Antimicrobial Susceptibility Studies with Varioskan

This application note describes the optimization of antimicrobial susceptibility test using Thermo's Varioskan spectrophoto/spectrofluorometer. The possibility to monitor bacterial growth during 24 hour incubation at +37°C with continuous optical density measurement is shown. Also evaporation and condensation effect during the incubation is considered.

For more information contact:
info.microplateinstruments@thermo.com
USA: 866-9-THERMO

www.thermo.com

Kari Kreander (M.Sc., microbiol.), Laura Riihimäki (M.Sc., pharm.) and Pia Vuorela (Ph.D., pharm.), Viikki Drug Discovery Technology Center, Faculty of Pharmacy, University of Helsinki, Finland

Abstract

Successful discovery of novel natural product antimicrobials has necessitated the development of new bioassay techniques and protocols that allow the detection of small amounts of biologically active chemicals, which should be selective enough to determine optimum target pathogens (Ranta et al., 2000, Ojala et al., 2000, Fyhrquist et al., 2002). For fast screening of new active compounds, microplate method has shown to be the best technique (e.g. Eloff 1998).

In 96 well cell based method the antimicrobial effect of studied compound can be measured as function of time using optical density measurements (Skyttä and Mattila-Sandholm, 1991). However, condensation of water onto the microplate lid has caused difficulties in continuous measurements. This article shows that when Thermo Varioskan is used, it is possible to make long time measurements as the instrument heat the lid to the slightly higher temperature than the other parts of the plate thus avoiding condensation.

Experimental

96 well microplates were used in all the tests. The first aim was to evaluate the evaporation of water from the microwells during 24 h measurement at +37°C using Thermo Varioskan. For the test 300 µl water/well was added, the temperature adjusted to +37°C and the measurement was performed for 24 h as described below. The lid of the microplate was left on (this is obligatory in long time cell measurements) to evaluate possible condensation of water onto the lid.
When bacteria were included, an

overnight grown *Streptococcus pyogenes* ATCC 12351-strain was diluted to 1:10 in nutrient broth, and 260 µl/well was applied. Then 40 µl of diluted antibiotic was added in each well and 40 µl H_2O in control wells instead of antibiotic. The sample plate with lid on was incubated at +37°C inside the instrument and measured using 620 nm wavelength in 10 min cycle for 24 h. Before every measurement the plate was shaken for 1 second (240 rpm).

Results and Discussion

According to the results the evaporation in edge wells of the microplate is significant (Figure 1), whereas the inner wells do not show significant loss of water. The measurements can be performed during 24 h with the lid on but it is recommended to ignore the results of the edge wells. However, it is important to fill the edge wells, because otherwise the edge effect would move in to the second row. The bacterial growth was easy to measure for 24 h (Figure 2) and in the susceptibility tests of antibiotic compounds the differences between control wells and antibiotic wells were easily detectable. As a result, Thermo Varioskan is suitable for susceptibility evaluation of antimicrobials on bacteria.

References

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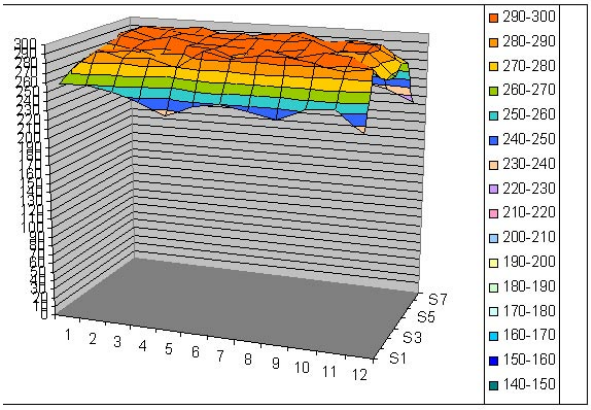

Figure 1. Evaporation of water in 96 microplate during 24 h incubation at +37°C

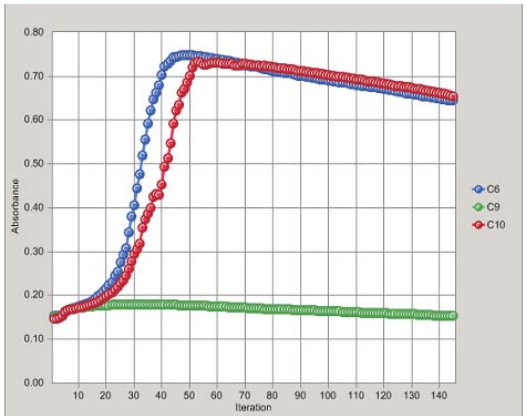

Figure 2. Growth curve for *Streptococcus pyogenes* ATCC 12351. C6; control (no antibiotic), C10 ; 0.0133 µg/ml erythromycin C9 ; 0.133 µg/ml erythromycin. X-axis describes measurements at 10 min intervals.

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