UNIVERSITI TEKNOLOGI MARA

THE EFFECT OF INCUBATION TIME ON ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

ANISAH BINTI HAJI MUSA

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Faculty of Health Sciences

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ABSTRACT

A typical Enzyme-Linked Immunosorbent Assay (ELISA) needs 2 to 3 hours to complete the test. The aim of this study is to analyze the effect of incubation time on ELISA. There are 3 incubation steps in ELISA procedures. Only 2 of the steps are involved in this study which are, the incubation for antibody in the serum react with anti-human IgM coated in microwells and incubation for antigen-Mab complex react with remaining antibody after washing procedure. Three incubation times which are 60 minutes, 45 minutes and 30 minutes were tested. The assay used E-DEN01M test kit with the manufacturers' protocol as the reference. Two strips of assays wells comprise of 11 samples, a negative control, a positive control, and triplicates calibrators were performed in each run. From the assay, the cut-off value (COV) was calculated. The results showed a significant difference of absorbance values for shorter incubation time. The COV reduced as the time was reduced. The test method with shorter incubation time cannot be applied as the results were invalid. The 60 minutes is the optimum incubation time for this ELISA.

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CHAPTER 1

INTRODUCTION

Enzyme-Linked Immunosorbent Assay (ELISA) is a widely known diagnostic method for the detection of antigen or antibody (Gibbs, 2001). Traditionally, the hemagglutination inhibition (HAI) assay has been used as the gold-standard serological test, but the ELISA has been proposed as a simpler and more rapid alternative (Cuzzubbo *et al.*, 1999). ELISA test is very sensitive and can be used in detecting *Salmonella* serotype Dublin in infected cattle that frequently have continuously high immunoglobulin levels in serum and milk. Therefore, ELISA that detects immunoglobulins has been suggested as good alternatives to bacteriological culture (Nielsen *et al.*, (2004). The ELISA technique is a solid-phase serological assay which can be used to detect, and if appropriate standards are run, to quantify any known antigen or antibody (Lai, 2005).

ELISA still being the reference method although the detection of antigen and antibody can be utilize from other methods. ELISA is always included in studies as reference or comparison with other techniques such as polymerase chain reaction (PCR) and dot immunobinding assay (DIA) (Wang, *et al.*, 1999). The ELISA is a highly sensitive and complex protocol that requires careful preparation of assay reagents and strict adherence to protocol (True 2001).

The ELISA test kit contains reagents and materials for antigen-antibody reaction such as Anti-human IgM Coated Microwells, Horseradish Peroxidase Conjugated