MEDICAL MICROBIOLOGY PRACTICES FOR 3RD YEAR STUDENTS
Academic year 2020/2021: Semester 1 (Fall semester)

Week 1	**08, 09, 10, 11 September**

1. Introduction:
   - Important information
   - Types, set-up, and organisation of microbiological laboratories
   - **Rules and instrumentation of the safe handling of microbes**
   - **Safety in the laboratory**

2. Microscopic morphology: Microscopic examinations and their information content

A. Making native preparations
   - Preparation of **Wet-mounts** and **hanging-drops** from bacterial (*Proteus sp.*), fungal (baker's yeast) suspensions. **Vital staining**.

B. Making stained preparations

Preparation of smears (*Escherichia coli, Staphylococcus epidermidis, Bacillus cereus)*
   - Simple staining (**methylene blue**)
   - **Gram-staining** on *E. coli, S. epidermidis, B. cereus* smears

3. Ready made smears: Gram stained (Gram-positive and Gram-negative) bacteria

Week 2	**15, 16, 17, 18 September**

Cultivation of bacteria

1. Bacteriological culture media: types, as well as rules of the preparation of different media
   - **Liquid and solid, transport, enrichment media**
   - **Dextrose bouillon**, Holman bouillon, demonstration of bacterial growth in liquid media
   - Preparation of solid media: **high- and slant agar, agar plate**, as well as **blood** and **chocolate agar**
2. **Parameters** and **instrumentation** of the **aerobic culture**
3. **Parameters** and **instrumentation** of the **anaerobic culture: anaerostat**, **wax seal (thioglycolate, Holman)**, special methods (gas-pack systems CO_2, H_2/CO_2), anaerobic chamber
4. **Differentiating and Selective media**

   Study on the lactose metabolism of bacteria:
   - The **eosin-methylene-blue medium** (EMB)
5. **Inoculation** of bacteria onto different media
   - Definition of a **pure culture**, a bacterial **isolate,** as well as **strain**
   - Definition of a transport medium, as well as a transport-culture medium: **Stuart-transport medium** and the **Uricult Plus-system**
   - **Inoculation into** bouillon, agar plate, slant, as well as high agar from *Staphylococcus epidermidis*, *Escherichia coli* cultures
6. Examination of the morphology of bacterial colonies:
   - Definition and methods of the determination of the **germ-count**, as well as the **colony-forming unit (CFU)**
   - Determination of the number of cell-divisions, as well as the **generation time**

Sample cultures: ***S. aureus, S. epidermidis, Bacillus cereus*** **(agar plate, blood agar);** ***Streptococcus pyogenes, S. mitis, S. pneumoniae*** **(blood, chocolate agar);** ***Klebsiella sp., Proteus sp., Serratia sp., Pseudomonas aeruginosa,*** **(agar plate. EMB);** ***Haemophilus influenzae*** **(chocolate agar)**

Testing the germ-content in the laboratory air on blood agar: 2 blood agar, 2open for for 60 minutes, then incubation on 20 and 37 degrees.

Week 3	22, 23, 24, 25 September

Sterilisation

1. Safe handling, storage, labelling, and annihilation of infectious materials of common and of hospital origin
2. Demonstration of the methods of sterilisation
   (a) Chemical: **gas sterilisation**, **plasma sterilisation**
   (b) Physical (**dry heat chamber**, **hot saturated steam (autoclave)**, **γ-radiation**)
   (c) Parameters of inactivation of prions
3. **Filtration of bacteria,** preparation of **pyrogen-free** solutions
4. Quality control of the process of sterilisation
   (a) Physical: monitoring of the parameters of sterilisation (thermometers, manometers)
   (b) Chemical (heat-sensitive dye, paper strips),
   (c) (Micro)biological (*Bacillus/Geobacillus spp.* spores)

Disinfection

1. **Methods of hand and skin disinfection** (hygienic *versus* surgical hand disinfection, disinfection of the skin before operation)
2. **Decontamination of medical instruments**, **annihilation of single-use instruments** (**containers for infectious waste**, **needle and syringe destructor**, **and incineration**).
3. **Decontamination of infectious materials of the patients**
4. Inoculation from the skin and underneath the nails onto blood agar before and after hand disinfection

Preservation, conservation
Microbiological control of drugs, sterility tests, LAL-test

Evaluation of the plates from last week, description: germ-count, colony morphology

Week 4	29, 30 September; 01, 02 October

Antibiotics and antimicrobial chemotherapy

1. Evaluation of the inoculations from last week, description: colony morphology (Worksheet-3)

- Antibacterial chemo- and antibiotic therapy*:*
  A. Methods of the study of the effect of chemotherapeutic agents, as well as antibiotics
  - Dilution methods: macro- and micro-dilution, liquid, as well as agar break point, determination of the minimal inhibitory and the minimal bactericidal concentration **(MIC, MBC)** with macro and micro-dilution
  - Diffusion methods: punching and paper disc diffusion, E-test
  - Microbiological monitoring of the antibiotic drug level of the serum

  B. Demonstration of a commercial disc diffusion method: **„Kirby-Bauer"**
  Examples for the:
  - **Natural sensitivity:** penicillin disc on *S. pyogenes* culture
  - **Natural resistance**: penicillin disc on *E. coli,* vancomicyn disc on *H. influenzae* cultures
  - **Cross-resistance**: penicillin, oxacillin, cephalosporin discs on methycillin sensitive *versus* methicillin resistant *S. aureus* (MSSA *vs.* MRSA) cultures
  - **Poly-resistance**: *P. aeruginosa, E. faecalis* cultures

  C. Demonstration of the tube and micro-plate dilution methods
  D. L-form in a native preparation or slide
  E. Determination of the antibiotic susceptibility of different bacteria with the **paper disc and E-test method** (*S. aureus, E. coli, Proteus sp.* on Mueller-Hinton agar)

Week 5.	06, 07, 08, 09 October

Interpretation of the EUCAST breakpoints based on www.eucast.org (excel table)
Characterisation of the most important antibiotic resistant bacteria: MRSA, MRSE, ESBL, VRE

Serological reactions

1. Evaluation of the inoculations from last week – antibiogram
2. Serological reactions
   A. Agglutinations
      (a) Qualitative: **slide agglutination** (agglutinating *E. coli* or the "Wellcogen" antigen detection test), latex agglutination
      (b) Quantitative: tube agglutination (**Widal's type tube agglutination**)
   B. Precipitation
      In liquid medium: **disc precipitation**, quantitative,
      Agar-gel (**immune-diffusion method**)
   C. Immunofluorescent (IF) assays: **direct-** and **indirect-IF**
   D. Radioimmunoassay (**RIA**)
   E. Enzyme linked immunosorbent assay (**ELISA**)
   (a) Evaluation of the results of serological reactions (**fresh *versus* past infections**: a "pair of sera")
   Typisation methods: serotyping, phage-typing, MALDI-TOF, PFGE

Week 6	13, 14, 15, 16 October

Gram-positive cocci I.

Staphylococcus

1. Identification of strains of *Micrococcus sp.* and *Staphylococcus sp.*:
   (a) Based on the **nitrofurantoin resistance** (*Micrococcus sp.*) **/ susceptibility (***Staphylococcus sp.*)
   (b) Voges–Proskauer (theory): *Micrococcus sp.* (-) and *S. aureus* (+)
   (c) O-F culture media
2. Sample cultures:
   S. aureus, S. epidermidis*, S. haemolyticus, S. saprophyticus*: **agar** and **blood agar** medium, *S. aureus* in dextrose bouillon
3. Biochemical tests characteristic for staphylococci:

Evaluation of the inoculations from last week (own nose)

(a) **Catalase-reaction** catalase test/own sample
(b) Coagulase tests: tube-, slide **(clumping factor)**, commercial kits: *S. aureus* (+) and *S. epidermidis* (-) clump test/own sample
Demonstration of the **novobiocin** resistance / susceptibility of ***S. saprophyticus*** (R) and ***S. epidermidis*** (S)

4. Demonstration of antibiotic susceptibility tests („Kirby-Bauer" method): MRSA *vs.* MSSA
5. Theoretical foundation and practice of the **phage -typing**
6. Sample slides: ***Staphylococcus sp***. and *Micrococcus sp.* with **Gram-stain,**

Week 7	20, 21, 22 October; 23 Public Holiday

Gram-positive cocci II.:

Streptococcus

1. ***Streptococcus pyogenes*** and ***S. agalactiae***
   (a) Sample cultures: on **blood agar** media,
   S. pyogenes in bouillon
   (b) Lancefield typing
   (c) **CAMP**-test: S. agalalactiae (+), S. pyogenes (-)
2. ***S. pneumoniae*** and ***S. mitis***
   (a) Sample cultures: on **blood agar** media
   (b) Testing the **optochin susceptibility**: ***S. pneumoniae*** (S) and ***S. mitis*** (R)
   (c) Serological identification of *S. pneumoniae* with slide agglutination ("Slidex Pneumo" kit)
   (e) **Vaccines** ("Pneumovax 23" and Prevenar 13 package and package insert)
3. *E. faecalis*
   (a) Sample cultures: on blood- and chocolate agar media
   (b) Demonstration of the growth of *E. faecalis* in 6.5 % NaCl containing medium at 45 ^{o}C
   (c) ***E. faecalis*** on **Uricult Plus** dip-slide
5. **AST, CRP**
6. Antibiotic susceptibility tests ("Kirby-Bauer" method):
   (a) *S. pyogenes* (Note: penicillin S)
   (b) *E. faecalis* (Note: natural cephalosporin resistance, aminoglycoside R or, vancomycin S)
7. Sample slides**: *S. pyogenes, S. pneumoniae*** with **Gram-stain**

Week 8	27, 28, 29, 30 October

Identification of Gram-positive aerobic rods

1. Irregular, non-spore-forming Gram-positive rod
   (A) ***Corynebacterium diphtheriae*** on **Löffler** and **Clauberg** media
   (B) Testing the sugar break-down (pathogenic *versus* apathogenic Corynebacterium spp.)
   + ~~API Coryne strip (demonstration)~~
   (C) Testing the virulence (**Elek-test,** positive slide only): toxin producing *versus* non-producing *C. diphtheriae* strains
   (D) Methods and instrumentation of sampling
   (E) **Vaccines** (DPT, DT), as well as **antitoxins**
   Sample slides: ***C. diphtheriae*** with **Neisser** and **Gram-stain** in fixed smear
   Neisser-staining/Albert staining (steps on ready smear)

Regular Gram-positive rods

(A) *Lactobacillus*
   (a) Sample culture: Rogosa-agar
   (b) "Bonolact" product, pro and prebiotics
   (c) Sample slide: *Lactobacillus sp.*
(B) *L. monocytogenes* on agar and blood agar medium
(C) *Erysipelothrix rhusiopathiae* (slides only)

Week 9	03, 04, 05, 06 November

Inoculation of own nose or throat sample on blood-agar, chocolate agar and Clauberg medium

Gram negative cocci

Neisseria genus and *Moraxella*

(a) *N. gonorrhoeae, N. meningitidis, N. pharyngitidis* cultures (on agar, blood agar, chocolate and special media).

(b) Sealed *N. gonorrhoeae* sample culture with the oxidase reaction

(c) **Oxidase test** with Pseudomonas aeruginosa *sp.* on agar medium

(d) The "Gonoline" sampling and culture system (package, as well as description of the kit)

(e) Sample slides: ***Neisseria sp.*** (from pure culture) with **Gram-stain**, **gonorrhoeal discharge with methylene-blue stain**

(f) Wellcogen video (detection of meningitis antigens)

Gram negative coccobacilli

1 *Haemophilus* genus

(a) Sample cultures: ***H. influenzae*** **blood agar medium**

(b) **Satellite phenomenon on blood agar medium:** ***H. influenzae*** with *S. aureus*

(c) ***H. influenzae*** (XV+) and *H. parainfluenzae* (XV+, V+) with **X- and V- factor-discs** (Sims-discs) on agar medium

(d) Demonstration of the **vancomycin resistance**, as well as ampicillin and amp+clav susceptible *versus* resistant ***H. influenzae*** strains

(e) **Vaccines** (e.g. package and package insert of the "Act-HIB", "Hiberix", "PedvaxHIB" vaccines)

(f) Sample slide: ***Haemophilus sp.*** with **Gram-stain**

2. *Bordetella* genus

(a) Sterile Bordet-Gengou medium for the cultivation of *Bordetella spp.*

3. *Brucella* genus

(a)**Wright reaction**

4. *Francisella* genus and *Y. pestis*: ppt-slides only

Gram negative pleomorph rod

Acinetobacter genus - sample culture

Gram negative aerobic rods

1. *Pseudomonas* genus

(a) Sample cultures: ***P. aeruginosa*** on **agar-** and **blood agar** medium

(b) **oxidase reaction** on glass slides

(c) pigment extraction with chloroform

(d) *P. aeruginosa* on OF-medium (O+F-)

(e) Antibiotic susceptibility tests: carbenicillin S and R, as well as poly-resistant *P. aeruginosa*

2. *Legionella* genus

(a) Sterile BCYE (Buffered Charcoal Yeast Extract) agar and *Legionella pneumophila* on BCYE, BinaxNow test

Vaccines: (Obligatory: DPT, Meningococcus, HiB; possibility: tularaemia, brucellosis), Calendar

Week 10	**10, 11, 12, 13 November**

Evaluation of the inoculations from last week (own ear and throat), simple tests – catalase, clump, oxidase

1. *Enterobacteriaceae*
   A. *E. coli, Klebsiella sp., Proteus sp., Serratia sp.:*
      (a) ***E. coli* on agar** and **EM** media
      (b) ***Proteus sp.*** on **agar** and **EM** media
      (c) ***Klebsiella sp.*** on **agar** and **EM** media
      (d) *S. marscescens* on agar medium (red pigment!)
   B. *Salmonella sp., Shigella sp., Yersinia sp.* strains
      (a) ***S. typhi*** with ***E. coli*** on **brilliant-green** and ***S. typhi*** on **bismuth-sulphite** media
      Characteristic biochemical reactions of *Salmonellae,*
      (a) **Gruber-Widal** reaction
      (b) **Vaccine:** package and package insert of the monovalent typhus vaccine
      (c) ***Shigella sp.*** and ***E. coli* EM**, as well as on **DC** media
      (d) *Y. enterocolitica* on DC medium
      (e) Sampling methods and instruments
   C. Biochemical reactions and special media
      (b) urease activity: Christensen and UI medium
      (c) indole test: *E. coli* + in UI medium
      (d) H_2S production: Fe-high agar,Bi-sulphite medium, *Proteus* and *Salmonellae* +
      (e) Sugarfermentation, gas-production and H_2S production together: TSI
2. *Vibrionaceae*
      (a) **TCBS medium**, sterile
      (b) **Vaccine**: Package and description of the cholera vaccine. Critics of the vaccination

Sample slides: ***E. coli*** with **Gram-stain**, L-form, demonstration of the capsule with India ink stains (*Klebsiella sp.*)

Slide agglutination (*E. coli*), serotyping polyvalent and specific sera

Week 11	17, 18, 19, 20 November

3. Spore-forming bacteria

(A) Gram-positive aerobic spore-forming rods: *Bacillus* genus

(a) ***B. cereus*** **on agar, blood agar and egg-yolk media (**lecitinase +).

Sample slides: ***Bacillus anthracis*** or ***B.cereus*** with **Gram-stain**

Dia positive-slides

(B) Gram-positive anaerobic spore-forming rods: *Clostridium* genus

(a) ***C. tetani*** and **gas-gangrene clostridia** on Zeissler-plate

(b) **Holman** and **thyoglycholate** media

(c) *C. difficile* on SABA

(d) C. difficile on CCFA agar and rapid toxin detection tests

(e) **Vaccines** (DPT, DT, TANAT)**, antitoxins** (TETIG 500), antitoxic sera against gas-gangrene, "Serum Antibotulique"

(f) Sample slides: ***C. tetani*** and **gas gangrene clostridia** with Gram-stain

Making smear, Gram staining and Spore-staining (ZN, *B. cereus*)

1. Microaerophilic bacteria

A. Methods and **parameters** of **microaerophilic cultivation**

B. *Campylobacter, Helicobacter*

(a) Sample cultures: *Campylobacter sp.* and *Helicobacter pylori*

(b) Presentation of commercially available rapid diagnostic tests

(c) Radioactive urea/CO_2 Expiration test

2. Non-sporeforming strict anaerobic bacteria

Bacteriodes-group, *Prevotella*, *Porphyromonas*, *Fusobacterium* and *Peptostreptococci (Finegoldia magna)*

(A) Methods of sampling and culture in infections caused by obligate anaerobic bacteria: anaerobic sampling systems, **anaerostat**, **wax seal, Holman and thioglycolate media**

(B) *Peptostreptococcus spp. o*n selective anaerobic blood agar (SABA)

(C) *Bacteroides fragilis* on SABA

(D) Identification according to the fatty-acid spectrum, MALDI-TOF

(E) Antibiotic susceptibility tests of anaerobic by the agar plate dilution, E-test method

(F) Sample slide: Plaut-Vincent disease

(G) Videotape: anaerobic infections

Week 12	24, 25, 26, 27 November

Systematic Bacteriology VII.

Acid-fast rods

1. *Mycobacterium* genus
   - ***M. tuberculosis*** on **Löwenstein**, **Sula**, Sauton, Dubos media
   - Apathogenic mycobacteria on Löwenstein medium
   - Application of the **PCR-technique in the rapid diagnosis of tuberculosis**

   Performing the **acid-fast- or Ziehl-Neelsen stain** on pre-fixed smears of sputum from tuberculosis patients

   Sample slide: direct **Koch-positive sputum** with **Ziehl-Neelsen-stain**
   - **Vaccine** (Package and description of the BCG vaccine)
   - **Tuberculin reaction** (theory and evaluation of the test)
   - **Sampling kits**

2. *Nocardia* genus - Petri dish
3. *Streptomyces sp*.

   Sample culture: *Streptomyces sp.* on agar medium

4. Anaerob: *Actinomyces sp. – Petri dish*

Week 13	01, 02, 03, 04 December

Spirochaetes

1. *Treponema* genus
   (a) Syphilis serology: presentation of the (CL, RP), **RPR**-reactions, **FTA, TPHA**
   (b) Molecular diagnostics of syphilis
   (c) Sample slide: Plaut-Vincent disease

2. *Leptospira* genus
   (a) fixed **leptospira smear** with **silver-impregnation**
   (b) **Sterile Korthof** medium and **leptospira culture in Korthof** medium

3. *Borrelia* genus
   (a) Ticks on ppt-positive slides, **the tick-forceps**, rules of the **state of art removal of ticks** from the surface of the skin
   (b) ELISA and PCR in the diagnosis of Lyme's disease

Week 14	08, 09, 10, 11 December

1. Rickettsiae
   Rickettsia prowazekii
   (a) The body louse on ppt-positive slides
   (b) Propagation (embryonated chick-eggs: opening the eggs, demonstration of the culture sites)
   (c) **Weil-Felix** reaction
2. Chlamydiae
   (a) **Sampling techniques and instruments**
   (b) Propagation (sterile McKoy-cells)
   (c) IF-pictures on ppt-slides
   (d) ELISA

3. Mycoplasmatales:
   (a) Culture: sterile and inoculated liquid mycoplasma media (BEA, BEG),
   (b) Detection of antibodies: ELISA, IF

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