# UNIVERSITI TEKNOLOGI MARA

# DEVELOPMENT OF A THERMOSTABILISED PCR-BASED DETECTION KIT FOR PATHOGENIC FAMILIAL HYPERCHOLESTEROLAEMIA VARIANTS IN MALAYSIA

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Thesis submitted in fulfillment
of the requirements for the degree of
**Master of Science**
**(Medicine)**

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# AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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# ABSTRACT

Familial hypercholesterolaemia (FH) is an inherited disease that causes an elevation of plasma low-density lipoprotein cholesterol (LDL-C) level, leading to increased risk of premature coronary artery disease. Next-generation sequencing (NGS) is currently used to detect FH variants molecularly among patients. However, this method is expensive, laborious, and time-consuming. Thus, a simpler method using a tetra-primers amplification refractory mutation system (T-ARMS) PCR was developed for detection of 10 most common pathogenic variants in Malaysia. The kit was designed to detect 9 pathogenic variants of the *LDLR* gene and 1 *APOB* gene pathogenic variant. These variants were selected by analysing their pathogenicity and their frequency among molecularly confirmed FH cases from previous published and unpublished data. The ratio of inner and outer primers' concentration of each variant and the annealing temperature were optimised to achieve optimal results. The optimised PCR was then evaluated with 154 clinical samples to determine the diagnostic performance of this kit. Limit of detection (LoD) was performed using synthetic DNA targets as well as extracted patient DNA. The diagnostic performance of the kit showed 100% for sensitivity, specificity PPV, NPV and accuracy. The LoD was $1.0X10^{-2}$ ng for synthetic DNA and 10.0 ng for the extracted DNA from FH and non-FH patients. A prototype was developed by using a 96-well PCR plate with lyophilised primers of each variant dispensed into different wells. The stability of the prototype was analysed using the Q10 accelerated aging method. This method showed the kit was stable at room temperature for up to three months. This thermostabilised T-ARMS PCR prototype provides a simple-to-use kit that can be performed using a simple PCR thermocycler for the rapid screening of pathogenic FH variants. It may also be useful for molecular confirmation of FH zygosity in the regional Asian countries. Easy identification of pathogenic FH variants will allow prompt and early intervention, thus reducing the risk of coronary artery disease among the population.

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