# MOLECULAR DETECTION OF AVIAN PATHOGENIC *Escherichia coli* (APEC) USING VIRULENCE GENE OF *iutA* VIA POLYMERASE CHAIN REACTION (PCR) ASSAY

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This Final Year Project Report entitled "Molecular Detection of Avian Pathogenic *Escherichia coli* (APEC) using Virulence Gene of *iutA* via Polymerase Chain Reaction (PCR) Assay" was submitted by Khairunnajwa Binti Abu Haiyan, in partial fulfilment of the requirements for the Degree of Bachelor of Science (Hons.) Biology, in the Faculty of Applied Sciences, and was approved by

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## ABSTRACT

### MOLECULAR DETECTION OF AVIAN PATHOGENIC *Escherichia coli* (APEC) USING VIRULENCE GENE OF *iutA* VIA POLYMERASE CHAIN REACTION (PCR) ASSAY

Avian Pathogenic *Escherichia coli* (APEC), which is the principal cause of morbidity and mortality in broiler chickens has caused a significant economic impact, particularly in a country that relies on broilers as the primary source of protein. The mass production of broilers in Malaysia makes it difficult to implement countermeasures for the prevention of disease transmission. This study aims to isolate and detect APEC from broiler chickens using a precisely designed primer for *iutA* gene of APEC under an optimized polymerase chain reaction (PCR) condition. The first phase of this study entailed culture-based assay, involving the isolation of *E. coli* from 35 cloacal swabs of broilers using Sorbitol MacConkey and Eosin-Methylene Blue agar. Following that, the Gram-staining showed pink-coloured, rod-shaped *Bacillus* which specified the traits of Gram-negative bacteria, whereas biochemical assay using Microgen GnA-ID kit detected seven samples as pure culture of *E. coli*. The culture-based assay, however, were tedious, laborious and time-consuming. Therefore, the molecular-based assay was approached in the second phase of the study, in which a primer specific to the *iutA* gene was designed using bioinformatic tools like BLAST, Primer3, ClustalW and *In Silico* PCR. As a result, the *iutA* gene of APEC was specifically identified using a primer that has 55-60% G-C content of 20 nucleotide bases, with the absence of primer dimer nor secondary structure. The annealing temperature of 55.6 °C and the $MgCl_2$ concentration of 6 mM were selected as the optimized condition for the PCR assay. This optimized condition was able to enhance the primer to work specifically toward the targeted sample of APEC. Subsequently, the amplicon size of 198 bp was detected via visualization of 1% agarose gel electrophoresis at 90V for 70 minutes, therefore concluding that molecular-based assay is more advantageous in terms of specificity and rapidness in detecting the virulence gene within APEC sample.

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