Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs ™

Prediffusion method (2+18 or 2+22 hours) for antimicrobials diffusing poorly on agar

Introduction

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.

Principle

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 (see enclosed regression lines)

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.