

بسم الله الرحمن الرحيم

Sudan University for Science and Technology

College of Graduate Studies

**Impact of Using Liquid Organic Cleaners as
an Alternative of**

Phenol in Ziehl Neelsen Staining Method

**اثر استخدام المنظفات العضوية السائلة عوضا عن
الفينول فى صبغة زيل نلسون**

By

Hisham Mohammed Ahmmed Ishage

B.Sc. in Medical Laboratory Sciences

Omderman Ahlia University

(2000)

**AThesis submitted for partial fulfillment of
requirement for master
degree**

Supervisor

Dr. Elwaleed Mohamed Elamin

PHD in Medical Laboratory Sciences

University of Khartoum

(2003)

August 2007

Contents

Dedication	VIII
Aknowledgements	IX
Abstract (Arabic)	X
Abstract(English)	XI
List of Abbreviations	XII,XIII
Chapter I Introduction and literature review	1
1-General introduction	1
Tuberculosis world wide .1-1-4	2
3. Tuberculosis in Africa	3
1-2. Tuberculosis in Sudan	3
1-5.Tuberculosis And HIV/AIDS	4
1-6. Common microbiologic characteristics of the genus	4
1-7. Staining characteristics	5
1-7-1. Acid –fast bacilli and gram’s stain	6
Transmission .1-8	7
1-9. Progression	8
1-10. Bacterial species	9
1-11. Pathogenesis	10
1-12. Stages of the Disease	12
1-12-1. Stage 1	12
1-12-2. Stage 2	12
1-12-3. Stage 3	12
Stage 4 .1-121-4	13
1-12-4-1. Exudative lesions	15
1-12-5. Stage 5	15
1-13. Diagnosis	16
1-13-1. Clinical diagnosis	16

1-13-2. Laboratory diagnosis	17
1-13-2-1. Microscopy	17
1-13-2-2. Culture	21
1-13-3. Immunological diagnosis	22
1-13-3-1. Serological tests	22
1-13-3-1-1. Agglutination	22
1-13-3-1-2. Elisa	22
1-13-3-1-3. Tuberculin skin test	23
1-13-4. Molecular diagnosis	23
1-13-4-1. PCR	23
1-13-4-2. Recombinant DNA technology and cloning	24
1-13-5. X-ray examination	24
1-14. Treatment	24
1-15. Prevention	26
1-16. Vaccines	26
Objectives	30
Chapter II	31
Materials and methods	31
2-1. Study design	31
2-2. Study population	31
2-3. Sampling technique	31
2-4. Sample size	31
2-5. Section preparation	31
2-6. Control	31
2-7. Staining	32
2-7-1. / Traditional ZN stain	32
2-7-1-1./ Traditional method with direct flaming of stain	32
2-7-1-1-1. Preparation of carbol fuchsin	32
2-7-1-1-2. Preparation of 3 % acid alcohol	33
2-7-1-1-3. Preparation of 0.25 % acidified methylene blue	33
2-7-1-2 Traditional ZN by omitting flaming and staining in oven at 65°	33
2-7-2. /Modified basic fuchsin using Fairy	34
2-7-2-1. Preparation of carbol fuchsin	34
2-7-2-1-1. Modified basic fuchsin procedure	34
2-7-2-2/ Modified basic fuchsin using Alfa	35
2-7-2-2. Preparation of carbol fuchsin	35

2-7-3. /Modified basic fuchsin using clorax	35
2-7-3-1. Preparation of carbol fuchsin	35
2-8.Traditional method by using 0.3%basic fuchsin and 1%acid	36
2-9. Modified Basic fuchsin by using 0.3 % basic fuchsin and 1% acid alcohol	36
2-10.Examination	36
Chapter IV	37
Results	37
4-1. / Traditional ZN stain	37
4-1-1 Traditional ZN stain with direct flaming	37
-1-2/ Traditional method by staining in coblin jar in oven at 65°	37
4-3. / Modified basic fuchsine using organic cleaners	37
4-3-1/ Modified basic fuchsine using organic cleaner(Fairy)	37
4-3-2 / Modified basic fuchsin using organic cleaner (Alfa)	37
3-3-3 Modified basic fuchsin using organic cleaner (Clorax)	37
4-3-4.Traditional method by using 0.3 % basic fuchsin and 1% acid alcohol	37
3-4-5. Modified basic fuchsin method by using 0.3 % basic fuchsin and 1% acid alcohol	38
3-4-6. Comparison between traditional method using direct flaming and coblin jar	38
3-4-7. Comparison between the different liquid organic cleaners	38
3-4-6. Comparison between traditional and modified method	38
Figures	39
Figure 1,2	39
Figure 3,4	40
Figure 5,6	41

Figure 7,8	42
Figure 9,10	43
Chapter IV	44
Discussion	44
Conclusion	46
Recommendation	46
Chapter V	47
References	47

Dedication

To my father soul

who died 21 years ago

of heart arrest

in saudia Arabia.

Acknowledgments

With affection and deep appreciation I acknowledge my indebtedness to the friends, family and colleagues who have been part of this work.

Dr Elwaleed mohammed Elamin my supervisor his invaluable insights ,wise counsel,skillful review of the pages,attention to the important details in writing proposal and throughout this thesis and his positive approach to errors.

Alawia Abdelazeez and Salih Abdelazeez my mother and uncle for their enthusiastic encouragement which have great impact in doing this work.

Mr Ahmed Elmostafa from Soba university hospital,Mr Magdi from National Laboratory Health for their great help.

My colleagues in Soba university hospital and National Laboratory Health for their unlimited help.

الخلاصة

يعتبر السل من الامراض الخطيرة التى تصيب معظم اعضاء الجسم البشرى وفى العموم تصيب الرئتين و تسببها جرثومة مقاومة للاحماض و الكحول بعد صباغتها بالصبغة التقليدية. السل من الامراض التى يمكن معالجتها باخذ الادوية المضادة, كما يمكن الوقاية منه.

هذه الدراسة تركز على استخدام المنظفات العضوية السائلة عوضا عن الفينول المستخدم فى الصبغة التقليدية الخاصة بصبغ هذه الجرثومة. تم اخذ عينات انسجة ارشيفية تم تشخيصها على احتوائها على جرثومة السل.

بهذه الطرق امكننا فحص جرثومة السل فى النسيج و عند مقارنة هذه الطرق المستحدثة بالطرق التقليدية وجدنا ان هذه الطرق الجديدة تصبغ الجرثومة بنفس كفاءة الصبغ التقليدية و فى نفس الوقت هذه المنظفات العضوية رخيصة السعر و غير سامة عكس الفينول المستخدم فى الصبغ التقليدية اذ انه سام و اكثر تكلفة عند شراءه.

Abstract

Tuberculosis (TB) is a potentially fatal contagious disease that can affect almost any part of the body but is mainly an infection of the lungs. It is caused by a bacterial microorganism, the tubercle bacillus or *Mycobacterium tuberculosis*. TB can be treated, cured, and can be prevented if persons at risk take certain drugs.

This study concerned mainly in finding an alternative material to phenol used in traditional Ziehl Neelsen stain for staining acid fast bacilli and excluding its highly toxicity from the staining solution hence developing safer staining method. Certain selective lipophilic agents, liquid organic detergents, Clorax, Fairy and Alfa were used.

Archival Paraffin blocks that suggestive of TB Infection upon diagnosis were reviewed, sectioned and stained.

By these methods acid fast bacilli were detected and stained red in blue background. Result obtained compared very favourably with traditional Ziehl Neelsen stain. In addition Detergents are efficient lipophilic agents and safer to handle than phenol.

In brief, the methods described here stains acid fast bacilli as efficiently as traditional carbol fuchsin method. Liquid organic cleaners are considerably cheaper.

List of Abbreviations

Abbreviation	Definition
TB	Tuberculosis
WHO	World Health Organization
HIV	Human Immune Virus
AIDS	Acquired immuno Deficiency Syndrome
MTB	Mycobacterium Tuberculosis
CMI	Cell Mediated Immunity
AMI	Antibody Mediated Immunity
PMNs	Polymorphonucleocytes
INF	Interferon
TNF	Tumour Necrosis Factor
IL	Interleukin
ZN	Ziehl Neelsen
AFB	Acid Fast Bacilli
PCR	Polymerase Chain Reaction
LCR	Ligase Chain Reaction
DNA	Deoxynucleic Acid
RNA	Ribonucleic Acid
CDC	Centre for Disease Control and Prevention
NIAID	National Institute of Allergy and Infectious Disease
CWC	Cell wall Composition

MDR.TB	Multi Drug Resistance Tuberculosis
XDR.TB	Extensively Drug Resistance Tuberculosis