

UNIVERSITI TEKNOLOGI MARA

OPTIMIZATION OF IN HOUSE LYSIS REAGENT
CONCENTRATION TOWARDS THE
PERFORMANCE OF *Sus scrofa* DNA DETECTION
SYSTEM

NUR ARINA BINTI AHMAD SPAIN

Dissertation submitted in partial fulfillment of the requirement
for the

PHM 555
RESEARCH INSTRUMENTATION
(JULY – NOV 2007)

Faculty of Pharmacy

October 2007

ACKNOWLEDGEMENT

Alhamdulillah, all praise to Allah S.W.T., the Most Merciful and the Most Gracious. With His helps and blessings, this study has been done successfully. Praises to our Prophet Muhammad, the greatest creation who brought light and peace throughout the universe.

I would like to express my sincere gratitude to my supervisor, Madam Norazlina Ahmad and also my co-supervisor, Mr Khairul Adzfa Radzun for giving me the opportunity to run this project and spending a lot of their time giving me advices, knowledge, guidance throughout this project. If not for their helpful comments and critiques, I am sure that this thesis cannot be completed.

Special thanks to Dr. Kalavathy Ramasamy the coordinator of PHM 555, Research Instrumentation final year project for giving support and very convincing advices to me and all my classmates. I would also like to express my deepest gratitude to Professor Dr Abu Bakar Abdul Majeed, Dean of Faculty of Pharmacy for his supports and encouragement.

I am also thankful to all staff in Life Sciences Research Laboratory especially for the help and teaching in dealing with equipments, constructive advices and supervision during this project in Life Sciences Research Laboratory. I would also like to express my appreciation to my research partner, Miss Hannah Md Mahir for always being there for me and support me through ups and downs.

Last but not least, I am mostly thankful to my beloved family and all my friends for their love and support which has indeed given me the strength and motivation to stay focused throughout completing this project.

TABLE OF CONTENTS

	PAGE
TITLE PAGE	
ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iii
LIST OF FIGURES	vii
LIST OF ABBREVIATIONS	ix
ABSTRACT	x
CHAPTER ONE (INTRODUCTION)	1
CHAPTER TWO (LITERATURE REVIEW)	
2.1. <i>Sus scrofa</i> and it's detection	4
2.2 <i>Sus scrofa</i> in pharmaceutical products	6
2.3 Polymerase Chain Reaction	8
2.4 DNA Extraction	10
2.5 Cytochrome <i>b</i> genes of mitochondrial DNA	12
2.6 Species Spesific Primer	13
CHAPTER THREE (MATERIALS AND METHODS)	
3.1 Materials	16

ABSTRACT

The purpose of this study were to design an optimized DNA extraction method which can produce optimum DNA yield and purity and to link the optimized DNA extraction method and PCR reaction in order to develop a porcine DNA detection system that can be commercialized for *halal haram* authentication of pharma-cosmeceutical products. There are two testing parameters used in this study and comparison between these two testing parameters and standard parameter of in house DNA extraction method was made in terms of efficiency to extract genomic DNA. This study was begun with in house DNA extraction using the all parameters as well the standard, followed by running nucleic acid gel electrophoresis to determine the size of the extracted genomic DNA. Next, the efficiency of all parameters used was confirmed with PCR assays. PCR assays will amplify the target DNA sequence, therefore its sensitivity and specificity is very important. At the final step, nucleic acid gel electrophoresis was run to determine to size of the PCR products as well as to verify the efficiency of the two parameters. In conclusion, the two testing parameters used in this study have same efficiency as the standard parameter, and therefore can be used to replace the standard parameter. The results show that all pharma-cosmeceutical samples tested are free from porcine DNA. The efficiency of the optimized in house DNA extraction method is proven by the successive PCR assay.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Consumers nowadays are very particular and selective in what they buy, consume and used. The concept of *halal* is not limited to food only but is applicable in every aspect of man's life. The importance of this relevance cannot be overlooked. Rising area where *halal* products have made progress is pharma-cosmeceutical products. Since pharma-cosmeceutical products are manufactured mostly by non Muslim country, the status of these products is questionable and need to be verified. Although in the case of a life-threatening emergency, one is allowed to consume prohibited (*haram*) products, it does not absolve Muslims from their religious and ethical duties to seek for the lawful or *halal* products. This has lead to the increase demand of Muslim consumers to use and consumed only products that have been certified *halal*. *Sus scrofa* (porcine) is considered *haram* in Islam. Therefore, any products that contain porcine sources are *haram*. Example of pharma-cosmeceutical products that contain porcine sources are capsules, gelatine, vaccine, insulin and heparin. Therefore, it is important to develop a reliable methods and procedures for porcine DNA detection system in pharma-cosmeceutical products.