Pre-diffusion Method (2 + 18 or 2+22 hours) for Antimicrobials Diffusing Poorly on Agar

High molecular weight antimicrobials (Vancomycin, Teicoplanin, Daptomycin, Colistin) diffuse poorly on agar media, resulting in difficult to interpret results when using the disc diffusion method. Rosco Diagnostica has developed a 2+18 or 2+22 hour prediffusion technique, permitting an easier differentiation between susceptible and resistant strains when testing against these antimicrobials.

Procedure

One Neo-Sensitabs of the antimicrobial to be tested is placed on an uninoculated plate containing the susceptibility test medium (Mueller-Hinton plain or BHI Agar + 5 % blood).
The plate is then placed in the incubator at 37°C for 2 hours. After 2 hours the tablet (disc) is removed (by knocking the plate against the table) and the plate is maintained at room temperature for further 18 or 22 hours (overnight).

The plate is now inoculated with the strain to be tested using a McFarland 0.5 inoculum.

Additional antibiotic discs (Neo-Sensitabs) may be added now (in different locations on the agar plate) using a dispenser (if MH agar is used) and thereafter the plate is incubated overnight at 35-37°C.

The zones of inhibition are then measured. Zone breakpoints are tentative and for research use only.

Notice: In the laboratory, the prediffusion plate can be prepared the day before it is inoculated, in which case there is no loss of time and results are obtained within 24 hours.

Interpretation

IA) Detection of Visa/GISA/hVISA strains (medium BHI + 5 % blood or MH plain

VISA/GISA/hVISA strains will show the following zones of inhibition:

BHI+5% blood			MH plain
Teicoplanin 30 µg:	inhibition zone < 20 mm	and/or	Teicoplanin R< 20 mm
Vancomycin 30 µg:	inhibition zone < 20 mm		Vancomycin R≤ 20 mm

IB) Detection of GISCN/hGISCH strains (medium BHI + 5 % blood)

Teicoplanin 30 µg: inhibition zone < 20 mm

GISCN = Glycopeptide intermediate staphylococci, coagulase negative (2).

Strains showing zones of inhibition < 20 mm around Teicoplanin 30 µg should be reported as heteroresistant to both Teicoplanin and Vancomycin.

IC) Detection of VanA, VanB and VanC in enterococci

Use Mueller-Hinton Agar (without blood added), McFarland 0.5 inoculum.

Vancomycin 30 µg (2+18 hours' prediffusion method):

Susceptible:	zone > 16 mm (sharp edge)
VanB:	zone < 16 mm (hazy edge)
VanC:	zone < 12mm (sharp edge)

The VanA genotype will show **no zone** of inhibition in the current diffusion test with Vancomycin 30 µg Neo-Sensitabs.

ID) Detection of VRE, vanB phenotype and vanA genotype

Use Mueller-Hinton Agar, Mc Farland 0.5 inoculum

Vancomycin 30 ug: no zone

Teicoplanin 30 ug: zone < 16 mm (MIC 4-12 ug/ml). Report as R

II) Daptomycin testing (medium used Mueller-Hinton plain)

a) Staphylococci:

Daptomycin 30 µg Neo-Sensitabs: Susceptible zone ≥ 22 mm (corresponding to an MIC of ≤ 1 µg/ml). Resistant zone <20 mm (MIC ≥ 2 µg/ml)

b) Enterococci:

Daptomycin 30 µg Neo-Sensitabs: Susceptible zone ≥12 mm (corresponding to an MIC of ≤ 4 µg/ml).

III) Colistin testing (medium used Mueller-Hinton plain)

Colistin 10 µg Neo-Sensitabs: Susceptible zone ≥15 mm (corresponding to an MIC of ≤ 2 µg/ml).

References:

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4) Borda N. et al: Comparison of methods: diffusion (DF), prediffusion (PDF) and E-test on isolates of Ac. baumannii-calcoaceticus complex (Abc) against colistin. 2007 (in press).
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6) Koeth L. et al: Multisite evaluation of the Daptomycin Neo-Sensitab prediffusion method against 20 S. aureus. ECCMID, Milan 2011.
7) Boyen F. et al: Disk prediffusion is a reliable method for testing Colistin susceptibility in porcine E. coli strains. Vet. Microbiol. **144**, 359-362, 2010.

Note:
Detailed description of the prediffusion methods for Vancomycin, Teicoplanin, Daptomycin and Colistin see: "Detection of resistance mechanisms using Neo-Sensitabs and Diatabs" documents 7.1.0; 7.2.0; 7.3.0; 7.4.0 and 7.5.0.