Institute of Medical Microbiology
Syllabus of the Practical Classes 2nd Semester 2019/2020

Week 1 **Feb 3, 4, 5, 6, 7.**

Medical mycology: microbiological diagnosis of fungal infections

1) Fungal infections on slides
2) Methods and instrumentation of sampling, preparation of the specimen for further investigations
3) Ready-made microscopic preparations:
   - ***Candida albicans*** from culture with **methylene-blue staining**
   - *Candida albicans* in direct smear (**vaginal discharge**)
   - *Aspergillus, Mucor, Penicillium* in block cultures
   - Pneumocystis (lung tissue, silverimpregnation)
4) *Candida albicans*: preparation of vital staining with methylene-blue dye
5) **Preparation of a fixed smear and methylene-blue staining of** *Candida albicans* from culture
6) Sample cultures: ***Candida****, Aspergillus, Mucor, Penicillium* on **Sabouraud** medium and on chromagar
7) Inoculation of *Candida albicans* onto **Sabouraud** medium
8) Examination of the susceptibility/resistance of fungi towards antifungal agents:
   - With ε-test (e.g. *Candida sp*. Diflucan ε-test)
     Ambisome film

Week 2 **Feb 10, 11, 12, 13, 14.**

PROTOZOOLOGY 1.

Microbiological diagnosis of infections caused by Protozoa

1) Evaluation of the cultures prepared at the previous class
2) Instrumentation and methods of sampling: **faeces container, gastro-duodenal-tube, glass slides for the diagnosis of malaria**
3) Ready-made microscopic preparations: *Giardia lamblia* in duodenal fluid***, Trichomonas vaginalis*** **in vaginal discharge**, **malaria plasmodia** and t**rypanosomes in blood thin-film,** as well as **thick-film** („long" **Giemsa** staining), Cryptosporidia and Cyclospora with ZN staining (stool, AIDS patient), Leishmania
4) Antiprotozoal drugs (tables)

Films: Trypanosoma (14 min), Leishmania (12 min), Chagas (9 min), Malaria (15 min.), Trypanosoma locomotion, Amoeba moving, Trichomonas moving

Week 3 **Feb 17, 18, 19, 20, 21.**

Protozoology I. and II. – For groups from Wednesday and Thursday

PROTOZOOLOGY II. – Continuation

Week 4	**Feb 24, 25, 26, 27, 28.**

HELMINTHOLOGY I.
Microbiological diagnosis of worm infections I.

Instrumentation and methods of sampling: **faeces container**, cellotape

1. Ready-made microscopic preparations: worm eggs in native preparations (*Taenia, Hymenolepis, Ascaris, Trichuris* and ***Enterobius vermicularis* egg cellotape** prep), Loa loa in thick blood film
2. Macroscopic preparations
3. Antihelminthic drugs (tables)

Ppt slides and Films **about medically important worms (Zentel), Filariasis (8+9+27 min); Onchocerca (8 min); Schistosoma (15 min),** „Human wildlife" **– Spektrum (45 min), Scrotum elephantiasis (5mp); Anisakis (kb. 2,5 min.); Ascaris – bile, - duodenum, - gut (kb. 2+2+1,5 min.); Echinococcus ; Enterobius (kb. 1 min.), Enterobius moving (music, 40 mp); Fasciola (kb. 1,5 min); Loa-loa (kb. 1,5 min); 3 Trichinella short (össz. kb. 1 min)PARASITOLOGY 3.**
Microbiological diagnosis of worm infections I-II

Week 5	**March 2, 3, 4, 5, 6.**

HELMINTHOLOGY II. – Continuation
Parazitozoonoses: Toxocara, Trichinella, Dirofilaria

Week 6	**March 9, 10, 11, 12, 13.**

General virology
Morphology, direct detection, propagation, and identification of viruses

1) Propagation of viruses: cell and tissue cultures, embryonated egg, laboratory animals, characteristic cytopathic effects (CP)
2) Direct effect of viruses on red blood cells**: haemagglutination (HA)**, **haemadsorption (Had)**
3) **Titration of viruses, as well as bacteriophages**
4) Projector slides (morphology, methods)
5) Instrumentation and methods of sampling clinical specimens, virus transport medium

Serological detection of viruses, as well as viral diseases: Detection of viral antigens, as well as antibodies raised against viral antigens:

1) latex agglutination
2) gel-precipitation
3) virus neutralisation tests (NT)
4) Haemagglutination inhibition (**HAI**), haemadsorption inhibition
5) Complement fixation (**CF)**
6) Immune electrophoresis
7) **Immune fluorescent techniques (IF)**: direct and indirect
8) Enzyme linked immunosorbent assay (**ELISA**)

Week 7	**March 16, 17, (18, 19 – no teaching), 20.**

Clinical virology diagnostics 1 DNA viruses I.

Associated diseases (projector slides)

Adenovirus

- Propagation on HeLa cells, CP
- HA and HAI
- IF, ELISA

Papillomavirus, Pox viruses, culture, CP on projector slides

Clinical virology diagnostics 1B (CVD-1), DNA viruses II.

Herpes viruses

- HSV 1,2, VZV (culture, CP – slides, IF, PCR)
- EBV (Paul-Bunnel reaction, anti-VCA and anti-EBNA (IgG and IgM) ELISA test)
- CMV (human fibroblast cells, CP on projector slides, IF, CMV-ELISA (IgG and IgM)
- HHV-6 and –7

HHV-8 (KSHV)

For group from Friday the Week 6 and 7 is the topic.

Week 8	**March 23, 24, 25, 26, 27.**

CVD-2: RNA viruses

Microbiological diagnosis of the most important respiratory pathogenic viruses

1) Viruses and clinical pictures (projector slides)
2) Influenza (embryonated eggs, HA, Had, HAI, CF, IF)
3) Parainfluenza, RSV
4) Rubivirus (HAI)

Microbiological diagnosis of the hepatitis virus infections

1) Clinical pictures (projector slides)
2) Serological differential diagnosis of viral hepatitis (ELISA)
3) The use of PCR techniques in the diagnosis of viral hepatitis

Microbiological diagnosis of the most important enteral pathogenic viruses

1. Viruses and clinical pictures (projector slides)
2. Rotavirus (electron-microscopy, latex agglutination: Rotalex pamphlets)
3. Calici-, corona, echo-, Coxsackie, Reo, Astroviruses

Microbiological diagnosis of the most important viruses causing central nervous system diseases

1) Clinical pictures (projector slides)
2) Thick-borne encephalitis: Flavi- and togaviruses (CF, HAI)
3) LCM
4) Polioviruses (culture, CP, vaccination!)
5) Rabies virus (Negri bodies, vaccination!)

The human immune deficiency viruses (HIV-1 and –2), as well as the AIDS

HIV-1, HIV-2 (propagation, ELISA, Western blot, PCR)

4) Opportunistic pathogens in AIDS (tables)

Week 9	**March 30, 31, Apr 1, 2, 3.**

Clinical microbiological diagnostics 1 (CMD-1): Skin, wound, and eye infections

1) The most frequent pathogens (**colonial morphology, microscopic preps**)
2) Clinical pictures (projector slides)
3) **Collection of clinical specimens**
   - The Bacteriological Investigation Request Form
   - Instrumentations, methods
   - Transport, and nutrient transport media
   - Storage and transport of the specimens
4) **Preparation of a fixed smear, Gram-staining**
5) Investigation of the normal flora of the skin and its appendices: inoculation onto blood (B), chocolate (CH), and eosine-metylene-blue (EMB) media

Pus, wound discharge, contact lens storage fluid: inoculation onto B, CH, EMB media

Materials to be presented			
Microscopic preps	Sampling of specimens, culture media, cultures	Biochemical tests	Serological reactions
S. aureus, *S. pyogenes,* *S. pneumoniae,* *B. cereus,* *C. perfringens,* *C. teteani,* *E. coli*	Cotton wool swab, Stuart transport medium, Methods and instruments of anaerobic cultivation, Necrotic tissue on Holman and thyoglycolate medium: *C. perfringens,* *C. tetani*		

Week 10	**April 6, 7, 8, 9, 10 – Spring holiday**

Week 11	**April 13 – No teaching, 14, 15, 16, 17.**

CMD-2: Upper and lower respiratory tract infections

1) Evaluation of the cultures done at the previous class (CBD-1) (**macroscopic colonial morphology, coagulase, catalase, oxidase reactions, indol test, antibiotic susceptibility tests**)
2) The most frequent pathogens in respiratory tract infections (table, **colony morphology in sample cultures, ready-made microscopic preps**)
3) Clinical pictures (projector slides)
4) **Collection of specimens** in case of follicular tonsillitis, acute and chronic medial otitis, bronchitis
5) **Discussion of the clinical cases**

Materials to be presented			
Microscopic preps	Sampling of specimens, culture media, cultures	Biochemical tests	Serological reactions
C. diphtheriae, *H. influenzae,* *M. tuberculosis* acid fast staining	Agar*: P. aeruginosa* B*: S. aureus,* *S. epidermidis,* *S. pyogenes,* *S. pneumoniae,* *B. cereus,* Apathogenic *Neisseria sp.* EMB: *E. coli* *Klebsiella sp. Proteus sp.* Löwenstein-Jensen, Sula: *M. tuberculosis* Löffler, Clauberg*:* *C. diphtheriae* Antibiotic susceptibility tests with disc diffusion: *S. aureus,* *S. epidermidis,* *P. aeruginosa,* *E. coli*	coagulase, catalase, oxidase, indol, sterile, as well as inoculated ATB panels	Elek test

Week 12	**April 20, 21, 22, 23 – No teaching, 24.**

CMD-3: Urogenital, abdominal and enteral infections, food-poisonings

1) The most frequent pathogens of the urinary tract (table, **colony morphology in sample cultures, ready-made microscopic preps**)
2) Sexually transmitted diseases (STD) caused by bacteria (table)
3) Abdominal infections (table)
4) Bacterial (gastro)enteritis, food-poisoning (table)
5) Clinical pictures (projector slides)
6) **Rules of the collection of specimens from the males' and females' urethra, and from the vagina**
7) Transport systems for *N. gonorrhoeae*
8) Sample slides: **gonorrhoeal discharge with methylene-blue staining**, ***Neisseria sp.* with Gram-staining**
9) **Rules of collection of urine for microbiological diagnostic purposes. The Uricult-plus system**
10) **Discussion of the clinical cases**
11) **Uricult-plus** video film

Materials to be presented			
Microscopic preps	Sampling of specimens, culture media, cultures	Biochemical tests	Serological reactions
Performing the Gram-staining, Gonorrhoeal vaginal discharge with methylene-blue-, *Neisseria sp.* with Gram-staining	CH*: S. pneumoniae* *H. influenzae* *S. enteritidis* and *E. coli* on Br and *S. typhi* on Bi, as well as *Shigella sp.* with *E. coli* on DC Sterile TCBS Bacteriophage typing, Sterile Uricult-plus	coagulase, catalase, oxidase, indol, ATB, Russell, TSI, Christensen, lesser sugar sequences	Gruber-Widal*, E. coli* slide agglutination, Wassermann, VDRL, RPR

Week 13	**April 27, 28, 29, 30, May 1 – No teaching**

CMD-4: Bacteriaemia, fungaemia, sepsis, osteomyelitis, endocarditis, meningitis

1) The most frequent causative agents of sepsis, endocarditis, meningitis (tables, **colony morphology in sample cultures, ready-made microscopic preps**)
2) Clinical pictures (projector slides)
3) **Collection of specimens**: cerebrospinal fluid (CSF), blood
4) **Different blood-culture flasks**. Rules of the collection of blood-cultures
5) Sample slide: ***Leptospira sp.* with silver-impregnation**
6) Video film about blood-cultures
7) **Discussion of the clinical cases**

Materials to be presented			
Microscopic preps	Sampling of specimens, culture media, cultures	Biochemical tests	Serological reactions
Leptospira sp. with silver impregnation	Antibiotic susceptibility tests with disc diffusion: *S. faecalis,* *B. cereus* *S. pyogenes* *S. agalactiae* (also bacitracin!) *S. epidermidis,* *S. saphrophyticus,* (also novobiocin!) *P. aeruginosa, Proteus* *E. coli* *Leptospira sp.* (Korthof medium) B: *L. monocytogenes* *Acinetobacter sp.* B, CH: *S. mitis* CH: *N. meningitidis* Sabouraud: *Candida albicans,* Sterile and positive blood culture flasks	coagulase, catalase, oxidase, indol, ATB	*E. coli* slide agglutination, Wellcome (Wellcogen) antigen detection rapid test for CSF samples

Week 14	**May4, 5, 6, 7, 8.**
Review: presentation of the complete material of the practical examination	

Week 15	**May 11, 12, 13, 14, 15.**
Practical examination	

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