UNIVERSITI TEKNOLOGI MARA

DETERMINATION OF OPTIMAL GROWTH PHASE AND INOCULUM SIZE OF *Klebsiella pneumoniae* (ATCC 13883) FOR LONG-TERM STORAGE (STOCK CULTURES)

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Project submitted in fulfillment of the
requirements for the degree of
Bachelor in Medical Laboratory Technology (Hons)

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledge as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby declare that I have been supplied with the Academic Rules and Regulations for Under Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Klebsiella pneumoniae (K. pneumoniae) is a Gram-negative, rod-shaped, and is one of common bacteria used for teaching purposes. It is often correlated as the cause of nosocomial urinary tract, burn, and wound infections. The cost to purchase *K. pneumoniae* (ATCC 13883) stock cultures from United States of America is expensive. Since budget for education had been reduced by the government, microbiology laboratory in Centre of Medical Laboratory Technology, Universiti Teknologi MARA Puncak Alam need to reduce the cost for constant purchasing of bacterial stock cultures. Thus, this study was performed to develop proper preservation method of *K. pneumoniae* (ATCC 13883) stock cultures to enable long-term storage with maintained cells viability. The rationale of this study was to determine the recovery potential of *K. pneumoniae* (ATCC 13883) stock cultures after one month storage in 20% glycerol and cryobeads at 4°C, -20°C and -80°C. The optimal growth phase was determined by performing growth curve; meanwhile the optimum inoculum size was determined by performing colony count. Two simple preservation methods were used in this study, where stock cultures were stored in 20% glycerol and cryobeads at 4°C, -20°C and -80°C. Late exponential phase of *K. pneumoniae* was reached at three hours growth in tryptic soy broth at 35°C, where the bacterial cells were harvested at the optimal growth phase. The corresponding colony count at third hour was 4.619×10^8 CFU/ml. After one month of storage, all stock cultures of *K. pneumoniae* were successfully recovered as mucoid colonies on 5% sheep blood agar and lactose-fermenter on MacConkey agar. The stock cultures were free of contamination and the bacterial species was confirmed as *K. pneumoniae* (ATCC 13883) as results of identification and confirmation test which were A/A with gas on triple sugar iron agar, citrate positive, methyl red negative, and Voges-Proskaeur positive. In summary, all stock cultures of *K. pneumoniae* (ATCC 13883) were recovered as pure cultures after one month of storage in 20% glycerol and cryobeads at 4°C, -20°C and -80°C.

Keywords: *Klebsiella pneumoniae*, stock culture, glycerol, cryobead