učební texty Univerzity Karlovy v Praze

Oto Melter
Annika Malmgren

PRINCIPLES AND PRACTICALS IN MEDICAL MICROBIOLOGY

KAROLINUM

Principles and Practicals in Medical Microbiology

MVDr. Oto Melter, Ph.D.
Annika Malmgren (medical student)

Reviewed by
MUDr. Eliška Bébrová
MUDr. Tamara Bergerová

Published by Charles University
Karolinum Press
as a teaching text for the Second Faculty of Medicine
Cover by Kateřina Řezáčová
Typeset by Karolinum Press
First Edition

ISBN 978-80-246-2413-6
ISBN 978-80-246-2545-4 (online : pdf)

Charles University
Karolinum Press 2018

www.karolinum.cz
ebooks@karolinum.cz

CONTENTS

Introduction 9

1 Laboratory safety rules 11
1.1 Purpose of the safety principles 11
1.2 Principles 11

GENERAL MICROBIOLOGY

2 Specimen collection & diagnostic principles 13
2.1 Specimen collection & transport 13
2.2 Material & methods 13
2.3 Conditions for specimen collection & transport 14
2.4 Request form 14
2.5 Diagnostic principles in medical microbiology 15
2.6 Light microscopy 15
2.7 Gram staining – basic staining procedure in clinical microbiology 16
2.7.1 Smear preparation 16
2.7.2 Quality of cell wall & Gram staining procedure 16
2.8 Practical part – specimen collection & diagnostic principles 17
2.9 Lab quiz 18

3 Direct detection & typing of infectious agents 19
3.1 Definition of agent detection & typing 19
3.2 Application of microscopy technics 19
3.3 Application of nucleic acid based methods 21
3.3.1 Principle of DNA amplification by PCR methods 21
3.3.2 Principle of DNA hybridization methods 22
3.3.3 Principle of sequence based methods 22
3.4 Other methods in direct antigen detection 22
3.4.1 Immunochromatographic method 22
3.5 Practical part – direct detection & typing of infectious agents 23
3.6 Lab quiz 24

4 Cultivation of infectious agents 25
4.1 Cultivation – definition 25
4.2 Cultivation of bacterial, viral & parasitic agents 25
4.3 Media for bacterial cultivation 26
4.4 Examples of bacterial cultures 27
4.5 Conditions for cultivation 28
4.6 Special staining procedures 28

4.7 Practical part – special stainings & cultivation of infectious agents . . . 28
4.8 Lab quiz . . . 30

5 Antibiotic susceptibility testing . . . 31
5.1 Antimicrobials; mode of action & resistance . . . 31
5.2 Antimicrobial susceptibility testing . . . 31
5.3 Basic methods . . . 32
5.4 Interpretations of the results . . . 32
5.5 Other methods; procedures & interpretation . . . 33
5.6 Practical part – special stainings & cultivation of infectious agents . . . 34
5.7 Lab quiz . . . 36

6 Identification of infectious agents . . . 37
6.1 Definition of identification of infectious agents . . . 37
6.2 Phenotypical methods . . . 37
6.3 Application of phenotypical methods . . . 40
6.4 Genotypical methods & application . . . 41
6.5 Practical part – identification of infectious agents . . . 41
6.6 Lab quiz . . . 43

7 Detection of specific antibodies . . . 45
7.1 Immune response . . . 45
7.2 Antigens & antibodies . . . 45
7.3 Basic serological methods . . . 46
7.4 Serological methods & application . . . 48
7.5 Practical part – detection of specific antibodies . . . 49
7.6 Lab quiz . . . 50

8 General mycology . . . 51
8.1 Fungi . . . 51
8.2 Classification . . . 51
8.3 Diagnostics . . . 52
8.3.1 Microscopy . . . 52
8.3.2 Cultivation . . . 53
8.3.3 Identification . . . 53
8.4 Susceptibility to antimycotical drugs . . . 56
8.5 Serological methods . . . 56
8.6 Direct detection of the agent in clinical material . . . 57
8.7 Practical part – general mycology . . . 57
8.8 Lab quiz . . . 58

9 General virology . . . 59
9.1 Definition of viruses . . . 59
9.2 History . . . 60
9.3 Electronmicroscopic evidence . . . 60
9.4 Virus detection by other methods . . . 60
9.5 Praktical part – general virology . . . 63
9.6 Lab quiz . . . 64

10 General parasitology . . . 65
10.1 Parasitism, parasite and host . . . 65
10.2 Classification . . . 65
10.3 Diagnostic methods . . . 66
10.4 Material and diagnostics . . . 67
10.5 Practical part – general parasitology . . . 68
10.6 Lab quiz . . . 69

11 Molecular microbiology & epidemiology ... 71
11.1 Definition ... 71
11.2 Bacterial cell wall synthesis and antibiotics ... 71
11.3 Antibiotic resistance genes ... 72
11.4 Regulation of structural genes for antibiotic resistance ... 72
11.5 Epidemiologic typing ... 73
11.6 Practical part – molecular microbiology & epidemiology ... 74
11.7 Lab quiz ... 75

SPECIAL MICROBIOLOGY

12 Staphylococci ... 77
12.1 General features ... 77
12.2 Virulence factors and pathogenesis ... 77
12.3 Infections ... 77
12.4 Treatment, prevention & control ... 78
12.5 Laboratory diagnosis ... 78
12.6 Practical part – staphylococci ... 80
12.7 Lab quiz ... 81

13 Streptococci & enterococci ... 83
13.1 General features ... 83
13.2 Classification ... 83
13.3 Virulence factors and pathogenesis ... 83
13.4 Infections and poststreptococcal diseases ... 84
13.5 Treatment, prevention & control ... 84
13.6 Laboratory diagnosis ... 85
13.7 Practical part – streptococci & enterococci ... 87
13.8 Lab quiz ... 88

14 Corynebacteria & listeria ... 89
14.1 General features ... 89
14.2 Virulence factors & pathogenesis ... 89
14.3 Infections & epidemiology ... 90
14.4 Treatment, prevention & control ... 90
14.5 Laboratory diagnosis ... 90
14.6 Practical part – corynebacteria & listeria ... 92
14.7 Lab quiz ... 94

15 Enterobacteria & enteric pathogens ... 95
15.1 General features ... 95
15.2 Virulence factors & pathogenesis ... 95
15.3 Infections & epidemiology ... 96
15.4 Treatment, prevention & control ... 96
15.5 Laboratory diagnosis ... 96
15.6 Practical part – enterobacteria and enteric pathogens ... 98
15.7 Lab quiz ... 100

16 Anaerobic bacteria ... 101
16.1 General features ... 101
16.2 Virulence factors & pathogenesis ... 101
16.3 Infections & epidemiology ... 102
16.4 Treatment, prevention & control ... 102
16.5 Laboratory diagnosis ... 102
16.6 Practical part – anaerobic bacteria ... 104
16.7 Lab quiz ... 106

17 ***Neisseria, Bordetella* & *Haemophilus*** 107
17.1 General features 107
17.2 Virulence factors & pathogenesis 107
17.3 Infections & epidemiology 107
17.4 Treatment, prevention & control 108
17.5 Laboratory diagnosis 108
17.6 Practical part – *Neisseria*, *Bordetella*, *Haemophilus* 110
17.7 Lab quiz 112

18 ***Pseudomonas aeruginosa* & other non-fermenters** 113
18.1 General features 113
18.2 Virulence factors & pathogenesis 113
18.3 Infections & epidemiology 114
18.4 Treatment, prevention & control 114
18.5 Laboratory diagnosis 114
18.6 Practical part – Pseudomonas and non-fermenters 116
18.7 Lab quiz 118

19 Mycobacteria 119
19.1 General features 119
19.2 Classification of mycobacteria 119
19.3 Virulence factors & pathogenesis 119
19.4 Infections & epidemiology 120
19.5 Treatment, prevention & control 120
19.6 Laboratory diagnosis 121
19.7 Practical part – mycobacteria 122
19.8 LAB QUIZ 124

20 Yeasts 125
20.1 General features 125
20.2 Virulence factors & pathogenesis 125
20.3 Infections & epidemiology 126
20.4 Treatment, prevention & control 126
20.5 Laboratory diagnosis 126
20.6 Practical part – yeasts 128
20.7 Lab quiz 130

21 Arthropods 131
21.1 General properties 131
21.2 Classification 131
21.3 Pathogenesis 131
21.4 Infecton caused or trasmitted by arthropods 131
21.5 Treatment and prevention 132
21.6 Laboratory diagnosis 133
21.7 Practical part – arthropods 134
21.8 Lab quiz 135

INTRODUCTION

Enormous amounts of new information in medical microbiology are becoming available on a daily basis. The amazingly huge quantity of new data is often focused on details and too complicate situations for both undergraduate medical students and physicians. This book has been created for medical students to ease the comprehension of the relations between theory and practice in current medical microbiology. The core comprehensive data is reviewed from prestigious publications in the field (Brooks et al: Jawetz, Melnick & Adelbergs' Medical Microbiology, 24th edition, LANGE, 2007; Lippincott's Illustrated Reviews, Microbiology, 2nd edition, Lippincott Williams & Wilkins, 2007; Murray *et* al, Medical Microbiology, 5h edition, Elsevier Mosby, 2005) and professional experience of the authors.

Each chapter contains a theoretical and a practical part. The practical part is divided into a few exercises which allows for the practice of some of the basic experimental procedures used in laboratories of medical microbiology. We believe that the original photographs and the diagrams that have been newly created by the authors will improve the understanding of the basic principles of microbiological diagnosis. Each chapter is complemented with a lab quiz intended to help students review their knowledge.

Acknowledgements

We would like to thank Adam Whitley and Florian Merkle, pregraduate Medical students, 2nd Faculty of Medicine of Charles University in Prague, for outstanding reviewing of the manuscript and for his suggestions. We also would like to thank specialists from our department for their help and preparation of the chapters of parasitology (Jan Urban, MSc., Ph.D.), virology (Petr Hubáček, M.D., Ph.D.) and mycology (Vanda Chrenková, M.D.). We also would like to thank both reviewers and outstanding clinical microbiologists: Eliška Bébrová, M.D., Department of Medical Microbiology of the 2nd Faculty of Medicine of Charles University in Prague, and Tamara Bergerová, M.D., Department of Microbiology, Faculty Hospital, Plzeň, for their critical suggestions and Bc. Vanessa Majeski for language revision.

1 LABORATORY SAFETY RULES

1.1 Purpose of the safety principles

The purpose of the safety principles is to reduce or eliminate exposure of potentially hazardous agents to

a) laboratory workers
b) other people
c) the outside environment

1.2 Principles

1. Use a protective lab coat and other appropriate items when handling potentially infected clinical materials or microbial cultures. Use the coat and the items only in the laboratory area.
2. Don't eat, drink or smoke and never touch your mouth, eyes or nose while in the laboratory.
3. Process clinical materials and cultures only in designated areas and never carry them away.
4. Keep your bench in order.
5. Sterilize bacteriological loops in a flame after usage, or use disposable ones.
6. After handling, cover petri dishes, tubes and flasks containing microbial cultures with a lid. (figure 1.1).
7. Disinfect laboratory glassware and other items with a disinfectant solution. If disposable, discard them into a special container.
8. Disinfect skin, mucosa and laboratory surfaces immediately after contamination with infectious agents.
9. After handling potentially infectious materials or cultures wash your hands in a disinfectant solution containing soap and rinse properly with running water.
10. All laboratory accidents should be reported immediately to the laboratory supervisor.
11. Respect the fire protection rules when working with fire.
12. Taking microbial cultures and laboratory animals away from the laboratory is strictly forbidden.

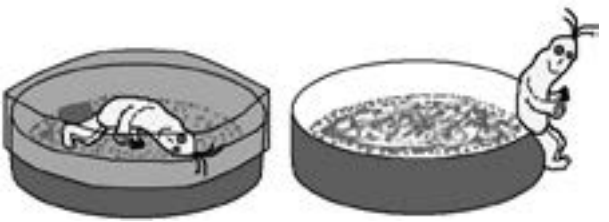

Fig. 1.1 Bacterial contamination. Make sure that you apply the laboratory safety rules to avoid spread of infection.

2 SPECIMEN COLLECTION & DIAGNOSTIC PRINCIPLES

2.1 Specimen collection & transport

The microbiological diagnosis is only as reliable as the quality of the specimen being tested (figure 2.1)!

Fig.2.1 There are three fundamental parts of microbiological a diagnosis: 1. specimen collection and transport, 2. results, 3. result interpretation

2.2 Material & methods

Specimens are collected from the patients using sterile tools such as swabs, tubes, containers etc. (figure 2.2). The rotating **swab** collects **surface specimen** (skin or underlying tissue) or specimen from **accessible mucosal surfaces** (e.g. pharyngeal swab) by direct contact with clinical material. The swab is then inserted into a transport medium. This is a medium without nutrients but with a preserving agent. **Blood, fluids and tissue samples** are collected into tubes and containers. Collected and labeled (errors may have disastrous consequences) specimen is sent with a **request form** to the lab as soon as possible (table 3.1).

Fig. 2.2 Material for collection of respiratory tract infection specimen
Swabs (1, 2), swabs and transport media (3, 4), spatula (5), container (6), anaerobic, aerobic and mycotic haemoculture containing liquid and solid culture media (7, 8, 9)

2.3 Conditions for specimen collection & transport

Table 2.1 Conditions for specimen collection and transport

AGENT	CONDITIONS	STORAGE, TRANSPORT
bacteria	swab and transport medium	RT*
viruses	swab, fluid, tissue culture medium	4–8 °C culture medium is used as transport medium
parasites/eggs	Three collections are made each two days apart. (container / tube)	RT* (storage 4–8 °C up to week)
anaerobes	fluid, tissue (avoid contact with oxygen)	RT*
fastidious bacteria	special conditions (e.g. *Neisseria* spp.)	various (if delay – freeze the sample)

Note: *RT – room temperature

2.4 Request form

All specimens should be accompanied by a request form (table 2.2).

Table 2.2 Example of request form

Record number	2489
Date of specimen collection	28.8.2008
Surname, first name & patient ID	Smile Frank 680811/1458
Ward	Surgery
Specimen	Sputum
Physician	Dr. Whitley
Clinical diagnosis	Pneumonia
Investigation required	Microscopy, culture, antibiotic sensitivity