

TITLE: WHAT IS THE IMPACT OF 96-WELL MICROPLATE PLACEMENT IN AN INCUBATOR ON THE MINIMUM INHIBITORY CONCENTRATION?

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ABSTRACT: Temperature is considered an important variable in microbiology. Thus, it is key to control the temperature in order to run tests for growth and antimicrobial sensitivities. Sensitivity testing in microdilution is essential for the definition of an effective antifungal therapy. However, a significant parameter to its reliable and reproducible results is the development of the microorganism, a factor that is highly dependent on incubation time and temperature. Considering this aspect, the present study evaluated the relation of the influence of temperature variation on the minimum inhibitory concentration (MIC) of fluconazole by mapping the internal temperature of an incubator measured by two different layouts. Four species of *Candida* (*C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei*) were used to determine MIC. The MIC point definition was compared between visual reading and the addition of vital resazurin dye. The MICs of the four yeast species tested showed significant changes ($p < 0.01$) as well as temperatures, showing that the position within the incubator is important influencing the MIC response. The use of resazurin eliminated the *trailing endpoint* effect typically found with triazole antifungals, avoiding the misinterpretation of MIC. This study revealed the need for careful validation of the effect of different thermal zones within a laboratory incubator.

Keywords: temperature-dependence, minimum inhibitory concentration, incubation temperature, *Candida* spp., resazurin.

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