High molecular weight antimicrobials (vancomycin, teicoplanin, daptomycin, colistin) diffuse poorly on agar media, resulting in difficulties in the interpretation of results when using the current disc diffusion method.

As a consequence, CLSI do not recommend the current diffusion method for vancomycin with staphylococci, daptomycin with staphylococci and enterococci and colistin with gram negative rods.

Teicoplanin has not yet been evaluated, but we expect it will perform like vancomycin.

ROSCO Diagnostica has taken on the investigation of this problem and has developed a 2 + 18 hours (or 2 + 22 hours) prediffusion technique, permitting an easier differentiation between susceptible and resistant strains when testing against these antimicrobials.

Detection of VISA, GISA, hVISA, VRE, Staphylococci Daptomycin Resistant, and Acinetobacter/Pseudomonas Colistin Resistant, using the Neo-Sensitabs prediffusion method.

S. aureus CB 182, Susceptible strain.
V = Vancomycin, T = Teicoplanin, D = Daptomycin
The principle of the prediffusion technique, was developed by a Danish microbiologist Frølund-Thomsen, several decades ago.

The idea is to give the high molecular weight antimicrobial a longer period of time to diffuse into the agar before bacterial growth takes place.

In a current disc diffusion minute colonies of growth are visible after approximately 8 hours incubation at 35 degrees. As a consequence the antimicrobial has only approximately 8 hours to diffuse into the agar, because when minute colonies are formed, further antimicrobial diffusion will not affect the size of the inhibition zone.

When using the 2 + 22 hours prediffusion the antimicrobial has 8 + 24 = 32 hours to diffuse into the agar, i.e. 4 times more than with the current diffusion method.

This results in a much larger zone size difference between 2 consecutive MIC values approximately 5 mm with the prediffusion method compared to 1.0-1.5 mm with the current disc diffusion method.

Another important point is that with the prediffusion method, the antimicrobial depot is eliminated after 2 hours.

From this moment no further antimicrobial is added to the agar and the antimicrobial that has diffused during the 2 hours will continue further diffusion without any pressure from the depot.

_S. aureus ATCC 700698 Daptomycin, susceptible hVISA strain._
PROCEDURE

1. One Neo-Sensitabs of the antimicrobial to be tested is placed on an uninoculated plate containing the susceptibility test medium, currently Mueller-Hinton Agar.

2. After 2 hours at room temperature, the tablet (disc) is removed (by knocking the plate against the table), but prior to this a short name (VAN or TEI or DAP or COL) is written on the back of the plate in order to make it possible to identify the antimicrobial.

3. Now the plate is maintained at room temperature for a further 18 to 22 hours (overnight).

4. The plate is now inoculated with the strain to be tested using a McFarland 0.5 inoculum. Additional antimicrobial discs (Neo-Sensitabs) may be added now, using a dispenser and thereafter the plate is incubated overnight at 35 degrees C.

5. The zones of inhibition are then measured and compared with the corresponding zone breakpoints.

Please note:
In the laboratory the prediffusion plate can be prepared the day before it is inoculated to avoid loss of time and results are available within 24 hours. Surplus of prediffused plates may be kept in the refrigerator for another 24 hours.

S. aureus ATS 403, GISA strain, Daptomycin susceptible.
DETECTION of VISA, GISA and hVISA
VISA, GISA and hVISA strains will show the following zones of inhibition using Mueller Hinton Agar and McFarland 0.5 inoculum and the prediffusion method:

Teicoplanin 30 ug inhibition zone < 20 mm and/or
Vancomycin 30 ug inhibition zone ≤ 22 mm

Please notice that Teicoplanin in general is the most sensitive drug to detect these isolates.

The current MIC methods and automatic systems are unable to detect hVISA strains, because they use too small inocula and consequently cannot detect heteroresistant isolates.

In the laboratory it may be useful to test vancomycin, teicoplanin and daptomycin together in order to be able to detect hVISA/VISA strains first and thereafter in order to find the best drug for treatment of MRSA and hVISA/VISA infections.

An 9 cm Mueller-Hinton Agar plate will be adequate for testing the 3 antimicrobials by the prediffusion method.

DETECTION of COAGULASE NEGATIVE GLYCOPEPTIDE INTERMEDIATE STAPHYLOCOCCI
The same procedure as above is used for Teicoplanin 30 ug.

Isolates showing zones of inhibition < 20 mm with Teicoplanin 30 ug (prediffusion method) should be reported as resistant to teicoplanin and possibly heteroresistant to vancomycin.

DETECTION of ENTEROCOCCI, VanA, VanB and VanC
Use Mueller-Hinton Agar (no blood added) and McFarland 0.5 inoculum.

Vancomycin 30 ug: 2+18 (or 2+22) hours prediffusion.

Susceptible: zone > 16 mm (zone sharp edge)
VanB: zone < 16 mm (zone hazy edge)
VanC: zone < 16 mm (zone sharp edge)
VanA: no zone of inhibition.

The VanA genotype will show no zone of inhibition in the current diffusion test with Vancomycin 30 μg Neo-Sensitabs.
DETECTION of VRE, VanB phenotype AND VanA genotype
Use Mueller-Hinton Agar and McFarland 0.5 inoculum.

Vancomycin 30 ug and Teicoplanin 30 ug (prediffusion method).

Vancomycin 30 ug: no zone
Teicoplanin 30 ug: zone < 20 mm (MIC 4 – 12 ug/ml).

DETECTION OF DAPTOMYCIN NON-SUSCEPTIBLE STAPHYLOCOCCI/ENTEROCOCCI
Use Mueller Hinton Agar and McFarland 0.5 inoculum and prediffusion method 2 + 18 (or 2 + 22 hours).

Staphylococci
Daptomycin 30 ug Neo-Sensitabs: Susceptible zone >= 22 mm (corresponding to MIC <= 1 ug/ml)

Enterococci
Daptomycin 30 ug Neo-Sensitabs: Susceptible zone >= 12 mm (corresponding to MIC <= 4 ug/ml)

DETECTION of COLISTIN RESISTANT ACINETOBACTER and P. AERUGINOSA
Use Mueller-Hinton Agar, McFarland 0.5 inoculum) and prediffusion method 2 + 18 (or 2 + 22) hours.

Colistin 10 ug Neo-Sensitabs: Susceptible zone >= 15 mm (corresponding to MIC <= 2 ug/ml).

S. aureus AST 408 GISA strain Daptomycin non-susceptible.
PRODUCTS MENTIONED IN THE BROCHURE INCLUDING REF NO:

NEO-SENSITABS

<table>
<thead>
<tr>
<th>Product code</th>
<th>CLSI potency Neo-Sensitabs</th>
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<tbody>
<tr>
<td>62812</td>
<td>Vancomycin 30 μg</td>
</tr>
<tr>
<td>61912</td>
<td>Teicoplanin 30 μg</td>
</tr>
<tr>
<td>80312N</td>
<td>Daptomycin 30 μg</td>
</tr>
<tr>
<td>41811</td>
<td>Colistin 10 μg</td>
</tr>
</tbody>
</table>

REFERENCES


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).