Week 1 11, 12, 13, 14 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-monts 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, Candida albicans).
      - Simple staining (methylene blue) on C. albicans smear
      - Gram-staining on E. coli, S. epidermidis, B. cereus smears

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

Week 2 18, 19, 20, 21 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 CO₂, H₂/CO₂), commercial anaerobic diagnostic systems (e.g. Sceptor)

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); Haemophilus influenzae (chocolate agar)

Testing the germ-content in the laboratory air on blood agar: 4 blood agar, 2 open for 5’, 2 open for 60’, then incubation on 20 and 37 degrees.
**Week 3  25, 26, 27, 28 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 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

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

**Week 4  02, 03, 04, 05 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, vancomycin disc on H. influenzae cultures
        - Cross-resistance: penicillin, oxacillin, cephalosporin discs on methicillin 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.
     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. 09, 10, 11, 12, October**

Interpretation of the EUCAST breakpoints based on www.eucast.org (excel table)

Characterisation of the most important antibiotic resistant bacteria: MRSA, MRSE, SBL, VRE

**Serological reactions**

1. Evaluation of the inoculations from last week – antibiogramme, worksheet-4
2. Serological reactions
   A. Agglutinations
      (a) Qualitative: **slide agglutination** (agglutinating *E. coli* or the "Wellcogen" antigen detection test)
      (b) Quantitative: tube agglutination (**Widal's type tube agglutination**)
   B. Precipitation
      In liquid medium: **disc precipitation**, quantitative, flocculation
      Agar-gel (**immune-diffusion method**): two-dimension, immunoelectrophoresis, immunosmophoresis
   C. Immunofluorescent (**IF**) assays: **direct-** and **indirect-IF**
   D. Radioimunoassay (**RIA**)
   E. Enzyme linked immunosorbent assay (**ELISA**)
      (a) Immune-cytolytic reactions: bacteriolysis and haemolysis, complement titration, complementfixation test (**CF** (**micro-litre plate**))
      (b) Evaluation of the results of serological reactions (**fresh versus past infections**: a “pair of sera”)

Typisation methods: serotyping, phage-typing, MALDI-TOF

**Week 6 16, 17, 18, 19 October**

**Mid-term exam I. (45 minutes)**: Topics of General Bacteriology and Immunology covered: Weeks 1–4 (lectures) and weeks 1–5 (practices)

*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 (**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** (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)

Inoculation of own nose sample on blood-agar, chocolate agar and Clauberg medium
**Week 7**

<table>
<thead>
<tr>
<th>23 Public Holiday, 24, 25, 26 October</th>
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<tbody>
<tr>
<td><strong>For Thursday and Friday groups next week topic must be included.</strong></td>
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<tr>
<td>Evaluation of own nose sample on blood-agar, chocolate agar and Clauberg medium.</td>
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<tr>
<td><strong>Gram-positive cocci I.</strong></td>
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<tr>
<td><strong>Staphylococcus</strong></td>
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<tr>
<td>1. Identification of strains of <em>Micrococcus</em> sp. and <em>Staphylococcus</em> sp.:</td>
</tr>
<tr>
<td>(a) Based on the nitrofurantoin resistance (<em>Micrococcus</em> sp.) / susceptibility (<em>Staphylococcus</em> sp.)</td>
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<tr>
<td>(b) Voges–Proskauer (theory): <em>Micrococcus</em> sp. (-) and <em>S. aureus</em> (+)</td>
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<tr>
<td>2. Sample cultures:</td>
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<tr>
<td><em>S. aureus</em>, <em>S. epidermidis</em>, <em>S. haemolyticus</em>, <em>S. saprophyticus</em>: agar and blood agar medium, <em>S. aureus</em> in dextrose bouillon</td>
</tr>
<tr>
<td>3. Biochemical tests characteristic for staphylococci:</td>
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<tr>
<td>Evaluation of the inoculations from last week (own nose)</td>
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<tr>
<td>(a) <strong>Catalase-reaction</strong> catalase test/own sample</td>
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<tr>
<td>(b) Coagulate tests: tube-, slide (clumping factor), commercial kits: <em>S. aureus</em> (+) and <em>S. epidermidis</em> (-) clump test/own sample</td>
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<tr>
<td>Demonstration of the <strong>novobiocin</strong> resistance / susceptibility of <em>S. saprophyticus</em> (R) and <em>S. epidermidis</em> (S)</td>
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<tr>
<td>4. Demonstration of antibiotic susceptibility tests („Kirby-Bauer” method): MRSA vs. MSSA</td>
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<tr>
<td>5. Theoretical foundation and practice of the <strong>phage-typing</strong></td>
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<tr>
<td>6. Sample slides: <em>Staphylococcus</em> sp. and <em>Micrococcus</em> sp. with Gram-stain,</td>
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<td>7. Inoculation of samples taken from the surface of the skin onto blood agar medium</td>
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**Week 8**

<table>
<thead>
<tr>
<th>30, 31 October, 01, 02 Nov – public Holiday</th>
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<tbody>
<tr>
<td><strong>For Tuesday group previous topic included.</strong></td>
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<tr>
<td>Evaluation of the inoculations from last week (own handskin/nail), description: colony morphology, simple tests (catalase, clump) Worksheet-6</td>
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<tr>
<td><strong>Gram-positive cocci II.:</strong></td>
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<tr>
<td><strong>Streptococcus</strong></td>
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<tr>
<td>1. <em>Streptococcus pyogenes</em> and <em>S. agalactiae</em></td>
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<tr>
<td>(a) Sample cultures: on blood agar media, <em>S. pyogenes</em> in bouillon</td>
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<tr>
<td>(b) Lancefield typing</td>
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<tr>
<td>(c) <strong>CAMP</strong>-test: <em>S. agalactiae</em> (+), <em>S. pyogenes</em> (-)</td>
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<tr>
<td>2. <em>S. pneumoniae</em> and <em>S. mitis</em></td>
</tr>
<tr>
<td>(a) Sample cultures: on blood agar media</td>
</tr>
<tr>
<td>(b) Testing the <strong>optochin susceptibility</strong>: <em>S. pneumoniae</em> (S) and <em>S. mitis</em> (R)</td>
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<tr>
<td>(c) Serological identification of <em>S. pneumoniae</em> with slide agglutination (&quot;Slidex Pneumo&quot; kit)</td>
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<tr>
<td>(e) <strong>Vaccines</strong> (&quot;Pneumovax 23&quot; and Prevenar 13 package and package insert)</td>
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<tr>
<td>3. <em>E. faecalis</em></td>
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<tr>
<td>(a) Sample cultures: on blood- and chocolate agar media</td>
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<tr>
<td>(b) Demonstration of the growth of <em>E. faecalis</em> in 6.5 % NaCl containing medium at 45 °C</td>
</tr>
<tr>
<td>(c) <em>E. faecalis</em> on Uricult Plus dip-slide</td>
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<tr>
<td>5. <strong>AST, CRP</strong> (transparency foil and package insert of the kit)</td>
</tr>
<tr>
<td>6. Antibiotic susceptibility tests (&quot;Kirby-Bauer&quot; method):</td>
</tr>
<tr>
<td>(a) <em>S. pyogenes</em> (Note: penicillin S, fluoroquinolon, )</td>
</tr>
<tr>
<td>(b) <em>E. faecalis</em> (Note: natural cephalosporin resistance, aminoglycoside R or, vancomycin S)</td>
</tr>
<tr>
<td>7. <strong>Sample slides</strong>: <em>S. pyogenes</em>, <em>S. pneumoniae</em> with Gram-stain</td>
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Week 9 | 06, 07, 08, 09 November, 10 November – Friday groups

For Friday group on 10th of November consultation, repetition – or week 8 topic

**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 apathogenic *Neisseria sp.* on agar medium

(d) The “Gonoline” sampling and culture system (transparency foil, and 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 ag-s)

**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*: dia-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

All: dia-slides

**Vaccines**: (Obligatory: DPT, Meningococcus, HiB; possibility: tularaemia, brucellosis), Calendar
Week 10  13, 14, 15, 16 November

**Spirochaetes**

1. *Treponema* genus
   - (a) Syphilis serology: presentation of the (CL, RP), VDRL-, RPR-reactions and transparency foils about the specific reactions (FTA, TPHA, TIT)
   - (b) Molecular diagnostics of syphilis
   - (c) Sample slide: Plaut-Vincent disease
   - (d) Dia-positive slides

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 dia-positive slides and transparency foils, 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
**Mid-term exam II. (45 min.)**
Topics of Systematic Bacteriology covered: weeks 6-10 practices; weeks 5-10 lectures

**Systematic Bacteriology VI/A.**

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/CO₂ 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.* on 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  27, 28, 29, 30 November

Sample collecting from external ear and inoculation onto blood and chocolate agar and Clauberg medium, as well of pharyngeal sample, + making smear and simple staining.

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 +).
      (b) Dia-positive slides
      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” and dia-positive slides
      (f) Sample slides: C. tetani and gas gangrene clostridia with Gram-stain

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

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
     (transparency foils)
   - Dia-positive slides
   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: dia-positive slide)
   - 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  04, 05, 06, 07 December
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) H₂S production: Fe-high agar,Bi-sulphite medium, Proteus and Samonellae +
      (e) Sugarfermentation, gas-production and H₂S 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 14  11, 12, 13, 14 December
Systematic Bacteriology IX.

1. Rickettsiae
   *Rickettsia prowazekii*
   (a) The body louse on dia-positive slides and transparency foils
   (b) Propagation (embryonated chick-eggs: opening the eggs, demonstration of the culture sites)
   (c) Weil-Felix reaction

2. Chlamydiae
   (a) **Sampling techniques and instruments** (also transparency foils)
   (b) Propagation (sterile McKoy-cells)
   (c) IF-pictures on dia-positive slides
   (d) ELISA
   (e) Dia-positive slides

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

REPETITION; RETAKES (midterm, practice)

1st of September 2018

Dr. Dóra Szabó  
Director

Dr. Ágoston Ghidán  
Tutor