OPTIMIZATION OF PRESERVATION CONDITIONS FOR THE DETECTION OF ADENOSINE DEAMINASE (ADA) ISOLATED FROM SALIVARY GLANDS OF FIELD-COLLECTED *Aedes albopictus*

By

NURUL SYAHIDA SYAMIRA BINTI RUSDI

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ABSTRACT

OPTIMIZATION OF PRESERVATION CONDITIONS FOR THE DETECTION OF ADENOSINE DEAMINASE (ADA) FROM SALIVARY GLANDS OF FIELD-COLLECTED *Aedes albopictus*

Dengue fever, caused by dengue virus (DENV) is a public health concern since it was first recognized in the 1780s. Adenosine deaminase (ADA) presents abundantly in the salivary glands of *Aedes albopictus*, the secondary vector of DENV. It is a protein essential for viral transmission into a human host following the blood-feeding process. ADA provokes the host antigenic and immunogenic responses thus favoring the transmission of DENV upon pathogenesis. Improper sample collection and handling might cause sample degradation leading to false detection of relevant proteins. Hence, this study aims to determine the optimum preservation media and storage conditions for the detection of salivary ADA extracted from *Ae. albopictus*. Samples were preserved in a number of media (i.e. phosphate buffered saline, PBS; phosphate buffered saline supplemented with protease inhibitor, PBS-Pi; cell lysis buffer, CLB) at various temperature (i.e. -20°C, 4°C and room temperature) prior to protein profile analysis by SDS-PAGE. Protein profiles revealed that all three media preserved at -20°C and 4°C can be used for salivary glands preservation as the putative protein of interest was detected at molecular weight of 53 kDa. Further experiments using affinity chromatography or protein sequencing shoul be done to confirm the identity of the putative ADA protein.

Keywords: *Aedes albopictus*, salivary glands, adenosine deaminase, SDS-PAGE

CHAPTER 1
INTRODUCTION

1.1. Background of the study

Aedes albopictus (*Ae. albopictus*), also known as 'Asian tiger mosquito' is a competent vector for a number of pathogens affecting humans including arboviruses such as dengue virus (DENV), chikungunya virus (CHIKV) and yellow fever virus (Doucoure *et al.*, 2014; Kraemer *et al.*, 2015). Nevertheless, DENV has become worldwide concern as growing of dengue cases were documented each year due to its high tolerance in adapting to new habitat (Guzman *et al.*, 2010). Four serotypes of DENV exist, namely DENV-1, DENV-2, DENV-3, and DENV-4. Serotypes vary by their antigenic groups making it hard to determine the mechanisms involved in the virus pathogenesis. Severe reaction in primary infection is expected if the host was infected with DENV-1 and DENV-3, whereas DENV-2 and DENV-4 has been reported to be frequently involved in secondary infection (Patramool *et al.*, 2011).

Infection of the female mosquito occurs during a blood feeding that supply for their nutrition, egg development, and survival on a DENV-infected host (Oktarianti *et al.*, 2015). DENV is taken up through the proboscis of the mosquito, passed along the salivary gland and multiply in the region. Following salivary gland infection, female mosquitoes are competent for DENV transmission for the extent of their lives (M. Zhang et al., 2013). Salivary gland secretes saliva that contains numerous anti-hemostatic and anti-inflammatory agents which benefit the vector since it enhances the transmission of DENV to other human hosts (Nguyen *et al.*, 2013).