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# SENSITIVITY AND SPECIFICITY TESTING OF A PCR BASED PHARMACOGENOTYPING METHOD FOR CYP2C9

ABDULLAH QUTUBUDDIN BIN RAMAN

**Dissertation submitted in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.)**

**Faculty of Pharmacy**

**Oktober 2007**

## ACKNOWLEDGEMENTS

First of all thanks to God and may His peace and blessings be upon all his prophets for granting me the chance and the ability to successfully complete this study. Then, I would like to express my gratitude to my supervisor Dr Teh Lay Kek, for her continuous support in completing this Research Instrumentation's project. Dr Teh had always listened and gave advice in the preparation in this project. Her wide knowledge in pharmacogenomic area has been of great value for me. Her understanding, encouraging and personal guidance have provided a good spirit to me to complete this course. Then, my thanks to Prof. Dr. Mohd. Zaki Salleh and Mr. Lee Wee Leng in helping me while doing the project. Lastly, special thanks to all postgraduate students and all my friends for their great cooperation and willingness.

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# ABSTRACT

PCR is a special technique used to amplify the DNA sequence of interest. A successful laboratory testing for PCR depends on sensitivity and specificity of PCR method itself. CYP2C9 is an important metabolizing enzyme responsible for 16% of drugs currently available in the market. This enzyme metabolized narrow therapeutic index drug such as warfarin, phenytoin and many others. This study aimed to evaluate a PCR pharmacogenotyping test kit that have been developed by the members of Pharmacogenomic Research laboratory. Two aspects of the kit were evaluated, that was the sensitivity and specificity of the test kit. DNA samples were selected randomly from the DNA bank from the laboratory. Ten DNA samples were selected for specificity test. For sensitivity test, 3 different samples with varying concentration of 0.1 ng to 1000 ng with 10 fold dilution were used. The detection limit was found to be 10 ng of DNA concentration. The kit is able to detect genetic variants of *CYP2C9* correctly. Thus, it would be useful to clinician in their attempts to personalize medicine for individual patients.

# CHAPTER 1

# INTRODUCTION

Polymerase Chain Reaction (PCR) is a sensitive technique used to amplify sequences of gene of interest *in vitro*. Prior to introduction of PCR, researchers use other techniques for gene analysis. In 1975, Southern blotting permitted rudimentary mapping of genes in unrelated individuals (insertions & deletions). In 1978, DNA sequencing required genes to first be cloned into plasmid or vectors. Gene library construction and screening could take many months and libraries had to be made for each individual analyzed. PCR was made possible by the discovery of *Taq polymerase*, the DNA polymerase that is used by the bacterium, *Thermus auquaticus* that was discovered in hot springs.

The sensitivity and specificity of PCR are major factors to optimize to get sophisticated laboratory test result. Analytical sensitivity represents the smallest amount of substance in a sample that can accurately be measured by an assay where else analytical specificity refers to the ability of an assay to measure one particular organism or substance, rather than others, in a sample (Alfred *et al.*, 1997). The PCR sensitivity can be achieved by considering some factors such as increasing concentration of primers, increasing concentration of *Taq DNA* polymerase or increasing number of cycles.