

Rapid Etest® MIC Testing Using CHROMagar™ And Mueller Hinton Agar For Gram Positive And Gram Negative Aerobes

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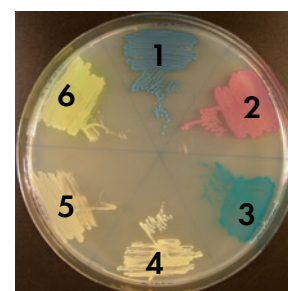
INTRODUCTION

Same day MIC results have significant clinical importance, as inappropriate and inadequate therapy of serious infections is associated with high mortality and morbidity. Rapid availability of resistant results allows de-escalation of broad spectrum empiric therapy and/or correction of therapy, saving lives and healthcare costs. Direct MIC testing of clinical specimens such as bronchial aspirates (VAP) and CSF (meningitis) with an agar-based and inoculum tolerant technique such as Etest® have been shown to be reliable and clinically useful.

We investigated the use of Etest with a prototype of CHROMagar™ (Mueller Hinton base) to generate rapid same day susceptibility results and organism identification. The colour of different species on CHROMagar (Figure 1) provides an indication of the pathogen involved.

Figure 1: Colour of major aerobes on CHROMagar

	Species	Colour
1	<i>Klebsiella pneumoniae</i>	Blue
2	<i>Escherichia coli</i>	Red
3	<i>Enterococcus faecalis</i>	Tuquoise-blue
4	<i>Staphylococcus aureus</i>	Colourless
5	<i>Stenotrophomonas maltophilia</i>	Colourless
6	<i>Pseudomonas aeruginosa</i>	Yellow-green metallic



MATERIAL AND METHODS

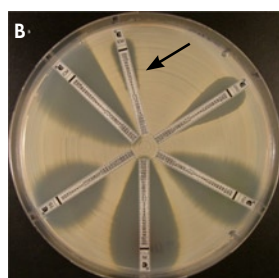
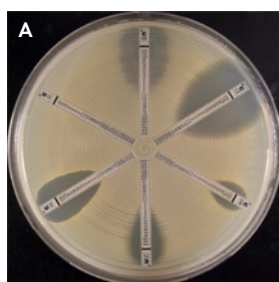
109 strains, including 9 ATCC® strains, and resistance phenotypes (MRSA, GISA/hGISA, VRE, ESBL, AmpC and OXA) comprised: staphylococci (18), enterococci (11), *E. coli* (17), *K. pneumoniae* (6), *E. cloacae* (5), *C. freundii* (5), *S. marcescens* (5), *P. aeruginosa* (16), *A. baumannii* (10), *S. maltophilia* (11), *B. cepacia* (5).

An Etest panel of 12 antibiotics for Gram + and Gram – aerobes (Figure 2) were used according to the manufacturers' instructions. Variables studied included: media (MHA vs CHROMagar); inoculum (0.5 vs 2 McFarland); incubation (6, 8, 10 and 16-18h).

Figure 2: Etest antibiotic panels

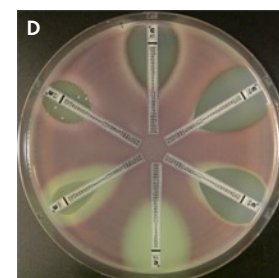
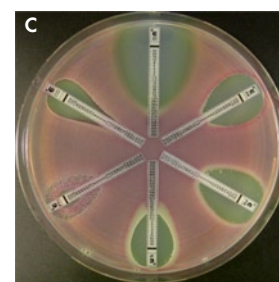
Gram positive (N29)

Antibiotic	Code
Oxacillin	OX
Vancomycin	VA
Linezolid	LZ
Daptomycin	DPC
Tetracycline	TC
Rifampicin	RI
Clindamycin	CM
Erythromycin	EM
Gentamicin	GM
Tigecycline	TGC
Trim/sulfa	TS
Ciprofloxacin	CI



Gram negative (N80)

Antibiotic	Code
Gentamicin	GM
Tigecycline	TGC
Trim/sulfa	TS
Ciprofloxacin	CI
Amikacin	AK
Tobramycin	TM
Aztreonam	AT
Piperacillin/tazo	PTc
Cefepime	PM
Cefotaxime	CT
Imipenem	IP
Colistin	CO

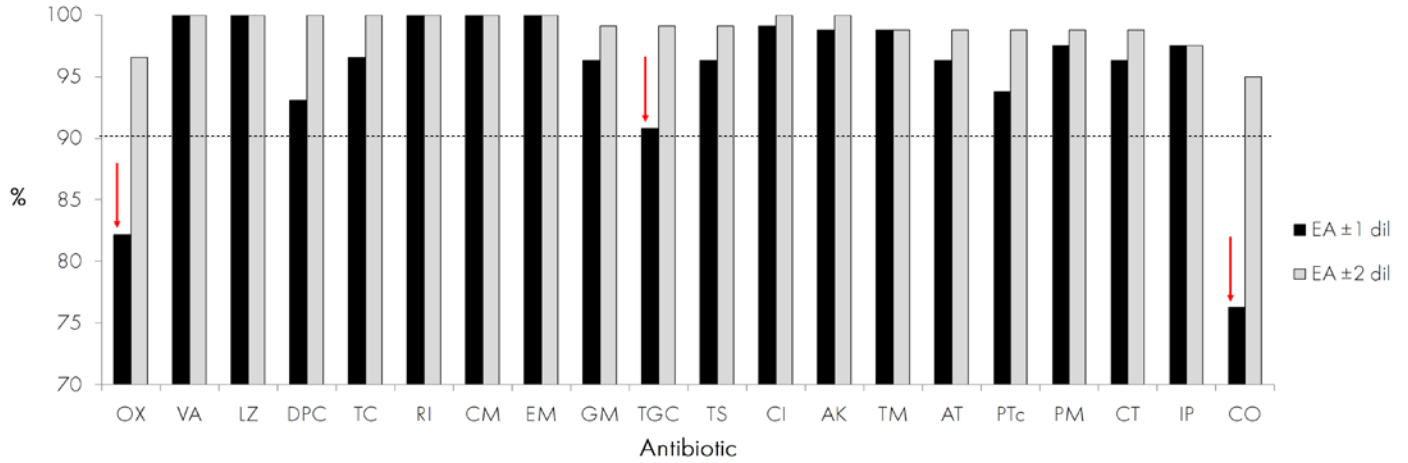


Gram + panels; EM/CM placed closer to detect MLS resistance (arrow-B)

Gram - panels; example of a mixed culture (C,D); MICs easily differentiated

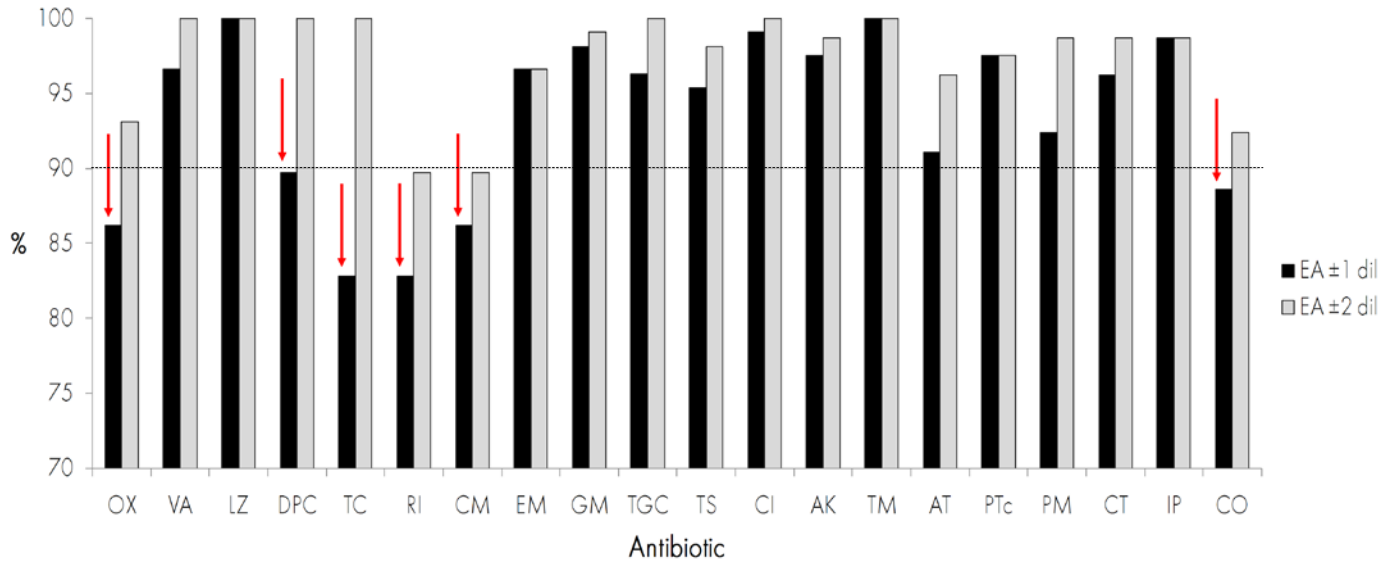
Figure 3: Different variables affecting MIC results

A: Medium CHROMagar vs. MHA (2 McF and 18h)



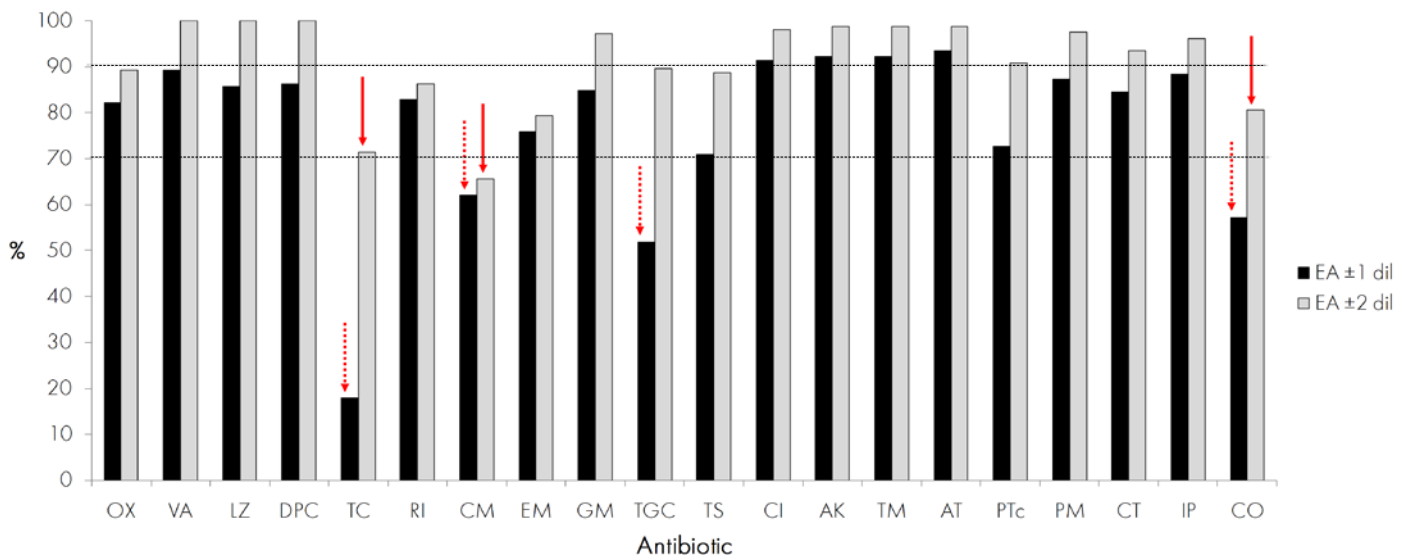
Media agreement (EA ±1 dil < 90 %) for oxacillin, tigecycline and colistin

B: Inoculum 0.5 McF vs. 2 McF (MHA and 18h)



Inoculum agreement (EA ±1 dil < 90 %) for oxacillin, daptomycin, rifampicin, clindamycin and colistin

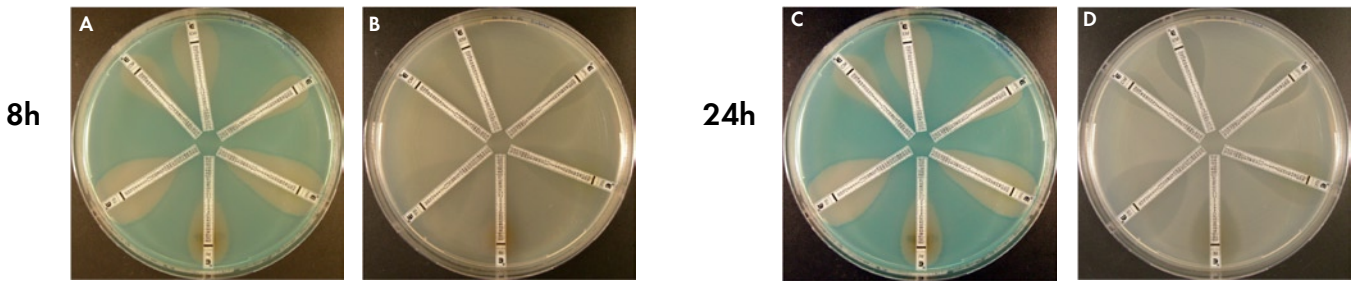
C: Rapid reading 8h CHROMagar (2 McF) vs. reference (MHA 0.5 McF/18h)



Rapid reading (EA ±2 dil < 90 %) for tetracycline, clindamycin and colistin

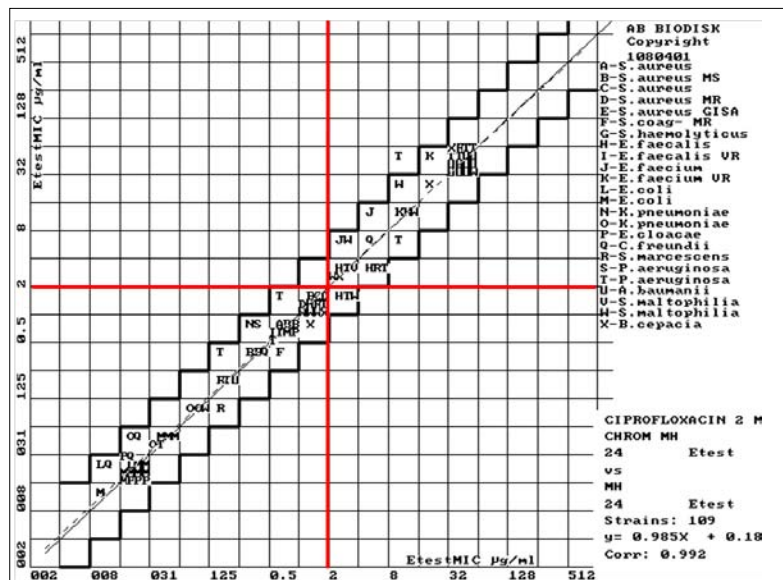
Rapid reading (EA ±1 dil < 70 %) for tetracycline, clindamycin, tigecycline and colistin

Figure 4: Rapid vs. overnight results for *E. faecalis* on CHROM vs MHA

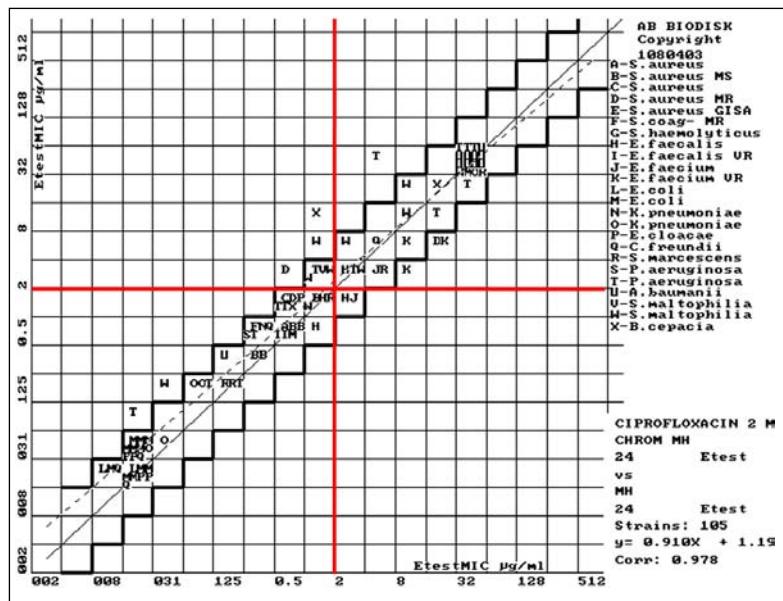


- A colour change was seen on CHROMagar after 8h for some species (Figure 4)
- MICs on CHROMagar was comparable to MHA (Figure 3A)
- Inoculum effects were minimal (Figure 3B)
- Rapid results on CHROMagar were comparable to reference MICs on MHA (Figure 3C)

Figure 5: MIC/MIC regression analysis – media and rapid reading vs. reference MICs



A: CHROMagar vs. MHA for ciprofloxacin



B: 8h on CHROMagar vs 18h on MHA for ciprofloxacin

Table 1: Category agreement for rapid reading vs. reference MICs and media comparisons

Antibiotics	8 h CHROM vs. reference MIC				CHROM vs. MHA – 2 McF (overnight)			
	CA %	VM	MA	MI	CA %	VM	MA	MI
Oxacillin	96.4	1			100			
Vancomycin	92.9			2	100			
Linezolid	100				100			
Daptomycin ¹⁾	86.2	1	3		96.6		1	
Tetracycline ²⁾	85.7	2	2		100			
Rifampicin	93.1	2			100			
Clindamycin	93.1	1		1	100			
Erythromycin	96.6	1			100			
Gentamycin	100				100			
Tigecycline ³⁾	-				-			
Trim/sulfa ²⁾	93.8	2	3		100			
Ciprofloxacin	97.1		1	2	100			
Amikacin	98.7			1	100			
Tobramycin	100				100			
Aztreonam	98.1			1	100			
Piperacillin/tazo	88.7	3		3	96.3	1	1	1
Cefepime	98.7		1		100			
Cefotaxime	94.8			4	100			
Imipenem	94.8	3	1		98.8		1	
Colistin ⁴⁾	79.2	16	1		95.0	3	1	

CA= Category agreement, VM= Very major, MA= Major, MI= Minor

¹⁾ Some calcium effects were seen for daptomycin

²⁾ Bacteriostatic agents (tetracycline & trim/sulfa) gave lower MICs at 8h compared to 18h

³⁾ No CLSI[®] breakpoints currently available for tigecycline

⁴⁾ Inducible resistance to colistin (*S. marcescens* & *S. maltophilia*) was not detected at 8h

CONCLUSIONS

- Etest used with a heavier inoculum on CHROMagar can provide same day results.
- Etest + CHROMagar can provide MIC results and species identification.
- Inoculum variations had minimal effects on Etest results.
- Media effects were antibiotic specific e.g. cation sensitive agents can be affected.
- Bacteriostatic agents can give underestimated MIC results with rapid reading.
- Inducible resistance (e.g. colistin) may go undetected with early reading.
- Etest specimen testing and rapid reading on CHROMagar deserves further study.