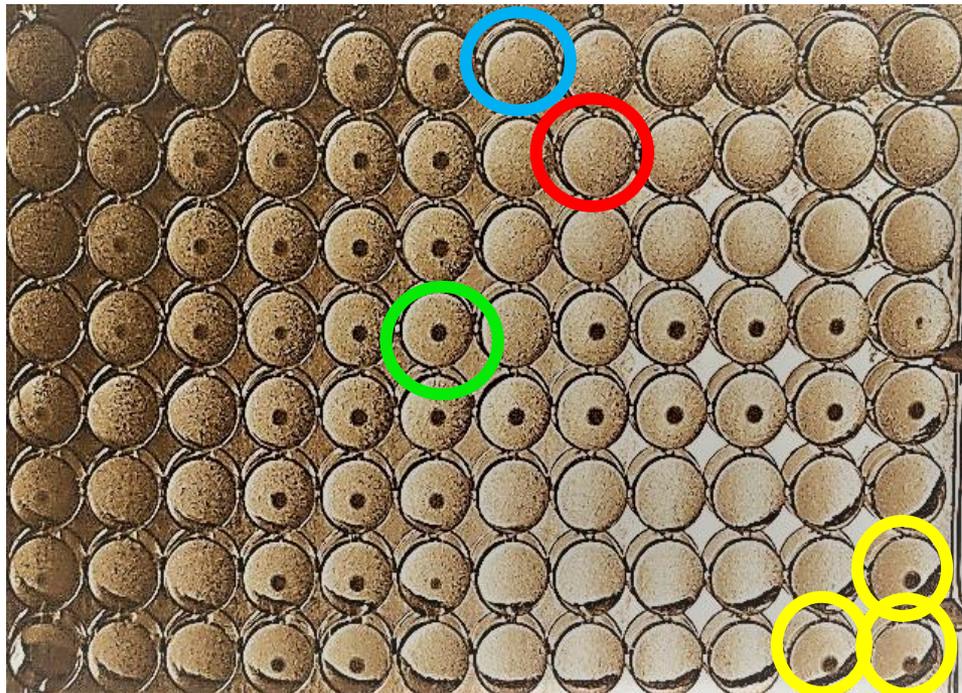


Reading guide

The MIC is the lowest concentration of antimicrobial that inhibits visible growth

For sulphonamides the MIC must be read as an 80-90% decrease in growth compared with the control well.

Occasionally a “skip” may be seen - a well showing no growth bordered by wells showing growth. A single skip can be ignored. However, never read the skip well as the MIC.



AMI 4	AZT 2	SXT 0.5/9.5	FEP 2	LEVO 1	CIP 0.25	MERO 1	DOR 0.12	DOR 0.25	DOR 0.5	DOR 1	DOR 2
AMI 8	AZT 4	SXT 1/19	FEP 4	LEVO 2	CIP 0.5	MERO 2	COL 0.25	COL 0.5	COL 1	COL 2	COL 4
AMI 16	AZT 8	SXT 2/38	FEP 8	LEVO 4	CIP 1	MERO 4	POL 0.25	POL 0.5	POL 1	POL 2	POL 4
AMI 32	AZT 16	SXT 4/76	FEP 16	LEVO 8	CIP 2	MERO 8	TAZ 1	TAZ 2	TAZ 4	TAZ 8	TAZ 16
TIM2 16/2	P/T4 8/4	GEN 1	TOB 1	DOX 2	MIN 2	FOT 1	FOT 2	FOT 4	FOT 8	FOT 16	FOT 32
TIM2 32/2	P/T4 16/4	GEN 2	TOB 2	DOX 4	MIN 4	TGC 0.25	TGC 0.5	TGC 1	TGC 2	TGC 4	TGC 8
TIM2 64/2	P/T4 32/4	GEN 4	TOB 4	DOX 8	MIN 8	ETP 0.25	ETP 0.5	ETP 1	ETP 2	ETP 4	POS
TIM2 128/2	P/T4 64/4	GEN 8	TOB 8	DOX 16	MIN 16	IMI 1	IMI 2	IMI 4	IMI 8	POS	POS

Colistin MIC \leq 0.25 $\mu\text{g/ml}$

Meropenem MIC \leq 1 $\mu\text{g/ml}$

Ciprofloxacin MIC $>$ 2 $\mu\text{g/ml}$

The verification of the method for Islab use

Isolates:

- ATCC 25922 E.coli, ATCC 27853 Pseudomonas aeruginosa
- 10 isolates provided by EUCAST Development Laboratory
 - E.coli, 3 isolates
 - Klebsiella pneumoniae, 2 isolates
 - Salmonella sp., 3 isolates
 - Pseudomonas aeruginosa, 2 isolates



504 MIC results

- two individual suspensions and plates for each isolate
- each plate had several blinded readings (2-4 persons)

Summary of the results

Technical performance:

- All positive control wells on all plates were positive
- All purity check plates contained the pure isolate only
- Most growth control plates hit the target (around 100 cfu), but some low counts were detected (minimum 28 cfu)
- Skips were detected 8 times (=1,6 % of the MIC results)
- ATCC strains: all MIC results within EUCAST MIC range

Summary of the colistin results

isolate	number	colistin ref. MIC (mg/L)	Sensititre reads, plate one				Sensititre reads, plate two			
E.coli	EDL 5	8	>4	>4	>4	>4	>4	>4	>4	>4
E.coli	EDL 9	4	>4	>4	>4	>4	>4	>4	>4	>4
K.pneumoniae	EDL 25	16	>4	>4	>4	>4	>4	>4	>4	>4
K.pneumoniae	EDL 26	8	>4	>4	>4	>4	>4	>4	>4	>4
Salmonella Dublin	EDL 30	1	1	1			0.5	1		
Salmonella Newport	EDL 34	0.5	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Salmonella Dublin	EDL 36	4	>4	>4	>4	>4	>4	>4	>4	>4
E.coli	EDL 52	>32	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
P.aeruginosa	EDL 2109	1	1	1			1	1		
E.coli	ATCC 25922	EUCAST target: 0.5-1, range 0.25-2	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
P.aeruginosa	ATCC 27853	EUCAST target: 1-2, range 0.5-4	1	1	1	1	1	1	1	1

Summary of the colistin results

isolate	number	colistin ref. MIC (mg/L)	Sensititre reads, plate one				Sensititre reads, plate two			
E.coli	EDL 5	8	>4	>4	>4	>4	>4	>4	>4	>4
E.coli	EDL 9	4	>4	>4	>4	>4	>4	>4	>4	>4
K.pneumoniae	EDL 25	16	>4	>4	>4	>4	>4	>4	>4	>4
K.pneumoniae	EDL 26	8	>4	>4	>4	>4	>4	>4	>4	>4
Salmonella Dublin	EDL 30	1	1	1			0.5	1		
Salmonella Newport	EDL 34	0.5	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Salmonella Dublin	EDL 36	4	>4	>4	>4	>4	>4	>4	>4	>4
E.coli	EDL 52	>32	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
P.aeruginosa	EDL 2109	1	1	1			1	1		
E.coli	ATCC 25922	EUCAST target: 0.5-1, range 0.25-2	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
P.aeruginosa	ATCC 27853	EUCAST target: 1-2, range 0.5-4	1	1	1	1	1	1	1	1

Summary of all results (1)

isolate	number	Sensititre results	> 1 dilution from ref.MIC
E.coli	EDL 5	all MICs \pm 1 dilution from ref.MIC	
E.coli	EDL 9	all MICs \pm 1 dilution from ref.MIC	
K.pneumoniae	EDL 25	all but one MICs \pm 1 dilution from ref.MIC	Trim-sulf: MIC=2, ref \leq 0.12
K.pneumoniae	EDL 26	all MICs \pm 1 dilution from ref.MIC	
Salmonella Dublin	EDL 30	all MICs \pm 1 dilution from ref.MIC	
Salmonella Newport	EDL 34	all MICs \pm 1 dilution from ref.MIC	
Salmonella Dublin	EDL 36	all MICs \pm 1 dilution from ref.MIC	
E.coli	EDL 52	2 weird MICs --> isolate problem?	colistin: \leq 0.25, ref >32 amikacin: \leq 4 / 8; ref >32
P.aeruginosa	EDL 2109	all MICs \pm 1 dilution from ref.MIC	
P.aeruginosa	EDL 2638	all MICs \pm 1 dilution from ref.MIC	
E.coli	ATCC 25922	all MICs within EUCAST range	
P.aeruginosa	ATCC 27853	all MICs within EUCAST range	

Summary of all results (2)

isolate	number	Sensititre results	categorical agreement
E.coli	EDL 5	all MICs \pm 1 dilution from ref.MIC	100%
E.coli	EDL 9	all MICs \pm 1 dilution from ref.MIC	100%
K.pneumoniae	EDL 25	all but one MICs \pm 1 dilution from ref.MIC	100%
K.pneumoniae	EDL 26	all MICs \pm 1 dilution from ref.MIC	100%
Salmonella Dublin	EDL 30	all MICs \pm 1 dilution from ref.MIC	100%
Salmonella Newport	EDL 34	all MICs \pm 1 dilution from ref.MIC	100%
Salmonella Dublin	EDL 36	all MICs \pm 1 dilution from ref.MIC	100%
E.coli	EDL 52	2 weird MICs --> isolate problem?	colistin, amikacin \rightarrow VME pip-tazo: \leq 8, ref 16 \rightarrow mE
P.aeruginosa	EDL 2109	all MICs \pm 1 dilution from ref.MIC	cefep: 4 (mE) and 8, ref 8 ceftaz: 4 (mE) and 8, ref 8 levoflox: \leq 1 (mE), ref 2
P.aeruginosa	EDL 2638	all MICs \pm 1 dilution from ref.MIC	100%
E.coli	ATCC 25922	all MICs within EUCAST range	100%
P.aeruginosa	ATCC 27853	all MICs within EUCAST range	100%

Comments by Sensitive responsible persons

- ② Easy house-keeping: 10 plates/package, long shelf life
- ② Technically easy and simple to perform
- ② Pipette: 8-channel pipette or automated serial pipette was considered better (personal opinions differed)
- ② Air bubble problems very rare
- ② Reading:
 - *Enterobacteriaceae* easy to read: sharp endpoints (except trimethoprim-sulfamethoxazole which may sometimes be difficult)
 - *Pseudomonas aeruginosa* also easy to read but somewhat fuzzier wells
- ② Eight clinical isolates analyzed so far with no delays or problems

In the future

Keeping it up:

- regular training (EQA distributions)
- teaching the method for newcomers keeps the responsible persons fit

New plate on the way: 3 isolates on one plate / 5 antimicrobials to each
→ control strain on the same plate

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EUCAST Development Laboratory