

# بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ





# شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



# جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

## قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها  
علي هذه الأقراص المدمجة قد أعدت دون أية تغييرات



## يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار





# بعض الوثائق الأصلية تالفة





# بالرسالة صفحات لم ترد بالأصل





## *Acknowledgement*

*First and foremost my gratitude and thanks should be submitted to God.*

*I should like to express my deepest gratitude and sincere appreciation to Prof. Dr. Ibrahim Khalil Ali Mohamed Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for his constructive supervision, great care and encouragement all through this work.*

*I would like to offer special thanks and appreciation to Prof. Dr. Nevine Nabil Kassem, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her honest assistance, meticulous supervision, valuable suggestions and constructive criticism throughout this work.*

*I would like to express my deepest gratitude to Prof. Dr. Malaka Zakaria Amer, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her close attention, encouragement, creative advices, and fruitful guidance during this study.*

*I am also deeply indebted to Prof. Dr. Manal Abd El-Alim Abd El-Satar Ahmed, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her constructive supervision, generous help, and valuable support throughout this study to emerge in the proper way.*

*I am also grateful to Prof. Dr. Fatma El-Sayed Metwally Mohamed, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her help, care, and valuable support during this study.*

*Finally, I would like to convey my warmest gratitude to my family for their everlasting care and support, also my colleagues and every member of the Clinical Pathology Department.*



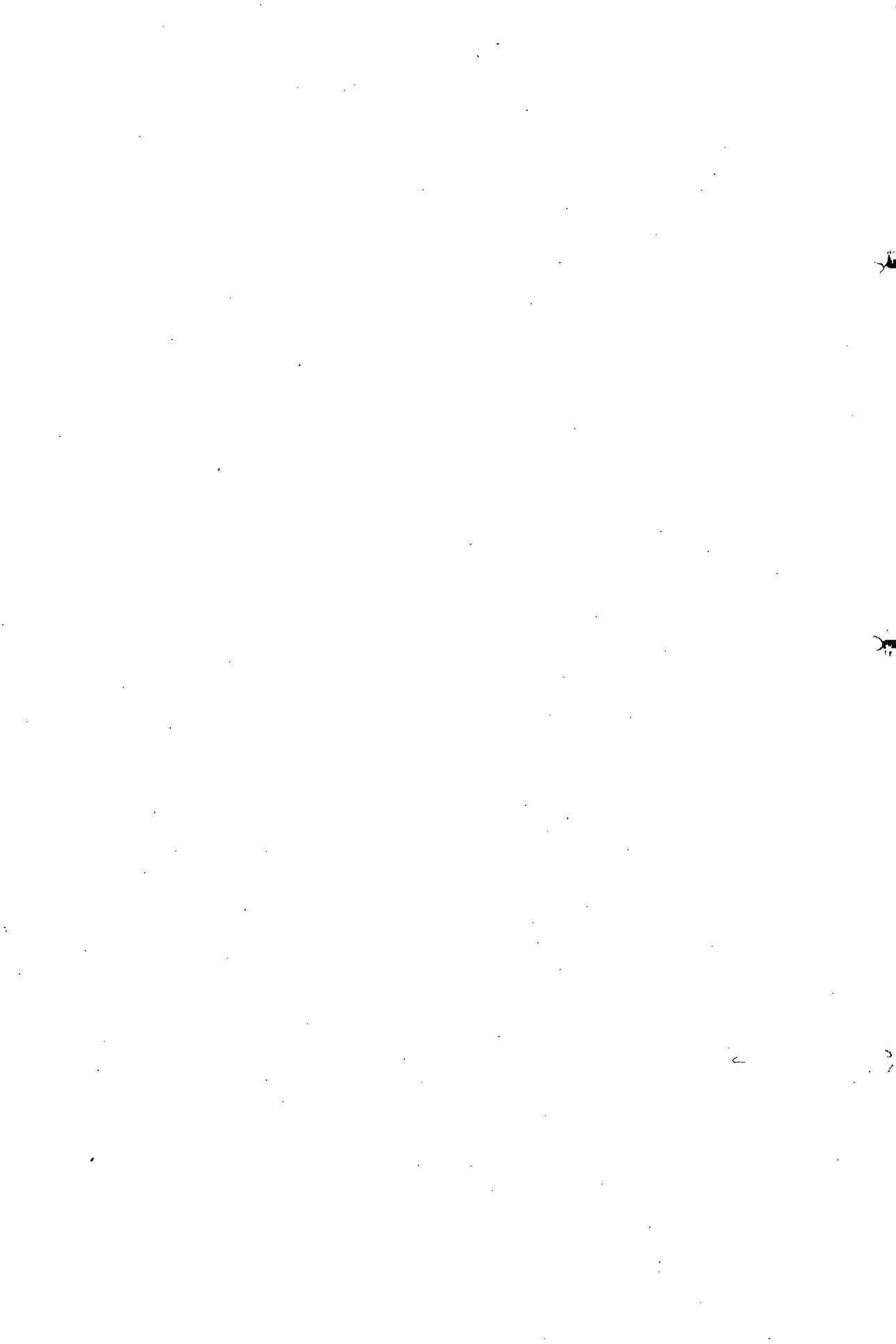
## **List of Contents**

<b>Introduction and aim of the work</b>	1
<b>Review of literature</b>	1
❖ <b>Candida Species</b>	3
▪ <b>Historical Aspects</b>	3
1. Taxonomy of Genus Candida	4
2. Classification of Candida species	6
3. Morphology and dimorphism:	7
4. Candida cell structure	8
5. Antigenic structure and extracellular products	9
6. Ecology and Epidemiology	11
▪ <b>Immunity To Candida Infection</b>	12
▪ <b>Pathogenicity and Virulence</b>	16
▪ <b>Pathogenic Candida species</b>	22
▪ <b>Clinical Aspects of Candida infections</b>	25
▪ <b>Laboratory diagnosis of Candida infection</b>	35
• <b>Specimen Collection and Transport</b>	35
• <b>Isolation and Identification of Candida</b>	38
<b>I. Direct methods of identification</b>	38
A. Direct microscopic examination	38
B. Culture	39
C. Antigen detection in serum	57
D. Nucleic acid detection techniques	61
E. Flow cytometry identification method	64
<b>II. Indirect methods</b>	64
❖ <b>Antifungal Agents</b>	66
▪ <b>General concepts</b>	66
I. <b>I. Polyene antifungal drugs</b>	68
1) Amphotericin B	68
2) Nystatin	71
3) Natamycin (Pimaricin)	71
II. <b>Azole antifungal drugs</b>	71
A. Imidazole	72
1) Ketoconazole	72
2) Miconazole	74

B. Triazoles	75
1) Fluconazole	75
2) Itraconazole	76
III. III. Antimetabolites	78
Flucytosine (5-fluorocytosine; 5-FC)	78
IV. . Allylamine	80
▪ Treatment of candidiasis	84
▪ Prophylaxis of invasive candidiasis and other opportunistic fungal diseases in immuno-compromised patients	91
❖ Flow cytometry	93
▪ Design and operation of flow cytometry	93
▪ Mechanism of staining by fluorescent dyes	96
▪ Parameters measurable by flow cytometry	98
❖ Antifungal susceptibility testing	100
▪ Problems with antifungal susceptibility tests	102
▪ Methods of antifungal susceptibility testing	104
I. Broth dilution methods (macro- and microdilution)	104
II. Agar dilution methods	109
III. Agar diffusion method	111
IV. Flow cytometry antibiogram	113
<b>Materials &amp; Methods</b>	118
<b>Results</b>	155
<b>Discussion</b>	169
<b>Recommendations</b>	179
<b>Summary &amp; Conclusion</b>	181
<b>References</b>	184
<b>Arabic Summary</b>	228

## List of Tables

<i>Table No.</i>	<i>Title</i>	<i>Page</i>
1	The major groups of medically important fungi and classification scheme of imperfect fungi.	5
2	Teleomorphic state of some <i>Candida</i> species	6
3	The most common <i>Candida</i> species implicated in human infections.	23
4	Fungal culture media and indications for use.	43
5	Morphology of different <i>Candida</i> species	51
6	The biochemical reactions of yeast and yeast-like fungi.	52
7	A summary of other antifungal drugs.	82
8	Different cellular parameters measurable by flow cytometry	105
9	Summary of M27 methodology	142
10	Steps of the broth microdilution method.	
11	<i>Candida</i> species isolated from different clinical specimens.	160
12	<i>Candida</i> isolates and their antifungal susceptibility determined by NCCLS M27-A & flow cytometry.	163
13	The range of MICs of <i>Candida</i> isolates obtained by both techniques.	165
14	Amphotericin B sensitivity pattern of isolated <i>Candida</i> species by M27-A and FCM.	166
15	Fluconazole sensitivity pattern of isolated <i>Candida</i> species by M27-A and FCM.	166
16	Ketoconazole sensitivity pattern of isolated <i>Candida</i> species by M27-A and FCM.	167



## List of Figures

<i>Figure No.</i>	<i>Title</i>	<i>Page</i>
1	Germination of <i>Candida</i> .	7
2	<i>Candida</i> cell structure.	9
3	(A) Chlamydo spores; between blastoconidia and terminal on the pseudohyphae, (B) Chlamydoconidia produced from true hyphae.	46
4	<i>Candida albicans</i> ; elongate pseudohyphal cells with large clusters of blastoconidia at junctions between cells. Sessile, intercalary, and many terminal chlamydo spores.	47
5	<i>Candida tropicalis</i> ; long branching pseudohyphae with blastoconidia anywhere along the pseudohyphae. Chlamydoconidia and true hyphae occasionally form.	47
6	<i>Candida (Torulopsis) glabarata</i> ; globose or oval cells with occasional short-branched chains.	47
7	<i>Candida parapsilosis</i> ; short, thin cells with pronounced curve, and occasional giant cells. Blastoconidia develop singly, in clusters and short chains on the pseudohyphae.	47
8	<i>Candida krusei</i> ; long, slender, straight cells with treelike branching and chains of blastoconidia from the junction between cells, resemble "crossed matchsticks".	48
9	<i>Candida guilliermondii</i> ; long, slender, slightly curved cells with pairs and small clusters of blastoconidia at junctions between cells, no terminal chlamydo spores.	48
10	<i>Candida kefyr</i> ; elongate, slender pseudohyphal cells with adjacent free blastoconidia, resembling logs in a stream.	48
11	Generalized fungal cell depicting the sites of action of the common antifungal agents.	68

<b>12</b>	the basic structure of a flow cytometer showing the fluid transport system, the optical system, the electronic system, and Cell sorting	95
<b>13</b>	Illustrates the relation of light scatter and cell size/structure	96
<b>14</b>	Diagram of the Microscan Rapid Yeast Identification Panel	123
<b>15</b>	Germ tube formation by <i>Candida albicans</i> .	133
<b>16</b>	<i>Candida albicans</i> with the characteristic chlamydospores, blastoconidia, true hyphae and pseudohyphae.	134
<b>17</b>	<i>Candida tropicalis</i> with blastoconidia formed at and between septa.	135
<b>18</b>	Microscan Rapid Yeast Identification Panel with <i>C. albicans</i> .	136
<b>19</b>	Microscan Rapid Yeast Identification Panel with <i>C. parapsilosis</i> .	137
<b>20</b>	Flowchart for identification of yeasts from clinical specimens	138
<b>21</b>	An inoculated microdilution plate.	144
<b>22</b>	Flow cytometric histogram profiles of one strain of <i>Candida albicans</i> stained with PI and containing viable and heat killed (nonviable) cells. SS, side scatter; FS, forward scatter. Cell count and log fluorescence (LFL2) are also seen, (A) 100% viable; (B) 50% nonviable; (C) 80% nonviable; (D) 100% nonviable cells.	148
<b>23</b>	Changes in flow cytometric parameters of <i>Candida</i> induced by amphotericin B after incubation for 2-h. (A) Growth control; (B) subinhibitory concentrations; (C) inhibitory concentrations; (D) maximal inhibitory concentration. SS, side scatter; FS, forward scatter; LFL2, log fluorescence.	152
<b>24</b>	Changes in flow cytometric parameters of <i>Candida</i> induced by fluconazole after incubation	153

	for 4-h. (A) Growth control; (B) subinhibitory concentrations; (C) inhibitory concentrations; (D) maximal inhibitory concentration. SS, side scatter; FS, forward scatter; LFL2, log fluorescence.	
<b>25</b>	Changes in flow cytometric parameters of <i>Candida</i> induced by ketoconazole after incubation for 4-h. (A) Growth control; (B) subinhibitory concentrations; (C) inhibitory concentrations; (D) maximal inhibitory concentration. SS, side scatter; FS, forward scatter; LFL2, log fluorescence.	154
<b>26</b>	The percent of <i>Candida</i> species isolated from different samples.	161
<b>27</b>	The different types of isolated <i>Candida</i> species.	162
<b>28</b>	Antifungal sensitivity pattern of <i>Candida</i> isolates by both M27-A and FCM.	168