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OPTIMIZATION OF STORAGE CONDITIONS FOR THE DETECTION OF HEAT SHOCK PROTEIN 70 (HSP70) FROM SALIVARY GLANDS OF FIELD COLLECTED *Aedes albopictus*

By

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Thesis Submitted in Partial Fulfillment of the Requirements for Bachelor of Medical Laboratory Technology (Hons), Faculty of Health Sciences, Universiti Teknologi MARA

2015

DECLARATION

I hereby declare that this thesis is my original work and has not been submitted previously or current for any other degree at UiTM or any other institutions.

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TABLE OF CONTENTS

ABSTRACT

OPTIMIZATION OF STORAGE CONDITIONS FOR THE DETECTION OF HEAT SHOCK PROTEIN 70 (HSP70) FROM SALIVARY GLANDS OF FIELD COLLECTED *Aedes albopictus*

Aedes albopictus is a significant vector capable of transmitting dengue virus (DENV) and Chikungunya virus (CHIKV). *Aedes albopictus* has heat shock protein 70 (HSP70) protein molecules that are receptor complexes associated with virus replication. Thus, it have been implicated in viral entry, propagation, and transmission of DENV. This study was performed to determine the optimum media and laboratory conditions for the detection of HSP70 extracted from salivary gland tissues from field collected *Aedes albopictus* mosquitoes. Proteins were subjected to a variety of temperature and media, and its 70 kDa profile, analysed by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Based on the protein profile analysis, protein band of 70 kDa was distinctly detected when preserved in phosphate buffered saline (PBS) and stored at 4°C for a period of 7 days. This protein is analogous to the protein synthesized by dengue infected *Aedes albopictus*. It is required for the entry of DENV in human cells such as in human monocytes and macrophages. Thus, further study utilising amino acid sequencing must be performed to confirm the presence of HSP70 which has been revealed by SDS-PAGE protein analysis.

Keywords: *Aedes albopictus*, salivary gland, HSP70, SDS-PAGE

CHAPTER 1
INTRODUCTION

1.1 Background of the study

The Asian tiger mosquito, also known as *Aedes* (Stegomyia) *albopictus* (Skuse), is a well recognized vector which capable of transmitting many arboviruses including dengue viruses (DENV), Chikungunya viruses (CHIKV), and recently, Zika viruses (ZIKV) as reviewed by Paupy *et al.* (2009), Grard *et al.* (2014) and Wong *et al.* (2013). However, previous findings have shown that DENV is one of the most important arbovirus which can cause mosquito-borne viral infection, particularly dengue infection which has been spread worldwide in recent years, (Paupy *et al.*, 2001; Wasinpiyamongkol *et al.*, 2012; Rohani *et al.*, 2005).

The World Health Organization has estimated the risks of the world's population is over 2.5 billion people annually (WHO, 2014) towards dengue infection. In Malaysia, as per March 2015, dengue viruses have caused 27,382 people to get sick from dengue fever since beginning of the year and 38,517 dengue cases in April as reported by World Health Organization for Western Pasific Region (WPRO, 2015). The number of dengue cases kept increasing as proved by recent data from WPRO which is the cases is 33.7% higher compared to the previous year on 2014.

As reviewed by Wasinpiyamongkol *et al.* (2012), DENV is one of the major arboviruses in which the transmission of DENV acquires the biting of an infected female *Ae. albopictus* to a human host. DENV is taken up through the proboscis of the mosquito and passed along the salivary gland region as mentioned by Rohani *et al.* (2005). The salivary gland of mosquitoes have significant roles in the transmission of DENV as the multiplication of DENV initiated in the salivary gland region (Zhang *et al.*, 2013; Wasinpiyamongkol *et al.*, 2012; Rohani *et al.*, 2005). Previous studies has reviewed on the potential of salivary gland as the site for the transmission of DENV. Thus, it is important to discover the optimum laboratory conditions for heat shock protein 70 (HSP70) isolated from field collected