OPTIMIZATION OF PRESERVATION CONDITIONS FOR THE DETECTION OF ENOLASE FROM MIDGUT TISSUES OF FIELD COLLECTED *Aedes albopictus*

By

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ABSTRACT

OPTIMIZATION OF PRESERVATION CONDITIONS FOR THE DETECTION OF ENOLASE FROM MIDGUT TISSUES OF FIELD COLLECTED *Aedes albopictus*

Aedes albopictus is a vector responsible for dengue outbreaks. The etiological agent, the dengue virus (DENV) must cross the vector's midgut epithelial cells to establish infection. In midgut tissues, enolase appears to be an important protein receptor which takes part in the entrance of DENV that can be detected by observing the protein profiles in sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Field collected samples are precious yet very fragile and can be easily degraded if not collected, handled or preserved properly. Important protein markers can remain undetected leading to false negative results. To date, the knowledge on the optimum preservation conditions of midgut proteins is currently limited, which forms the rationale of this study. Samples of *Ae. albopictus* collected from Kolam Basah 8, UiTM Puncak Alam were reared until adulthood under insectary conditions and dissected to obtain midgut tissues. The harvested tissues were then preserved in three medium namely; phosphate buffered saline (PBS), PBS supplemented with anti-protease cocktail (PBSpi), and cell lysis buffer (CLB) at -20°C, 4°C, and room temperature (RT) for 3 days. SDS-PAGE was performed after 3 days of preservation and the result revealed the protein of interest (Mr 57 kDa) at -20°C and 4°C when preserved in PBS, PBSpi, and CLB. However, the protein was not detected at RT in all 3 media. Findings of this study indicates that midgut tissues from field collected *Ae. albopictus* can be well preserved upon storage in PBS, PBSpi, and CLB media at laboratory conditions of -20°C and 4° for the purpose of enolase detection. The identity of enolase should be further confirmed in forthcoming studies using mass spectrophotometry, amino acid sequencing or Western Blot.

Keywords: *Aedes albopictus*, DENV, midgut, enolase, SDS-PAGE.

CHAPTER 1
INTRODUCTION

1.1 Background of the study

Aedes albopictus, also known as the Asian tiger mosquito is widely recognized as a vector of several viruses such as dengue viruses (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV) (Grard *et al.*, 2014). Each year, the reported cases of dengue fever has increased and has now become worldwide concern. One of the factors that contributes to its expansion is the high tolerance of DENV in adapting to new habitat. As reported by World Health Organization (WHO), the risks of the world's population is over 2.5 billion people annually due to dengue fever (WHO, 2014).

Female *Ae. albopictus* feed blood to obtain nutrients required for egg maturation and production of yolk proteins. Blood containing various pathogens, such as DENV may be taken up through proboscis during the feeding process. DENV starts to multiply as it cross midgut epithelial cells and salivary glands as these two organs are the principal organ for digestion of the blood meal (Rohani *et al.*, 2005). As reviewed by Saboia-Vahia *et al.* (2012), midgut represents one of the main immunologically active sites and the first barrier the pathogens must overcome to initiate infection in their host.

Proteins in the midgut tissues are important for the mosquito development as it provides energy, acts as a defense mechanism, take parts in metamorphosis, and is the major nutrient for oogenesis. (Saboia-Vahia *et al.*, 2012). To initiate the infection cycle, DENV must be able to attach to protein receptors of vector/host. The abundance and distribution of the receptor throughout the vector/host cell surface affects the binding of the DENV virus to these receptors (C. Cruz-Oliveira et al., 2015). A study by Muñoz *et al.*, (2013) stated that before DENV can infect other mosquito's organ, the virus must first interact with certain proteins on the midgut epithelial cells. Enolase is a 57 kDa protein receptor that appears to play a significant role in the entrance of virus into the midgut (Muñoz *et al.*, 2013). This protein often goes undetected if not handled or stored carefully. Thus, it