

Pharmaceutical Microbiology
LABORATORY PRACTICES
Spring semester of 2019/2020

1.

4 February

Introduction to basic microbiology, laboratory rules.

Microscopic examination of microbes

Wet mount, hanging drop, vital staining of yeast.

Preparation of smears (*S. epidermidis*, *E. coli*, *B. cereus*)

Simple and Gram staining.

2.

11 February

Cultivation of bacteria

Culture media (broth, agar, blood agar and chocolate agar plates, slant and stab agar)

Cultivation of anaerobic bacteria (supplemented thioglycolate and Holman (chopped meat) medium, GasPak jar)

Selective/differential culture media (Eosin-methylene blue: *E. coli*, *Proteus*)

Haemoculture.

Colony morphology of bacteria (*S. epidermidis*, *S. aureus*, *S. pyogenes*, *S. pneumoniae*, *B. cereus*, *E. coli*, *Klebsiella*, *P. aeruginosa*, *Proteus*, *Serratia*)

Preparation of pure culture (isolated colonies) of bacteria.

3.

18 February

Evaluation of the preparations of previous practice.

Methods for sterilization and disinfection

(Physical and chemical possibilities)

Control of sterility (culture media: thioglycolate and glucose containing broth media)

Examination of the effect of an antiseptic on the bacterial flora of the skin (before and after hand washing with disinfectant).

4.

25 February

Evaluation of the preparations of previous practice.

Antibiotic susceptibility of bacteria

Measurement of antibacterial activity:

dilution methods: broth and plate dilution tests

diffusion tests: filter paper disc methods; E-test (gradient test).

Examples for antibiotic susceptibility (*S. aureus*, *E. coli*, *Proteus*, *P. aeruginosa*)

Determination of gentamicin concentration in patient serum

Determination of antibiotic susceptibility of different bacteria (*S. aureus*, *E. coli*, *P. aeruginosa*)

5.

3 March

Evaluation of the antibiotic susceptibility tests from previous practice.

In vitro antigen-antibody reactions (serological methods)

Agglutination (qualitative: serotyping of *E. coli* on slide; quantitative: tube dilution test)

Precipitation (ring, agar gel diffusion test, immunoelectrophoresis)

Complement fixation (CF)

Enzyme-linked immunosorbent assay (ELISA)

Immunofluorescent methods

Inoculation of throat samples on blood agar.

6.

10 March

Midterm I.: General microbiology

Gram positive cocci (Staphylococci, Streptococci)

Cultures: broth cultures of *S. aureus* and *S. pyogenes*; agar plates: *S. epidermidis*, *S. aureus*;

blood agar plate: *S. epidermidis*, *S. aureus*, *S. pyogenes*, *S. pneumoniae*, *S. mitis*, *E. faecalis*;

chocolate agar plate: *S. pyogenes*, *S. mitis*, *S. pneumoniae*

Smears: *S. aureus*, *S. pyogenes*, *S. pneumoniae* (Gram stained)

Biochemical tests: catalase test on slide (*Staphylococcus*/*Streptococcus*)

coagulase test (*S. aureus*, *S. epidermidis*)

Serological tests: ASO, CRP

Antibiotic susceptibility of *S. aureus*, *S. pyogenes*, *S. pneumoniae*, *E. faecalis*

7.

17 March

Gram negative cocci

Culture: *N. gonorrhoeae* and *N. meningitidis* on chocolate agar plate

Smears: Gram-stained smear of *Neisseriae* and methylene-blue stained smear of endocervical exudate

Biochemical reaction: oxidase test

Gram-negative non-fermenting rods.

Pseudomonas

Legionella

Gram-negative coccobacilli

Haemophilus on blood agar (satellite phenomenon) and chocolate agar

Bordetella.

Brucella, *Francisella*.

Video: Latex agglutination in meningitis diagnostics.

8.

24 March

Enteric Gram-negative rods (Enterobacteriaceae)

Cultures: agar plates: *E. coli*, Klebsiella, Proteus

Differential and selective media: Eosin methylene blue (EMB): *E. coli*, Klebsiella, Proteus

Deoxycholate-citrate (DC) agar: Shigella and *E. coli*; Bismuth sulphite medium: *S. typhi*

Smears: *E. coli* (Gram stain), Klebsiella (capsule staining)

Serology: Widal test (tube dilution test: *S. typhi*)

Biochemical reactions: carbohydrate fermentation with and without gas production in solid media (*E. coli*, Proteus, Klebsiella, Shigella, *S. typhi* and *S. paratyphi*), urea fermentation (*E. coli*, Klebsiella, Proteus), H₂S production (Proteus), indol test: (*E. coli*)

Vibrio

Helicobacter, Campylobacter

9.

31 March

Mycobacteria

Cultures: Löwenstein-Jensen, Sula

M. tuberculosis and *M. bovis*, atypical and apathogenic mycobacteria

Stained smear of sputum (Ziehl-Neelsen staining)

Vaccine

Non-spore forming Gram-positive rods

Cultures: *C. diphtheriae* on Löffler's coagulated serum medium and on tellurite plate

Smear of *C. diphtheriae* (Neisser stain)

Vaccine

10.

6-10 April - Spring Holiday

11.

14 April

Endospore-forming Gram-positive rods

Cultures: *B. cereus* on agar plate

C. perfringens and *C. tetani* in liquid media (Holman and thioglycolate)

Smears: Gram-stained preparations of endospore-forming bacteria

Obligate anaerobic bacteria

Bacteriodes-group, Prevotella, Porphyromonas, Fusobacterium and Peptostreptococci.

Methods for cultivation of obligate anaerobic bacteria: anaerostat, Holman and thioglycolate media, GasPak jar.

Vaccination calendar

12.

21 April

Spirochetes

Treponema pallidum: morphology and disease

Serology: non-treponemal and treponemal antibody test (FTA)

Borrelia

Leptospira (cultivation in Korthof medium, silver impregnation)

Rickettsia, Chlamydia, Mycoplasma

Embryonated egg for cultivation

Serology: Weil-Felix reaction

13.

28 April

Midterm II: Bacteriology

Medically important fungi

Cultures: *C. albicans*, Penicillium, Aspergillus, Mucor on Sabouraud plate

Block preparations of different moulds

Smear: *C. albicans* (methylene-blue)

14.

5 May

General virology

Cultivation of viruses: embryonated egg, tissue cultures

Morphology of viruses

Cell-virus interactions (CP on tissue culture, HA in microplate)

Serological tests: HI, CF, IF

Tests for detection of nucleic acid (PCR, hybridization)

Bacteriophages (morphology and phage typing)

15.

12 May

Medical parasitology

Most important protozoa (microscopic preparations)

Most important helminths (macroscopic and microscopic preparations)

Videofilms.