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**DEVELOPMENT OF A MICRODILUTION METHOD TO A EVALUATE MYCOBACTERIUM  
TUBERCULOSIS DRUG SENSITIVITY**

**Scialino G.**, 2° anno di corso del Dottorato in Farmacologia, Chemioterapia e Microbiologia, XVI ciclo; durata del Dottorato in anni: 3. Sede di servizio: Dipartimento di Scienze Biomediche, via Giorgieri 7/9 34127 Trieste, Università degli Studi di Trieste.

Tuberculosis is the leading cause of death due to a single infectious agent; there is an urgent need for screening drug as new candidates to kill multiple drug-resistant bacilli and it is furthermore necessary a rapid determination of drug susceptibility of clinical isolates.

The aim of this study was to evaluate the minimal inhibiting concentration of the front-line drugs isoniazid, rifampin, ethambutol and streptomycin on *M.tuberculosis* reference strain H37Rv by a simple coloured test employing the dye resazurin as indicator of mycobacterial growth. Resazurin is a blue and non fluorescent dye which becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells and is used as indicator for the evaluation of cell proliferation and microbial growth. The growth condition of the strain, the growth incubation time of the culture, the concentration of the bacterial inoculum, the medium used for the microdilution assay, the incubation time of the microplate before and after the addition of resazurin have been evaluated. Results were compared to those obtained by the standard agar dilution method and the accuracy of the micromethod was confirmed by viable counting from the test wells. Visual observation of colour change from blue to pink was adequate and results have been assessed after 1 week, without instrumentation for photometric and fluorimetric reading. New molecules were also evaluated by the microdilution resazurin assay for their antimycobacterial activity.

The microplate method was furthermore evaluated on 13 clinical strain of *M.tuberculosis*, including sensitive and multiple drug-resistant, and results showed agreement with those obtained by means of the reference agar-proportion method. The microdilution plate resazurin assay is a rapid, low technology based procedure, suitable for susceptibility testing of first-line and second-line antitubercular drugs and for screening new antimycobacterial compounds on *M.tuberculosis* from clinical specimens.