

بسم الله الرحمن الرحيم

Sudan University of Science and Technology  
College of Graduate Studies

**Detection of *Salmonella typhimurium* in Stool Specimens by Real time  
Polymerase Chain Reaction**

الكشف عن السالمونيللا التيفية الفارية في عينات البراز بتقنية تفاعل البلمرة المتسلسل الزمني

A dissertation Submitted in Partial Fulfillment for the Requirement of M.Sc.  
in Medical Laboratory (Microbiology)

By  
LEMIA MOHAMED ALHASSAN ALTYEB

Supervisor  
Dr. Humodi Ahmed Saeed

2007

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الرَّحْمَنِ \* عَلَمَ الْقُرْآنَ \*

\* خَلَقَ الْإِنْسَانَ \* عَلَمَهُ الْبَيَانَ \*

صدق الله العظيم

سورة الرَّحْمن

# **Dedication**

*My great love, regards and every things to:*

*My country*

*Family*

*Teachers*

*Friends*

*Who taught me how to be optimistic and lead me in the road of success, to whom I will always be in dept.*

*Acknowledgment*

*Firstly I would like to thank Alla for blessing me and help me in completing this work.*

*All my gratitude and thanks to my supervisor Dr. Humodi A. Saeed, who gave me a lot of his time and real knowledge and guidance.*

*Deep thanks are extended to Dr. Mojahed M. Elhassan who greatly support me through this work and provided me directions needed for completion of the study.*

*I'm also grateful to Dr. Misk Elyaman A. A. Elmeki for her technical help .*

*Thanks are also extended to my colleagues in the university and hospitals for their help and continuous support .*

*Also I would like to thank every one who gave me any sort of help and assistance.*

## **Abstract**

This study was carried out in the period from May 2007- March 2008 to detect the presence of *Salmonella typhimurium* in stool of patients who were suffering from acute gastrointestinal tract infection. Forty seven stool specimens were collected from patients who were attended to Bashair Hospital, Omdrman Teaching Hospital and Haj Alsafi Hospital during the study period.

Bacterial DNA was extracted from each stool specimen. The DNA was runned using real time PCR technique to detect *S.typhimurium*. The result revealed that only two specimens were positive.

The study concluded that the real time PCR technique facilitates detection of *S.typhimurium* without bacteriological culture.

## الخلاصة

أجريت هذه الدراسة في الفترة بين مايو ٢٠٠٧ إلى مارس ٢٠٠٨ للكشف عن وجود باكتيريا السالمونيلة التيفية الفأرية في براز مرضى الالتهابات المعوية الحادة.

تم تجميع سبعة و أربعون عينة من مرضى ترددوا على مستشفى بشايرو و مستشفى امدرمان التعليمي و مستشفى حاج الصافى خلال فترة المستشفى.

تم استخلاص الحمض النووي الريبي منقوص الأكسجين من كل عينة من عينات البراز استخدمت تقنية متسلسل البلمرة الزمني للكشف على السالمونيلة التيفية الفأرية.

أظهرت النتائج وجود عينتين موجبتين في مرضى الالتهابات المعوية الحادة.

كما خلصت الدراسة إلى أن تقنية متسلسل البلمرة الزمني تسهل الكشف عن باكتيريا السالمونيلة التيفية الفأرية.

## Table of contents

Dedication	I
Acknowledgment	II
Abstract (English)	III

Abstract (Arabic)	IV
Table of contents	V
List of Tables	VII
List of figures	VIII
<b>Chapter One : Introduction and Literature Review</b>	
1.1 Introduction.....	1
1.1.1 The genus <i>Salmonella</i> .....	1
1.1.1.1 Classification.....	1
1.1.1.2. Definition.....	1
1.1.1.3. Pathogenesis.....	1
1.1.1.4. Pathology .....	2
1.1.1.5. Diseases.....	2
1.1.1.6. Host defenses.....	3
1.1.1.7. Immunity to reinfection.....	3
1.1.1.8. Diagnosis.....	4
1.1.2. <i>Salmonella enterica</i> serovar <i>typhimurium</i> .....	4
1.1.2.1 <i>Salmonella typhimurium</i> .....	4
1.1.2.2 The virulence of <i>Salmonella typhimurium</i> .....	5
1.1.2.3 <i>Salmonella typhimurium</i> antibiotic resistance .....	6
1.1.2.4 <i>Salmonella typhimurium</i> sequence .....	7
1.1.2.5. <i>Salmonella typhimurium</i> important strains.....	7
1.1.3. Polymerase Chain Reaction (PCR) .....	8
1.1.4. Real time PCR .....	9
1.2 Literature Review.....	12
1.3 Objectives.....	12
<b>Chapter Two : Materials and Methods</b>	
2.1. Materials.....	13
2.2. Study area.....	13
2.3. Subjects.....	13
2.4. Age group.....	13
2.5. Sample size.....	13
2.6 Sterilization.....	13
2.7. Experimental work.....	13
<b>Chapter Three : Results</b>	
3.1. Clinical specimen.....	17
3.2. DNA extraction .....	18
3.3 detection of <i>S.typhimurium</i> .....	18
<b>Chapter Four : Discussion</b>	
4. Discussion.....	22
<b>Chapter five :Conclusion and Recommendations</b>	
5. Conclusion and Recommendations	24
References.....	25
Appendices.....	27

## **List of Tables**

**Table 1. Distribution of specimens according to hospitals .**

**Table 2. Distribution of specimens according to age of patients.**

**List of figures**

**Figure 1. Shows the Quantica thermocycler.**

**Figure 2. Shows typical reading of the plate.**

**Figure 3. Shows the two positive specimens in duplicate against negative control.**