

سُودانِيَّةُ الْعُلُومِيَّةُ وَالْتَّكْنُوُلُوْجِيَّةُ

Sudan University of Science and Technology
College of Graduate Studies

**Detection of *Salmonella typhi* in Blood Specimens by
Real-time Polymerase Chain Reaction Technique**

سُودانِيَّةُ الْعُلُومِيَّةُ وَالْتَّكْنُوُلُوْجِيَّةُ

**A Dissertation Submitted in Partial Fulfillment for the
Requirement of M.Sc.**

In Medical Laboratory Science (Microbiology)

By
Mawahib Hassan Fath- Elrahman

Under Supervisor of
Dr. Humodi Ahmed Saeed

2008

بسم الله الرحمن الرحيم

قال تعالى:

﴿سَنُرِيهِمْ آيَاتِنَا فِي الْأَفَاقِ وَفِي أَنفُسِهِمْ حَتَّىٰ
يَتَبَيَّنَ لَهُمْ أَنَّهُ أَنَّهُ الْحَقُّ أَوْ لَمْ يَكُفِ بِرَبِّكَ أَنَّهُ عَلَىٰ
كُلِّ شَيْءٍ شَهِيدٌ﴾

صدق الله العظيم

(سورة فصلت، الآية 53)

Dedication

*To the soul of my lovely father,
who worked hard for us.*

To my great mother,

who taught me:

How I could be human.

*To my lovely brothers and sisters for
their support and kindness*

To my extended family

*To the people whom I loved, Respect
and appreciate....*

I dedicate this research.

Acknowledgement

First of all unlimited and great thanks and acknowledgement one due to **Allah**, the most Merciful, who blessed me with the courage for preparation and completion of this study.

My longest – standing debt is to my supervisor **Dr. Humodi Ahmed Saeed** (Dean Faculty of Medical Laboratory Science in Sudan University of Science and Technology) whose guidance and expertise helped the gradual development and improvement of the quality of this research. I want to express my deep thank to Dr. Mogahed, Ustaz. Mansoor and Dr. Misk Alyaman for their help and support.

Many people have helped in bringing this research to life, it would be impossible to name them all, but to each of them I extend my thanks and appreciation. In addition, I thank the staff of Microbiology lab in Faculty of Medical Laboratory Science in Sudan University of Science and Technology for their help.

Abstract

This study was carried out in Khartoum state during the period between May 2007_ March 2008 to detect the presence of *S. typhi* in patients with suspected enteric fever. Forty-seven blood specimens were collected from patient at three hospitals: Khartoum Teaching Hospital, 14 (29%), Omdurman Teaching Hospitals, 28(59%) and Alengath Medical Center 6 (12%). *S.typhi* DNA was extracted directly from each blood specimens using phenol - chloroform technique. Real time PCR technique was adopted to detect presence of *S. typhi*. The result revealed that only 7 (15%) specimens were positive and 40 (85%) were negative.

The study concluded that the real time PCR technique facilitates detection of *S.typhi* in blood of patients with suspected typhoid fever without bacteriological culture.

ملخص الأطروحة

أجريت هذه الدراسة في مدينة الخرطوم في الفترة بين مايو 2007 إلى مارس 2008. هدفت هذه الدراسة لتحديد وجود بكتيريا (السلمونيلا التيفية) في عينات دم مرضى حمى التيفود. تم تجميع سبعة وأربعون عينة دم من هؤلاء المرضى من ثلاثة مستشفيات، هي مستشفى الخرطوم التعليمي، 14 عينة وتمثل (15%)، مستشفى امدرمان التعليمي، 28 عينة وتمثل (59%) ومركز الإنقاذ الطبي، 6 عينات وتمثل (12%). تم استخلاص الحمض النووي لبكتيريا السلمونيلا التيفية من عينات الدم مباشرة باستخدام تقنية الفينول كلوروفورم. وقد استخدم تفاعل البلمرة المتسلسل الزمني للكشف عن بكتيريا (السلمونيلا التيفية) وأظهرت النتائج وجود سبعة عينات إيجابية وتمثل 15% من مجموع العينات بينما أربعون عينة وتمثل 85% كانت سلبية. خلصت الدراسة إلى أن تقنية تفاعل البلمرة المتسلسل الزمني لاكتشاف السلمونيلا التيفية من الدم مباشرة بدون اللجوء لتقنيات الاستزراع المخبري.

Table of contents

Acknowledgement.....	vii
Abstract.....	viii
ملخص الأطروحة.....	ix
List of Figures.....	i

List of Tables

Acknowledgement.....	vii
Abstract.....	viii
ملخص الأطروحة.....	ix
List of Figures.....	i

List of Figures

Figure (1) Plate layout showing typical amplification of real time PCR during reaction showing relative florescence in each well from A 1 – H 12.....	19
Figure (2) shows negative control.....	20
Figure (3) all positive result in duplicate.....	20
Figure (4) one positive result in duplicate the curve starting increase from cycle 35.....	21
Figure (5) one positive result in duplicate the curve starting increase from cycle 30.....	21
Figure (6) one positive result in duplicate the curve starting increase from cycle 31.....	22
Figure (7) one positive result in duplicate the curve starting increase from cycle 32.....	22
Figure (8) one positive result in duplicate the curve starting increase from cycle 28.....	23
Figure (9) one positive result in duplicate the curve starting increase from cycle 33	23
Figure (10) one positive result in duplicate the curve starting increase from cycle 35.....	24