

3736751 MTMT/M : MAJOR : MEDICAL TECHNOLOGY ; M.Sc.
(MEDICAL TECHNOLOGY)

KEY WORDS : ATP / BIOLUMINESCENCE / SUSCEPTIBILITY TEST /
CRYPTOCOCCUS NEOFORMANS

ORAWAN RATANAPISANWONG : ANTIFUNGAL SUSCEPTIBILITY TEST
OF *CRYPTOCOCCUS NEOFORMANS* USING ATP BIOLUMINESCENCE ASSAY.
THESIS ADVISORS: VIRAPONG PRACHAYASITTIKUL, Ph.D., SRISURANG
TANTIMAVANICH, Ph.D. 111 P. ISBN 974-664-108-5

An ATP bioluminescence assay, which employs the luciferin/luciferase reaction, was developed and evaluated for susceptibility testing of *Cryptococcus neoformans* to fluconazole and amphotericin B. A time and ATP concentration curve was calculated on cultivation of five selected isolates. The data indicated that the suitable time for determination of antifungal susceptibility test was 12 hours. The intracellular ATP concentration and relative light unit (RLU) of antifungal-exposed cell was expressed as a percentage of their own control growth (ATP index). The value of $\leq 40\%$ and $\leq 20\%$ was indicative value for MIC of fluconazole and amphotericin B, respectively. Similarly, an ATP ratio of ≥ 7 was used to reflect a MIC of amphotericin B whenever extracellular ATP was determined.

Susceptibility to the drugs of 25 isolates of *Cryptococcus neoformans* was determined by ATP assay and compared to the broth macrodilution and the broth microdilution methods. These comparisons of MIC pairs by the two methods were evaluated to obtain percentage of agreement. For fluconazole, excellent agreements (within four fold concentrations) were observed between MIC_{ATP} and MIC₈₀ of macrodilution (92%) and microdilution (100%). Comparison between MIC_{ATP} and MIC₉₀ exhibited good agreement of 72% for macrodilution and 92% for microdilution.

Comparative MIC determined for amphotericin B was 100% between the intracellular ATP assay and the macrodilution while that of the ATP assay and the microdilution was 92%. The MIC of the extracellular provided the lower degree of agreement to both of the macrodilution (87%) and the microdilution (65%) methods.

An overall result suggested a high potential to apply the ATP bioluminescence test as an alternative for antifungal susceptibility test in clinical laboratory.