1. Principles of LDG of SFI:
   - Hair
   - Nail
   - Skin
   - Mucosal
   - Eye
   - Ear

2. Principles of LDg of IFI:
   - agglutination, precipitation, ELISA, PCR, PH
   - invasive aspergillosis (IA)
   - invasive candidiasis (IC)
   - zigomycosis
   - Pneumocystis pneumonia

3. Methods for fungal identification
   Methods of antifungal susceptibility testing (AST)
   - Diffusion
   - Dilution
   - Etest

4. Epidemiology of FI
   - Risk groups of patients:
     Endemic FI (biphasic and subcutaneous mycoses)
   - Mycoallergens and mycoallergosis
   - Mycotoxins and mycotoxicosis
LDg and localization of FI

Deep mycoses
- Brain
- Lungs
- Heart
- Liver
- Spleen
- Kidney

Superficial, cutaneous, subcutaneous mycoses
- Superficial (hair, nail, skin)
- Cutaneous (hair, nail, skin)
- Subcutaneous
LDg of FI: METHODS

Classical / traditional / mycological (conventional) methods:
• DMP / PH
• CULTURE (isolation) the "golden standard"

Most fungi are cultivated on nutritious media (NM), except *Pneumocystis, Rinosporidium* ...
  - The problem - a low sensitivity (IFI)
  - analysis are long-lasting

- Immunodiagnosis (method of choice for IFI and endemic FI)
  • Detection of Ag (agglutination, diffusion, ELISA)
  • Ab detection (agglutination, diffusion, ELISA)
    The problem - interpretation of the results

- Molecular diagnosis (rapid and sensitive)
  • DNA detection (PCR)
    The problem - contamination standardization
**Classic / Traditional (conventional) method**

- 4-7/14-21 day (dermatophytes)
- Low sensitivity, slow

**Immunological methods**

- ~ up to 24 hours
- High sensitive, fast

**Molecular methods**
Superficial fungal infections (SFI)

- Proper selection and sampling:
  - prior to initiation of Th
  - the right place
  - in sufficient quantity
  - asepsis!

Direct microscopic examination (DME): A quick, inexpensive method
- Only reveals fungal elements
- It is not possible to identify

- Isolation of fungi: slow method, but the "golden standard"
- Identification of fungi, quantization, typing, susceptibility testing...
Hair - proper sampling
Microsporia - some strains of Microsporum spp. have the ability to autofluorescence under UV light
Hair – proper sampling

Favus
Instruments for sampling in suspected SFI
(nail, hair)
Nail – proper sampling

Psoriasis

Onychomycosis
Nail – proper sampling

Finger-nail scrapings

- DMP
- CULTURE
Nail – proper sampling
Skin – proper sampling

Skin squama

- DMP
- CULTURE
“Screeing” cornea of the eye - the proper sampling

Keratomycosis

The finding of hyphae in corneal scraping (KOH)
Ear – proper sampling

Isolated *A. fumigatus*

Isolated *C. parapsilosis*

The material is taken using a smear

Otomycosis – endoscopic findings
Mucous membranes - proper sampling

The material is taken using a smear

- Vulvovaginal candidiasis (VVC)
- About 10% of women have recurrent VVC (Th serious problem!!)

Oropharyngeal candidiasis (sore)
Direct microscopic preparation (DMP)

METHODS

- Saline
- KOH (potassium hydroxide 10%, 20%)
- LFCB (lactophenol-coton-blue)
- Ink, nigrosine
- FLUORESCENT DYES (Blankophor, Uvitec, Calcofluor white)
DMP- squama infected with fungi

- Hyphae in the sawdust of the skin (10% KOH and Parker shower) 100X
- It is not possible to identify fungi
- *Epidermophyton floccosum* identified on the culture

- Hyphae and spores in the sawdust of the skin (LFCB) 100X
- “Spaghetti and minced meat” – report
- Characteristic for *Malassezia* genus
Blankophor - linked to the fungal cell wall (nonspecific fluorescence 100X)
DMP – sensitivity is increased by using fluorescent dyes

KOH (20%) Fluorescent colors are binded to the fungal cell wall
DMP – increase of sensitivity by using fluorescent dyes

Squames from the skin - Malassezia

KOH 30% (100X)

Parker (100X)

Fluorescence (100X)
DMP – appearance of hair infected by dermatophytes

ectothrix  endothrix  Favus

Hair and dermatophytes - schematic (microscopic findings)
Nutrient medium (NM) for the isolation and differentiation of fungi

The most commonly used medium for the isolation of yeast / fungi / dermatophytes:
- Sabouraud dextrose agar (SDA), yeasts and molds
- SDA + AB yeasts and molds
- Potato dextrose agar (PDA) - molds
- Capek - molds
- SDA + AB + actidion - dermatophytes
- Dermatophyte test medium (DTM)-dermatophytes

Blood cultures:
- Brain heart infusion agar (BHIA)
- Commercial liquid or biphasic media

Differential / special NM:
- GACA (Cryptococcus) brown colonies
- CHROME Candida Agar (Candida) various colors
- mDixon modified agar (Malassezia)
- Leeming Notman agar (LNA) (Malassezia)
- Corn / rice agar (Candida) chlamydomspore
- Serum (Candida albicans) germ tube
- Serum (Cryptococcus) capsules

TEMPERATURE!!!
- ~ 25°C
- ~ 37°C

INCUBATION TIME!!!
3-7 days (yeasts and undermatophyte molds)
14-21 day (dermatophytes)
4-6 weeks (dimorphic fungi, eg. Histoplasma)
Dg *Pityriasis versicolor*

Isolated *Malassezia* on LNA
**Malassezia**: SDA (unlipophilic); mDixon (lipophilic) types

- **M. pachydermatis** (SDA)
- **M. furfur** (mDixon)

Identification - *Malassezia* microscopic characteristics (LFCB 100X)
Yeasts – cultural characteristics

*SDA – Candida*

*Preparation from the culture - 100X (FS)*

*Candida - pseudohyphae 400X*

*Candida - blastospores 400X*
Identification: *Candida albicans* (microscopic characteristics)
Identification: *Candida albicans* (microscopic characteristics)

- Hlamidospore on corn agar 100X
- Chlamydomspore on corn agar 400X
- Chlamydomspore EM 1000X
Molds: cultural/macroscopic and microscopic characteristics

SDA – culture *A. niger* (above)
*A. fumigatus* (below)

Microscopic preparation from the culture 400X (KOH): *A. niger* (above) *A. fumigatus* (below)
Dermatophytes - isolation and identification

MEDIA: SDA+Act, DTA (Petri dish)  
PDA (test tube)

Taking samples for microscopic identification  
"tape" method
Microsporum - cultures and microscopic findings

M. gypseum/ above and M. canis/below
Trichophyton - cultures and microscopic findings

T. violaceum/above and T. schönleinii/below
Identification of dermatophytes: culture and microscopic findings

Preparation from the culture
*M. canis*

Culture *T. mentagrophytes*

*M. canis* - EM

Preparation from the culture
*T. mentagrophytes*
Epidermophyton - cultures and microscopic findings

E. floccosum - culture

E. floccosum - microscopic findings (LFCB 100X)
Identification of mold - a problem!

“Contaminants” – Needs seriously treatment!!

- Identification is often problem for general microbiology laboratories
- Isolates of fungi, which are clinically significant and can not be identified, sent to:

Medical Mycology Reference Laboratory, Faculty of Medicine, University of Belgrade
www.mikologija.org.rs

CBS (Centraalbureau voor Schimmelmcultures) Utrecht, The Netherlands
www.cbs.knaw.nl