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Journal of Microbiological Methods 66 (2006) 381–389
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

Real-time monitoring of antimicrobial activity with the multiparameter microplate assay

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Received 5 October 2005; received in revised form 5 January 2006; accepted 9 January 2006
Available online 17 February 2006

Abstract

Kinetic measurements of the bacteriostatic, bactericidal and bacteriolytic activities of six model antibiotics (ampicillin, erythromycin, nalidixic acid, polymyxin B, tetracycline, and trimethoprim) against *Escherichia coli* as target bacteria were performed by bioluminescence, fluorescence, and optical density based real-time assay. Additionally, plate counting was used as a control measurement. The *gfp* and insect luciferase (*lucFF*) genes were cloned into cells used for measurements to enable fluoro-luminometric detection. Bacteria were exposed to antibiotics for 10 h, and the effects of antimicrobial agents were established. Inhibitory concentration of 50% (IC_{50}), minimum bactericidal concentration (MBC), and bactericidal concentration of 50% (BC_{50}) of each antibiotic were calculated from these procedures. Bacteriostatic, bactericidal or bacteriolytic actions of each antibiotic, as well the time interval from exposure to visible effect, were readily observed from kinetic data. No significant differences were observed between data obtained with the different methods employed. Ampicillin and polymyxin B were clearly bacteriolytic, nalidixic acid and tetracycline showed bactericidal effects, and erythromycin and trimethoprim were bacteriostatic drugs. The assay has the advantage of speed and accurately discerns between lytic, cidal and static compounds. Thus, this reliable and fully automated novel kinetic assay with high sample capacity offers new possibilities for real-time detection, making it suitable for diverse high throughput screening (HTS) applications.

Keywords: Fluoro-luminometric; Killing; Multiparameter; Real-time; Viability

1. Introduction

The search for new antimicrobial agents and characterization of the effects of potential antibiotic candidates are significant issues in the modern pharmaceutical industry. Accordingly, efficient techniques to evaluate the effects of various drugs used for therapy are of particular interest. A variety of methods are employed for measuring the susceptibility of bacteria to antimicrobial agents. Plate counting is the conventional technique (Li et al., 1996; Virta et al., 1994), but does not provide results on a real-time basis. Another frequently used in vitro technique is the microdilution method (Amsterdam, 1996), in which target bacteria are distributed into a microtiter plate, followed by varying concentrations of the drug. After a suitable incubation period (commonly overnight) at appropriate temperatures, the plate is evaluated photometrically for bacterial growth, and the effects of the selected drugs are assessed. Depending on the instruments used, approximately 1×10^6 cells per well are required for photometric measurements. To achieve this cell density, several

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doi:10.1016/j.mimet.2006.01.002

hours are required, particularly if the size of inoculation is small. Alternative methods to detect the effects of antimicrobial compounds have therefore been developed. We previously showed that fluorescence and bioluminescence-based techniques are practical choices for measuring bacterial viability and killing (Lehtinen et