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PRINCIPLES AND PRACTICALS IN MEDICAL MICROBIOLOGY

KAROLINUM

Principles and Practicals in Medical Microbiology

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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.

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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.

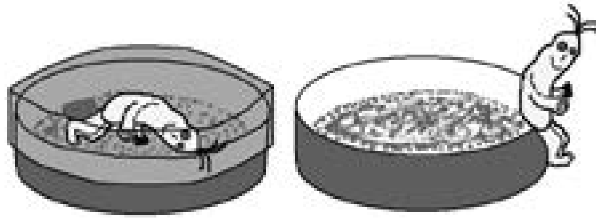


Fig. 1.1 Bacterial contamination. Make sure that you apply the laboratory safety rules to avoid spread of infection.

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)!

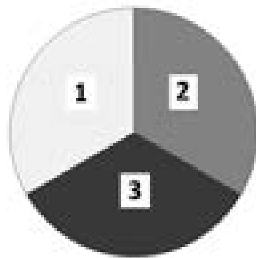


Fig.2.1 There are three fundamental parts of microbiological a diagnosis: 1. specimen collection and transport, 2. results, 3. result interpretation

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).

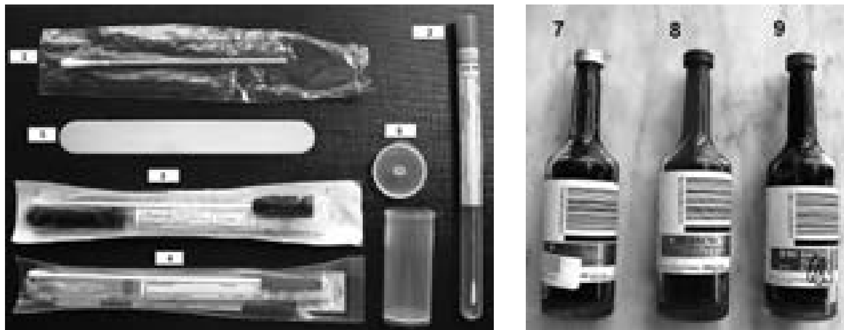


Fig. 2.2 Material for collection of respiratory tract infection specimen
Swabs (1, 2), swabs and transport media (3, 4), spatula (5), container (6), anaerobic, aerobic and mycotic haemoculture containing liquid and solid culture media (7, 8, 9)

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

2.5 Diagnostic principles in medical microbiology

Infectious agents can be detected in clinical material **directly** (microscopy, culture, confirmation of structural parts – DNA, RNA, others) or **indirectly** (specific antibodies – bodily response to the agent) (fig. 2.3).

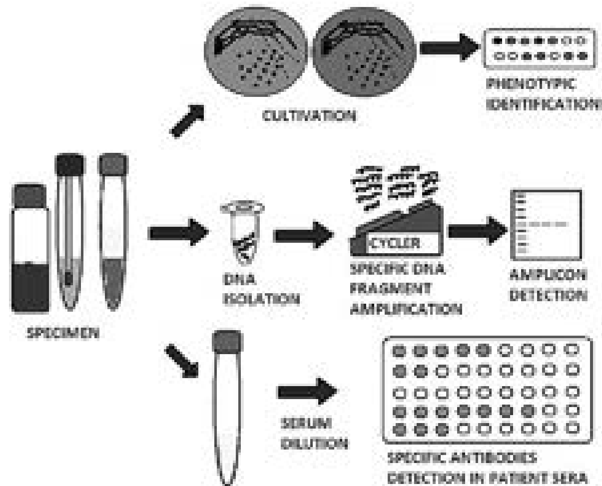


Fig. 2.3 Diagnostic principles used in medical microbiology**.** Note: viruses need living host cells for cultivation. Thus they are cultivated in tissue cultures.

2.6 Light microscopy

Principles of light microscopy: visible light transmitted through the sample and lenses allows a magnified view of the sample. The image is then detected directly by the eye, imaged on a photographic plate or captured digitally. Components of the light microscope are depicted in figure 2.4.

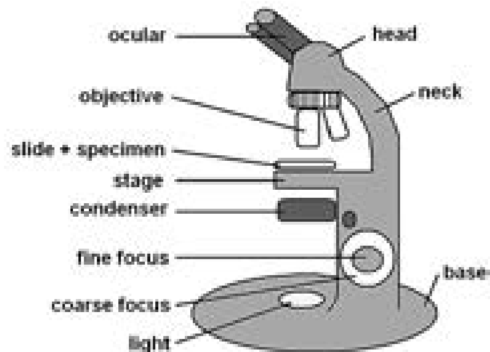


Fig. 2.4 Components of light microscope

2.7 Gram staining – basic staining procedure in clinical microbiology

2.7.1 Smear preparation

Smear preparation is depicted in figure 2.5.

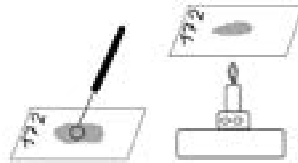


Fig. 2.5 Steps in smear preparation: 1. label the slide; 2. spread clinical material or culture in a thin film over slide; 3. air dry; 4. pass slide through flame to fix it

2.7.2 Quality of cell wall & Gram staining procedure

Gram-positive (on the left) and gram-negative (on the right) cell surface structures are depicted below (fig. 2.6).

Fig. 2.6 Diagram of cell wall and adjacent structures in gram-positive and gram-negative bacteria: 1. cytoplasmic membrane, 2. cell wall (peptidoglycan, which consists of multiple layers of polymeric saccharides cross-linked by amino acids), 3. outer membrane.

Gram staining procedure is depicted in figure 2.7.

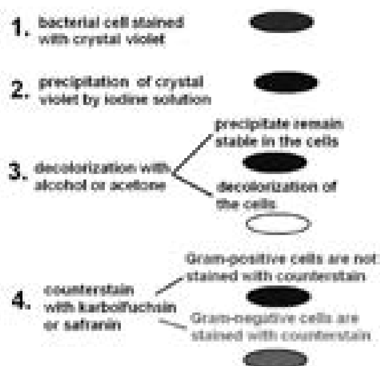
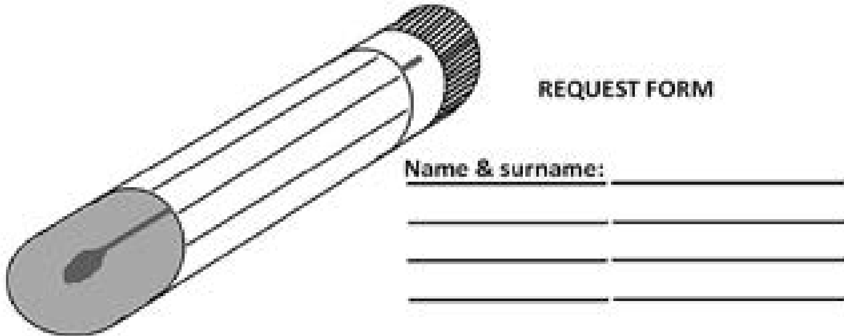


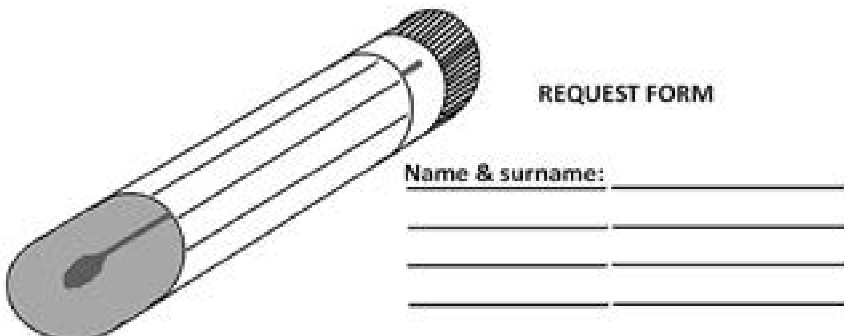
Fig. 2.7 Diagram of particular steps in Gram staining procedure and properties of gram-positive and gram-negative bacteria. Rinse the preparation after each step with water.

2.8 Practical part – specimen collection & diagnostic principles

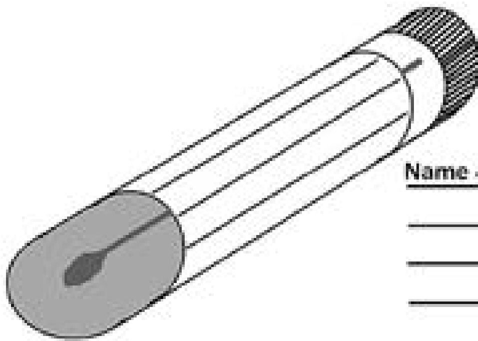
Exercise 2.8.1 *Press your colleague's tongue down with the spatula and rotate a sterile cotton swab against the tonsillar fossa. Specimen being tested for bacteriological analysis should be processed in the laboratory within 2 hours. If longer time is needed for the transport insert the swab into a transport medium and store it at room temperature. Label the specimen and prepare a request form for the specimen.*



Exercise 2.8.2 *Insert a cotton swab into the ear canal and rotate it gently against the mucosa. Such specimen should be processed in the laboratory within 2 hours. If longer time is needed for transport insert the swab into a transport medium and store it at room temperature. Label and prepare a request form for the specimen.*



Exercise 2.8.3 *Insert a cotton swab into a nostril and gently rotate it against the mucosa. Such specimen should be processed in the laboratory within 2 hours. If longer time is needed for transport insert the swab into a transport medium and store it at room temperature. Label and prepare a request form for the specimen.*



REQUEST FORM

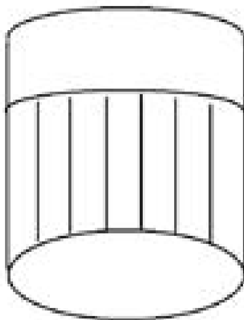
Name & surname: ____________

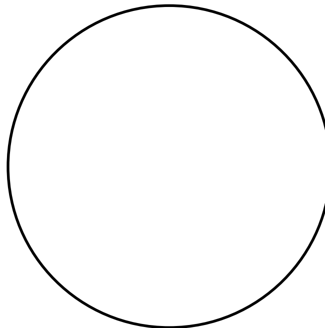
____________ ____________

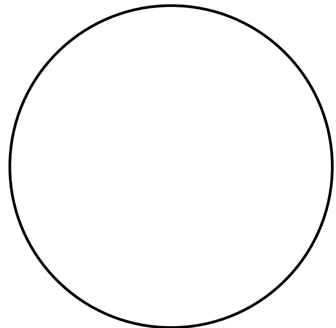
____________ ____________

____________ ____________

Exercise 2.8.4 *Prepare a smear slide from collected sputum. Fix and stain it as described above. Draw a magnified view of host cells and flora as you see it under the microscope and specify what you can detect.*







2.9 Lab quiz

2.9.1 Specify the three equally important parts of a microbiological diagnosis and state more details concerning the first fundamental part.
2.9.2 Describe procedures necessary for collecting patient sera for a serological analysis.
2.9.3 Describe the role of bacteriological transport media.
2.9.4 Do bacteriological transport media contain any nutrients?
2.9.5 Are all specimens potentially infectious?

3 DIRECT DETECTION & TYPING OF INFECTIOUS AGENTS

3.1 Definition of agent detection & typing

Infectious agents are detected **directly** in clinical material by the confirmation of their structural parts by using microscopy, culture or DNA amplification. They can also be detected **indirectly** by using specific antibodies. Typing means classifying clinical isolates into particular groups based on their biological or molecular properties (e.g. serotypes, clones, genotypes).

3.2 Application of microscopy technics

a) Bright field microscopy: see 2.6 light microscopy in Chapter 2.
b) Dark field microscopy: oblique light scattered by an object creates an image of high contrast.
c) Phase contrast microscopy: phase shifts in the light passing through a transparent specimen are converted into contrast changes in the image (fig. 3.1).
d) Fluorescence microscopy: structures of interest are stained with fluorochromes that emit fluorescent light (fig. 3.3).
e) Transmission electron microscopy: electron beams passing through an object creating an image of much higher resolution (0,05 nm) than light microscopy.
f) Scanning electron microscopy: visualizes details on the surfaces of cells and particles, nice 3D view.

A

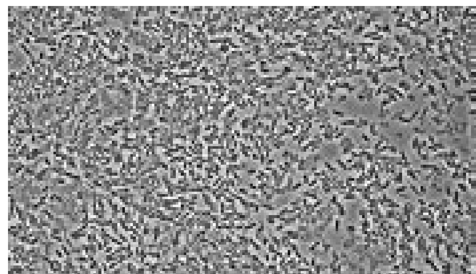

B

Fig. 3.1 Bright-field (A) and phase contrast microscopy (B), mesophilic bacteria in drinking water

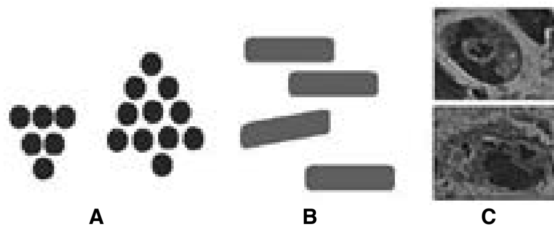


Fig. 3.2 Schematic diagram of the most common bacterial shapes and Gram properties as seen by bright field microscopy: gram-positive cocci (A, staphylococci), gram-negative rods (B, enterobacteriae) and direct detection of intracellular *Staphylococcus aureus* in neutrophils of sputum from a patient with cystic fibrosis (C)

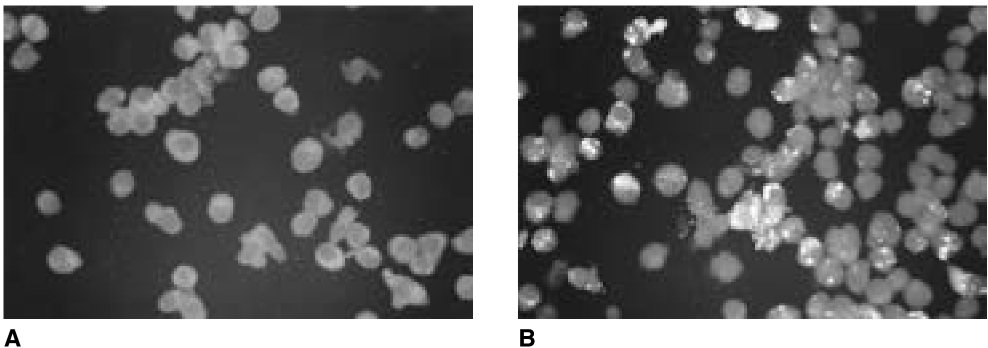


Fig. 3.3 Bacterial or viral agents can be detected directly after reaction with specific antibodies, which are labelled with fluorochromes. The emitted light is then detected by immunofluorescence microscopy.

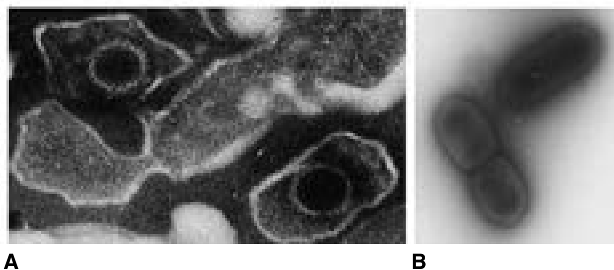


Fig. 3.4 Electron microscopy is especially useful for the detection of viral particles in clinical material. Depending on their structure, symmetry and size, viruses can be sorted into families. Two Epstein Barr virions loosely surrounded by membrane envelopes (A) (http://biestmilch-seven.com/archives/2009/08/18/chronic-epstein-barr-virus-infection-in-athletes-or-dare-to-take-a-break.html). Bacteria are usually observed by electron microscopy only for experimental reasons. *Listeria monocytogenes* from haemoculture of a febrile patient cultivated previously on a nutrient agar (B)