

MIC determination of *Staphylococcus aureus* – reading and interpretation

CRL course Copenhagen April 2009

21st April (morning)

Reading of the MIC-results.

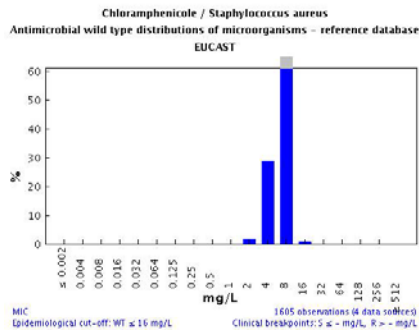
Protocol:

1. Check the purity control for contaminations
2. Check the growth control in the panel
Read panel only if growth control has growth and if weak and MICs seem low, do not read
3. Check the panel for skips in the test ranges.
4. Check pellets and growth pattern (observe if any signs of contamination)
5. Check volumes (if apparently the volume is less (due to dehydration or so- MIC will be too high)
6. Read the panel using the reading table
7. Annotate the endpoints:
consider MIC the smallest concentration causing inhibition of growth.
the pellet might be different close to endpoint (star-like pellet, absent but cloudy growth, splashed-out and apparently bigger
Sulphonamides and trimethoprim are bacteriostatic and reading differs in this case as the growth might not be totally inhibited and thus the growth inhibition should be around 20% of the growth in the control wells
8. Interpret the results using the recommended breakpoints. Results can be interpreted either using clinical breakpoints or cut-off values.

Clinical breakpoints are based on the resistance phenotype but taking in account the drug pharmacokinetic properties, to calculate the breakpoint used to predict treatment outcome whereas cut-off values are based on the distribution of MIC in the wildtype and non-wildtype populations, and are used mostly for monitoring purposes to increase the sensitivity of the detection of resistance determinants.

In this case, we will do the interpretation using the EUCAST cut-off values which are applied for resistance monitoring purposes.

For example, chloramphenicol epidemiological cut-off value is based on the distribution of the wildtype strains so, if $w_t \leq 16$, strains with $MIC > 16$ are non-wildtype



Source: www.eucast.org

Figure 1- Wildtype distribution of MIC of chloramphenicol.

In this case, we will do the interpretation using the EUCAST cut-off values which are applied for resistance monitoring purposes, using the attached reading table.

Evaluation of results

- Look at phenotype, is it expected? For example, the MRSA obtained from the screenings with selective media are expected to display resistance to beta lactam drugs.
- Are resistances observed rare?
 - Try to check for contamination
 - Check reading
 - Do some testing from the wells where growth would be unexpected
 - Check strain: purity, how long it has been on plate (culture should be fresh), morphology consistent with expected, re confirm ID with some phenotypic tests.
 - Re-test to confirm results
 - Look for possible genetic determinants that might explain the observed resistances.
- Discard contaminations
 - Purity control
 - Growth in the MIC panel
 - Resistance pattern
- Contamination can be avoided by:
 - Using pure cultures (if in doubt re-isolate)
 - Streak out well on a non selective agar so you can see if culture on plate is pure (single colonies can be observed and picked)



- Be careful when pipetting and transferring the suspension (use long tips to avoid contamination of the pipette)
 - Don't touch the tip of the dosing head when preparing for the autoinoculator
 - Perform all procedures of MIC panel inoculation in a LAF-bench and be careful not to contaminate the panel
 - Assure sterility of saline and broth.
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- Assure the quality of the testing results- Quality control procedures
 - As for any MIC determination run control strains to detect any problem with the testing procedure, the panels, the media or any deviation in results.

Results sheet- MIC panel

Panel used for: *Staphylococcus* / *Streptococcus* / *Listeria* / *Rhodococcus*

Medium: MH

Volume: 50µl pr. well

	1	2	3	4	5	6	7	8	9	10	11	12
A	PEN 0,06	PEN 0,12	PEN 0,25	PEN 0,5	PEN 1	PEN 2	PEN 4	PEN 8	PEN 16	TMP 32	SXT 16/304	SMX 512
B	TIA 0,25	TIA 0,5	TIA 1	TIA 2	TIA 4	TIA 8	TIA 16	TIA 32	CHL 64	TMP 16	SXT 8/152	SMX 256
C	ERY 0,25	ERY 0,5	ERY 1	ERY 2	ERY 4	ERY 8	ERY 16	SPE 256	CHL 32	TMP 8	SXT 4/76	SMX 128
D	TET 0,5	TET 1	TET 2	TET 4	TET 8	TET 16	TET 32	SPE 128	CHL 16	TMP 4	SXT 2/38	SMX 64
E	CIP 0,12	CIP 0,25	CIP 0,5	CIP 1	CIP 2	CIP 4	CIP 8	SPE 64	CHL 8	TMP 2	SXT 1/19	SMX 32
F	GEN 0,25	GEN 0,5	GEN 1	GEN 2	GEN 4	GEN 8	GEN 16	SPE 32	CHL 4	TMP 1	SXT 0,5/9,5	POS CON
G	FOX 0,5	FOX 1	FOX 2	FOX 4	FOX 8	FOX 16	FOX 32	SPE 16	CHL 2	TMP 0,5	SXT 0,25/4, 75	POS CON
H	FFN 1	FFN 2	FFN 4	FFN 8	FFN 16	FFN 32	FFN 64	STR 4	STR 8	STR 16	STR 32	STR 64

Code	Antimicrobial drug	Range (µg/ml)	Cut-off values (>)
FOX	CEFOXITIN	0.5-32	4
CHL	CHLORAMPHENICOL	2-64	16
CIP	CIPROFLOXACIN	0.12-8	1
ERY	ERYTHROMYCIN	0.25-16	1
FFN	FLORFENICOL	0,5-64	8
GEN	GENTAMICIN	0,25-16	1
PEN	PENICILLIN	0.06-16	0,125
SPE	SPECTINOMYCIN	16-256	128
STR	STREPTOMYCIN	4-64	16
SMX	SULPHAMETHOXAZOL	32-512	128
TET	TETRACYCLIN	0.5-32	1
TIA	TIAMULIN	0.25-32	2
TMP	TRIMETHOPRIM	0,5-32	4
SXT	TMP+SMX	0.25-16	0,5