DETECTION OF VISA, GISA AND hVISA USING THE NEO-SENSITABS PREDIFFUSION METHOD

1. One Neo-Sensitabs of each Vancomycin 30 µg and Teicoplanin 30 µg are placed on an uninoculated plate containing the susceptibility test medium 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) 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.

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

S. aureus CB 182, Susceptible strain. V = Vancomycin, T = Teicoplanin, D = Daptomycin

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.

Interpretation

DETECTION of VISA, GISA, hVISA and hGISA

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:

hVISA, hGISA	**VISA,GISA**
Teicoplanin 30 ug inhibition zone < 20 mm **OR** Vancomycin 30 ug inhibition zone ≤ 22 mm	Teicoplanin 30 ug inhibition zone < 20 mm **AND** 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.

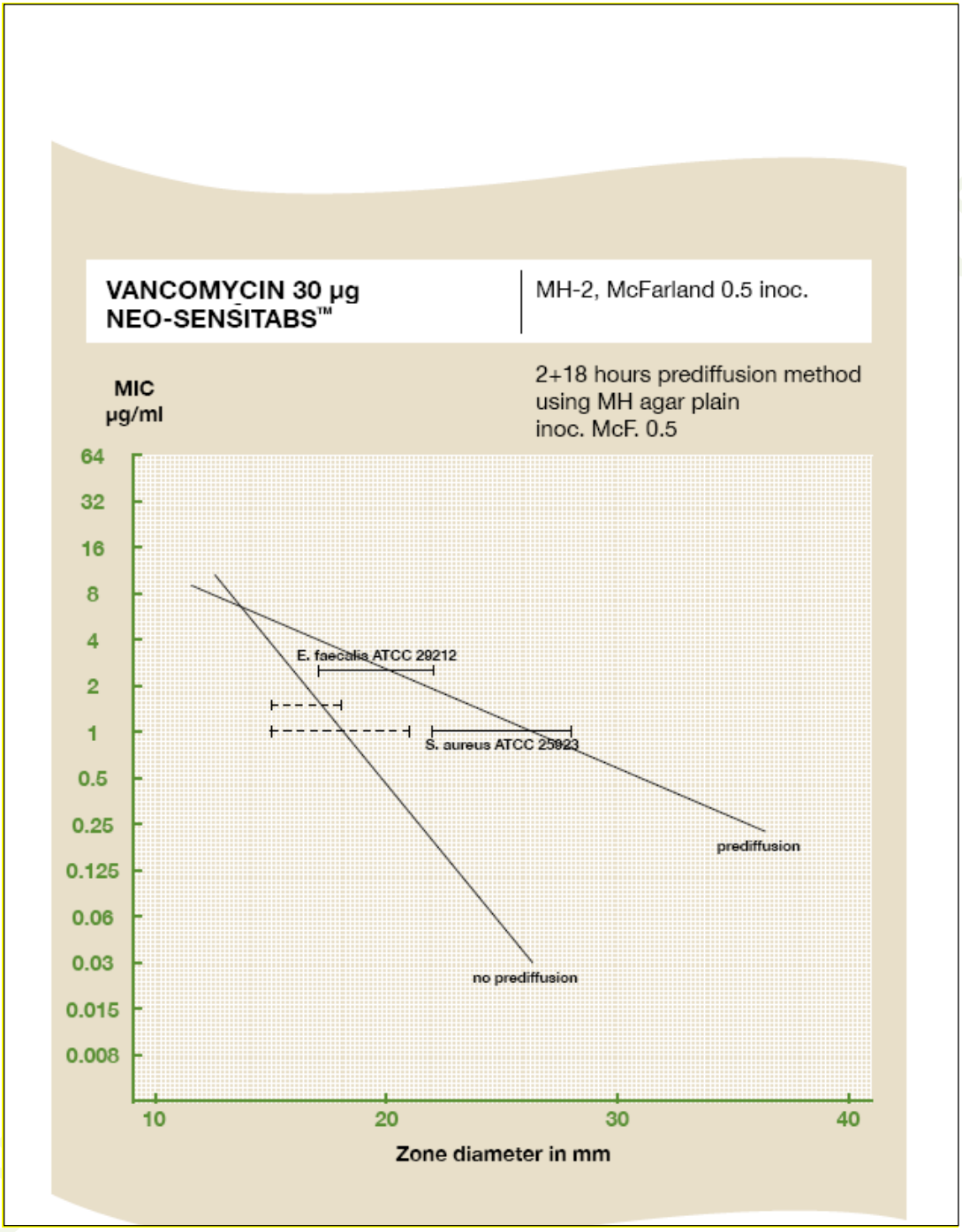
A 9 cm Mueller-Hinton Agar plate will be adequate for testing the 3 antimicrobials by the prediffusion method.

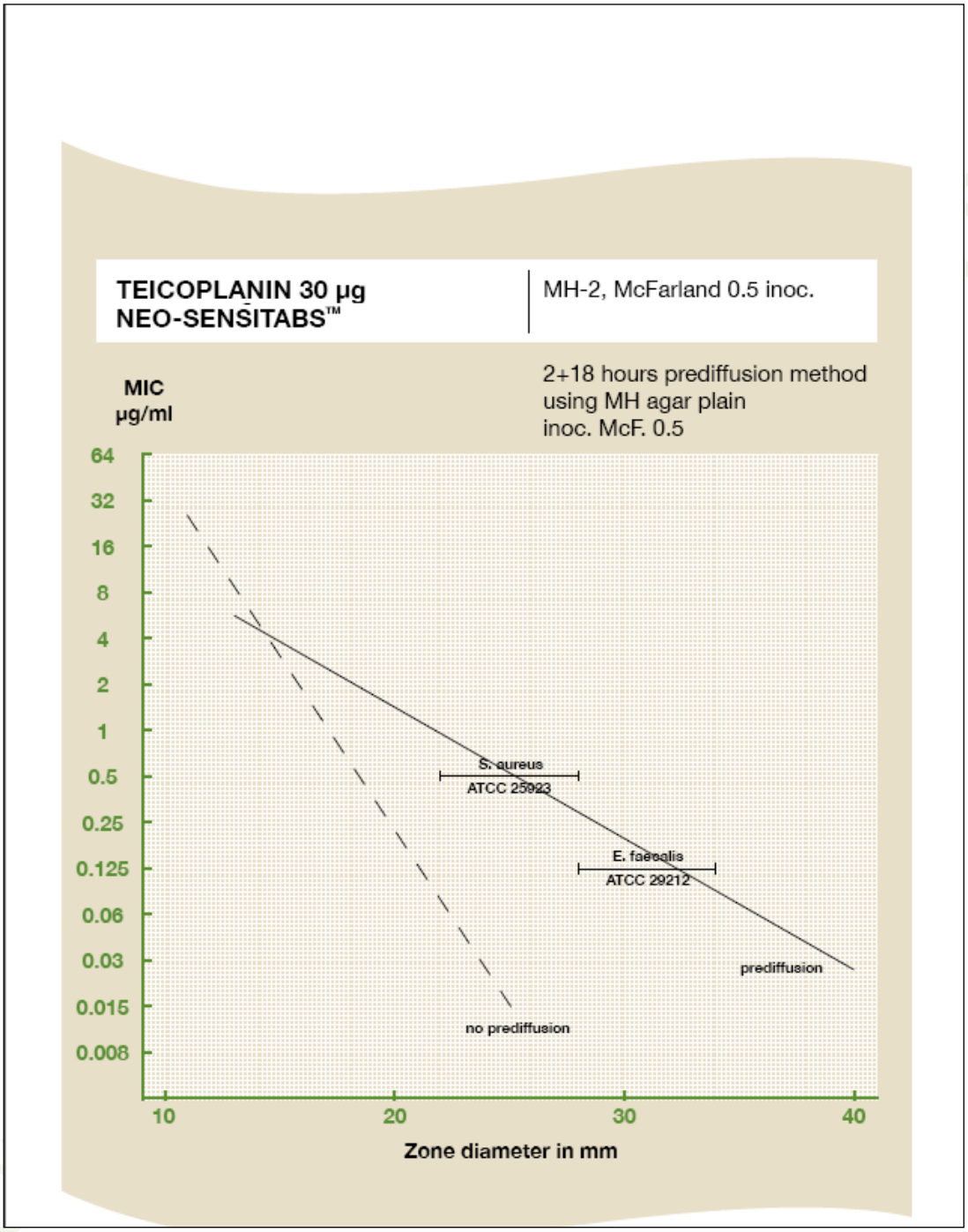
S. aureus ATCC 700698 Daptomycin, susceptible hVISA strain.

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.

S. aureus ATS 403, GISA strain, Daptomycin susceptible.


VANCOMYCIN 30 µg
NEO-SENSITABS™
MH-2, McFarland 0.5 inoc.
MIC
µg/ml
2+18 hours prediffusion method
using MH agar plain
inoc. McF. 0.5
64
32
16
8
4
2
1
0.5
0.25
0.125
0.06
0.03
0.015
0.008
E. faecalis ATCC 29212
S. aureus ATCC 25923
prediffusion
no prediffusion
10
20
30
40
Zone diameter in mm


TEICOPLANIN 30 µg
NEO-SENSITABS™
MH-2, McFarland 0.5 inoc.
MIC
µg/ml
2+18 hours prediffusion method
using MH agar plain
inoc. McF. 0.5
64
32
16
8
4
2
1
0.5
0.25
0.125
0.06
0.03
0.015
0.008
S. aureus
ATCC 25923
E. faecalis
ATCC 29212
prediffusion
no prediffusion
10
20
30
40
Zone diameter in mm

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