Fungal infections

Pathogenic fungi are classified as **yeasts**, **moulds** and **dimorphic fungi**. Fungal infections are known as **mycoses** and may affect different parts of the body: **superficial mycoses**, **cutaneous mycoses**, **subcutaneous mycoses** and **systemic mycoses**.

Candida

The genus Candida belongs to phylum of the yeasts, which are unicellular microorganisms without hyphae.

Pathogenic candida include the species *Candida albicans, C. auris, C. glabrata, C. krusei, C. famata, C. tropicalis, C. parapsilosis*, etc.

The most important pathogenic species is ***Candida alibicans***.

Candida albicans

Clinical Significance

Candida albicans is found within the skin, mouth, gastrointestinal and genitourinary tract and is mainly involved in systemic mycoses (65-70%). Infections with *C. albicans* are caused by conditions causing the reduction of indigenous bacteria at various anatomical sites where *C. albicans* is present; from these sites, they can enter into the bloodstream and cause systemic infections. *C. albicans* can cause mucosal and cutaneous infections known as **superficial candidiasis** or **systemic candidiasis** with renal, pulmonary, cardiac and CNS involvement.

Factors that favour the emergence of candidiasis are:

- **Physiological factors**: age, pregnancy
- **Local factors**: maceration, humidity, trauma, burns
- **Pathological factors**: diabetes, immunosuppression
- **Iatrogenic factors**: antibiotics, corticosteroids, immunosuppressive drugs, intravenous catheters

Laboratory Diagnosis

Collection

Collection depends on the site of infection and transport should be done as soon as possible, in order to prevent growth within the specimen. For semi-liquid specimens transport media should be used.

Microscopic examination

Microscopic examination is performed using the wet mount technique (e.g. without staining) and reveals yeasts that are oval in shape and range in diameter from 4-6 μm. Direct examination is done from specimens that were prior prepared with **Potassium hydroxide** (KOH preparation) – a clearing agent that dissolves keratin (skin, nails, hair) from specimens.

Additionally, *C. albicans* can be microscopically studied with Gram-staining. Gram-staining reveals ovoid microorganisms that stain blue to purple (same for all fungi) and is usually used in order to determine whether the culture is pure or not (i.e. with or without bacteria). Furthermore, Methylene blue or May-Grünwald-Giemsa staining can be performed.

However, direct examination and wet mounts are usually preferred, as they are performed faster.

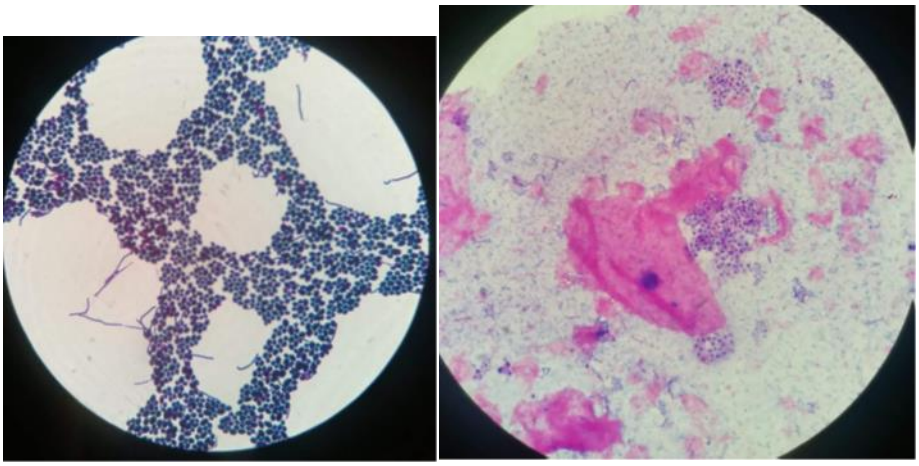


Figure 32. Microscopic examination of fungi: (A) culture - Gram stain, (B) biological product - Pick stain

Culture media and Identification

Inoculation of *Candida albicans* is performed on Sabouraud media. The colonies appear smooth, moist and creamy or mucoid with a transparent or white aspect. Even though some hints can be concluded by colonial and cellular morphology, biochemical tests are performed for a final diagnosis. Such biochemical tests, as discussed in the bacteriology section, are available in commercial kits – in this case for yeast identification with carbohydrate fermentations.

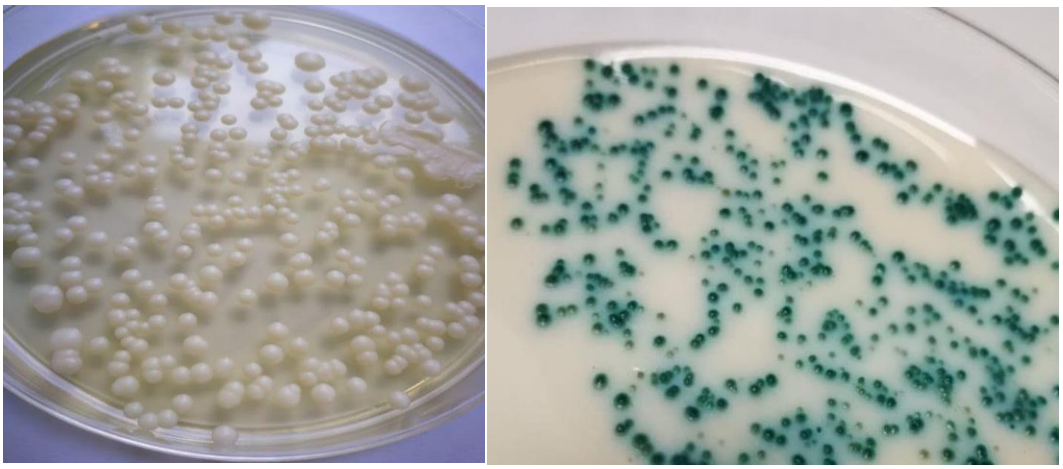


Figure 33. Fungi culture (A) Sabouraud environment, (B) Brilliance candida environment- *Candida albicans*

Treatment

Most cases of superficial candidiasis respond to topically administered nystatin, azole or amorolfin. In cases of systemic candidiasis fluconazole or itraconazole might be administered.

Essential to remember:

- Mainly involved in systemic mycoses
- Sabouraud media used for inoculation and growth

Viral infections

Laboratory diagnosis of viral infections

Methods:

A. Cytology

B. Electron Microscopy

C. Cultivation

D. Serology

E. Molecular methods (detection of genetic material i.e. nucleic acids)

Cytology

Cytology is a fast method that involves detecting the effects of viruses on cell structures: morphological changes, the appearance of multiple nuclei in infected cells, lysis infected cells, vacuolization, formation of syncytias through fusion between cells, the appearance of intranuclear / intracytoplasmic inclusions, ciliocytophoria (see below).

Cytology is applicable in a wide variety of viral infections. Thus, eye infections, respiratory, genital and urinary tract infections are locations that are well suited to collecting biological evidence for rapid cytological diagnosis.

Here are some examples of cytology applications in diagnostics of viral infections.

Urinary cytology may reveal cellular changes in cytomegalovirus (CMV) infections, herpes simplex virus (HSV), papovaviruses etc. CMV infection produces cytological effects such as: large volume cells, with large intranuclear inclusions and cytoplasmic inclusions basophils.

In pneumonia with measles virus, which can occur in immunosuppressed persons, it is possible observe multinucleated giant epithelial cells, with intranuclear eosinophilic inclusions and intracytoplasmic, but similar changes are also caused by infections with syncytial breathing virus.

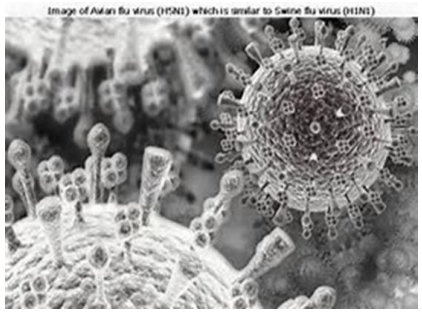
In adenovirus infections, cells exhibit large intranuclear inclusions and ciliocytophoria (cilia buds, remnants of the bronchial ciliated epithelium, present in different biological fluids, such as lung secretions).

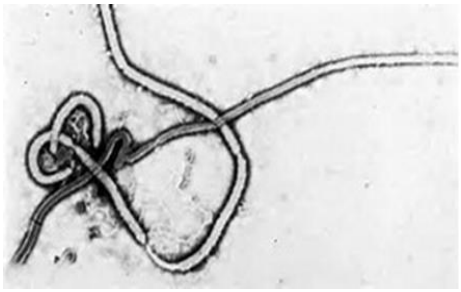
It should be noted that the cellular changes described in the above examples may be non-specific or common to several viral and non-viral conditions, so that the etiological diagnosis requires carrying out additional tests.

Electron microscopy

Electron microscopy allows the detection and identification of virions in clinical samples by direct visualization of viral particles. The method has a high specificity but it requires expensive equipment (both purchase price and maintenance), highly qualified and experienced personnel. The sensitivity of the method is relatively low (requires a large number of viral particles in the sample examined for a positive result), is laborious and supposes examination of a safe sample (as such is not applicable in routine diagnosis).

Applicability: visualization of rotaviruses in faecal samples, rabies virus in brain tissue samples, visualization of influenza virus virions, filoviruses (e.g. Ebola), etc.

A 

B 

Electron microscopy (A) Influenza virus, (B) Ebola virus

Cultivation

Viruses can be grown on cell cultures and on embryonated eggs.

1. Cell cultures

Some viruses can be cultured on cell cultures, the diagnosis is based on the effects of viral cell grow on culture cells.

The following types of cell cultures are used:

- Primaries - obtained from animal organs through enzymatic lysis and growth in monolayer at which we can add growth factors (for example, calf serum)

- Secondary - obtained by dissociation (with trypsin) of primary cell cultures followed by successive transfers (passages) of the viral inoculum to be investigated

- Cell lines - tumor cells, "immortalized" cells - the process involves a continues multiplication to be artificially induced for experimental purposes; they can support an infinite number of transfers (passages).

Examples:

- Primary culture of simian (monkey) kidney cells can be used for cultivation orthomyxoviruses, paramyxoviruses, enteroviruses, adenoviruses

- Fetal diploid cell cultures - used for the cultivation of herpesviruses, picornaviruses, adenoviruses.

2. *Chicken embryonated eggs*: used to grow influenza viruses.

Serology

Serological methods are useful for non-cultivable viruses for viruses with slow evolution of the infection as well as for evaluation of the immune response.

The main serological methods used are:

- Complement fixation reaction (RFC);
- Hemagglutinoin inhibition (HAI);
- The neutralization reaction;
- Direct and indirect immunofluorescence;
- Passive agglutination reactions: Latex-agglutination; passive hemagglutination;
- Immunoenzymatic assays: ELISA - "enzyme-linked immunosorbent assay"; Western blot;
- Radioimmune assays ("Radioimune assay" - RIA)

Viral hepatitis

The term "viral hepatitis" refers to acute or chronic inflammation of the liver caused by an infection with one of the "hepatitis viruses" identified so far. The main tropism for liver tissue is the only common feature of these viruses, otherwise they are different taxonomically, structurally, pathogenetically and evolutionarily. In the case of diagnosis of hepatitis (inflammation of the liver), clinically and biochemically supported, the viral etiology can be confirmed by specific assays. In the following they are played the main methods used in the etiological diagnosis of viral hepatitis.

Laboratory Diagnosis of viral hepatitis

The laboratory diagnosis of **hepatitis A (HAV**) infection is based on the following tests:

1. Anti-HAV IgM antibodies by ELISA. The test is highly sensitive and specific when is made from sera collected from people with typical symptoms of acute hepatitis (jaundice, acute digestive manifestations). Usually, anti-HAV IgM are already detectable with 5- 10 days before the onset of symptoms and remain at elevated serum levels for 4-6 months. After this period, the anti-HAV IgM titer starts to decrease in parallel with the increase of the anti-HAV IgG antibody titer that will generally persist throughout the serum patients who have gone through the infection. Also, anti-HAV IgG are also detected in the serum of vaccinated persons.

2. Electron microscopy allows visualization and identification of HAV virions in samples stool or blood in patients with viral hepatitis A. The method is highly sensitive and specificity, but it is expensive and rarely used for routine diagnosis.

3. Molecular methods use nucleic acid amplification (PCR) techniques from serum samples, stool and liver tissue. The sensitivity and specificity of these methods are they are also very high, but due to the high relative costs they are rarely used in routine diagnosis. They can be very useful in investigating epidemics.

The laboratory diagnosis of **hepatitis B (HBV**) infection is based on the following tests:

1. ELISA method for the determination of HBV antigens and specific antibodies against them:

a. The HBV surface antigen (HBsAg) is detectable in the serum at 2-10 weeks from infection. In the case of acute self-limiting infections that develop towards healing, HBsAg becomes undetectable after 4-6 months after infection. The persistence of this serum antigen for more than 6 months represents a serological marker of chronicization of HBV infection.

b. Antibodies against HBV surface antigen (anti-HBs) appear in several weeks after HBsAg becomes undetectable in serum and, in most patients, ensures long-term immunity ("for life"). Its presence in the serum represents the marker of cured HBV infection or serological evidence of HBV vaccination.

c. IgM antibodies against the "core" antigen of HBV (anti-HBc IgM) during the first weeks after the acute infection and remain detectable for 4-8 months. During the "serological window" period (from a few weeks to a few months) between the disappearance of HBsAg and the occurrence of anti-HBs AC, anti-HBc IgM detection may be the only way to make the diagnosis of acute HBV infection. Some patients with chronic HBV infection or some asymptomatic HBV carriers may present tests positive for anti-HBc IgM during the reactivation or reactivation phases of chronic infection. This makes for a positive anti-HBc IgM test always a marker relevant for the diagnosis of acute infection.

d. Anti-HBc total IgM + IgG antibodies are usually detectable in all patients who have been exposed to HBV (acute or chronic infections). These antibodies do not provide protection against HBV infection being only serological markers of the passage through infection.

e. HBV antigen "e" (HBeAg) is a soluble viral protein that appears in serum in the early stages of acute HBV infection and usually disappear shortly after maximum alanine level of serum aminotransferase. Its persistence for more than 3 months after the onset of the disease, it is a potential marker of chronic HBV infection. the sea most patients with chronic HBV and HBeAg positive have one active hepatic impairment.

f. Antibodies against "e" antigen of HBV (anti-HBe); Spontaneous seroconversion (HBeAg negativity and anti-HBe positivity) is usually associated with decreased HBV replication and a more favorable evolution.

2. Molecular methods - DNA-HBV can be detected by qualitative or quantitative tests. The level of DNA-HBV (viremia) is usually measured by PCR. The level of DNA-HBV is usually uses it to evaluate the possibility and opportunity of starting therapy antivirals as well as for evaluating the response to treatment.

Laboratory diagnosis of **hepatitis D infection - hepatitis D virus** (Delta agent) is a defective virus that can only infect with HBV. There are two possibilities:

- Coinfection: the patient is infected simultaneously with HBV and HDV

- Superinfection: HDV infects a patient with a pre-existing HBV infection (by usually a chronic infection); in this situation, the evolution of the infection is aggravated. The etiological diagnosis is based on ELISA detection of HDV serological markers (anti-HDV IgM antibodies) and on RNA-HDV detection in serum by molecular biology techniques (PCR).

Laboratory diagnosis of **hepatitis C infection**

In clinical practice, virological diagnosis and monitoring of HCV infection is based on mainly on the use of 4 markers:

1. total anti-HCV antibodies,

2. HCV core antigen,

3. HCV RNA

4. HCV genotype.

These markers are determined by ELISA-type immunoenzymatic serological methods (total anti-HCV antibodies and HCV core antigen) and molecular diagnostic methods (ARNHCV and HCV genotype).

1. The total anti-HCV antibodies become detectable after about 60 days, the so-called period of "serological window" (the period between the time of infection and the time in that the secretion of specific antibodies reaches the level at which they can be detected by the techniques laboratory; if during this period the HCV RNA and core HCV antigen are already present detectable). After the appearance in the serum, these antibodies persist for a lifetime in patients who develops a chronic HCV infection.

2. HCV "core" antigen can be detected in the serum of infected persons. It has been shown that the serum level of HCV antigen "core" (detectable by ELISA techniques) is correlated

significantly with the level of HCV RNA measured by molecular biology techniques. So, determining the “core” antigen of HCV may be an alternative to ARNHCV measurement, being an easier method, cheaper and less risk-free contamination of samples from some molecular biology techniques. However, detection the "core" antigen is less sensitive and the serum level of this marker may vary much from one individual to another.

3. Detection and quantification *of HCV RNA* in peripheral blood is a marker of HCV replication confidence. This marker is detectable at 1-3 weeks after the moment infectious, so about 1 month earlier than the positive of serological tests for total anti-HCV antibodies. RNA-HCV persistence after a period of 6 months denotes chronic HCV infection.

RNA-HCV detection and quantification is performed by 2 categories of biology techniques molecular weight:

- those based on target nucleic acid amplification (such as PCR) and

- those that involve signal amplification (such as branched DNA tests): these techniques use multiple oligonucleotides ("capture" nucleotides) with known sequences which hybridize to complementary regions of the nucleic acid to be tested; capture probes, attached to the surface of a microtiter plate (with wells) binds to the nucleotides of capture thus fixing the target nucleic acid to the plate.

4. Determination of *HCV genotype* (genotyping)

RNA-HCV chains (chains) are classified into 6 genotypes (1-6) and a large number of subtypes. Genotyping can be done by the so-called direct analysis of the sequence consisting of phylogenetic analysis of the sequences generated after PCR amplification of a portion of the genome and comparison with reference sequences. Another method is reverse hybridization of of amplicons (PCR amplification products) using probes. Determination of HCV genotype is necessary to establish the anti-therapeutic regimen HCV.

Laboratory diagnosis of **hepatitis E (HEV)** infection is based on serum detection of anti-HEV IgM antibodies by ELISA and RNA-HEV by PCR. It should be mentioned that in infections with hepatic viruses, in addition to the predominantly liver disease.

Other organs and tissues may also be affected. For example, chronic HCV infection can also cause extrahepatic manifestations: lymphoproliferative disorders (cryoglobulinemia), nephropathy, thyroiditis, idiopathic pulmonary fibrosis, late cutaneous porphyria, flat lichen, chronic arthritis. On the other hand, liver tissue can also be affected in the case of infections with viruses main tropism is not hepatocyte (CMV, EBV).

HIV infection

The etiological diagnosis of HIV infection is made by ELISA, immunoblot tests (Western blot) and molecular biology (PCR) techniques, according to algorithms tests based on the dynamics of the serological markers of the infection. Serological markers based who are diagnosed with infection, staging, therapeutic approach as well evaluating the response to treatment are: HIV-RNA, HIV p24 antigen, antibodies (IgM, IgG) anti-HIV-1 / HIV 2.

Laboratory Diagnosis of HIV infections

The diagnostic algorithm is based on the dynamics of these markers in the serum of individuals infected. After the infectious moment, the first detectable marker in serum is HIV-RNA (at approximately 10 days after infection), followed by positive assay for p24 antigen (after about 2 weeks) and the appearance of anti-HIV antibodies (about 20 days after the infectious moment). Anti-HIV IgM antibodies are detectable with current assays (ELISA-4th generation) 3-5 days after p24 antigen emergence and 10-15 days after ARNHIV occurrence; anti-HIV IgG antibodies subsequently appear and persist throughout the infection.

Based on this dynamics of HIV markers, the infection is classified into the following stages:

- The period of "eclipse" (or "serological window") - initial interval (after infection) in which does not detect any HIV markers in the serum

- Seroconversion period - the interval between HIV infection and the appearance of the first antibodies detectable; the duration of this period depends on the type of tests used for testing and their sensitivity

- Acute infection - the interval between the appearance of detectable RNA-HIV and the positivity of the assays detection of specific antibodies; the duration of this stage also depends on the type of tests used by their sensitivity.

- Established (chronic) HIV infection - this stage is characterized by a full response in IgG antibodies detected by ELISA and confirmed by Western blot assay. The main tests included in the diagnostic algorithm are ELISA, Western blot and viral load testing (viremia quantification).

1. ELISA tests detect anti-HIV1 and / or HIV2 antibodies (1st, 2nd, 3rd generation) and the p24 antigen of HIV1. Currently combined tests are available that offer the possibility concomitant detection of anti-HIV antibodies and HIV p24 antigen1. ELISA tests, even those of generation I and II, have a good sensitivity in the established (chronic) HIV infection but can not to detect recent infections during the "eclipse" or "serological window" period in which the infected person may have a high level of HIV viremia, with adverse consequences concerns the transmission of the infection.

2. Western blot tests are used to confirm repeated positive tests by ELISA. It should be mentioned that in the case of combined ELISA tests that detect anti-HIV 1 & 2 antibodies concurrent with the detection of p24 antigen of HIV 1, confirmation by Western blot is no longer required.

3. Viral load tests (viremia level measurement) quantify the level of HIV in the blood. They are usually used to monitor the effects of treatment or for diagnosis early in HIV infection. Three types of technologies are used:

a. Reverse transcription polymerase chain reaction (RT-PCR) - is a combination application PCR technology with reverse transcription, a process that involves the action of enzymes called reverse transcriptase which performs the conversion of RNA sequences into complementary DNA sequences (CDNA) which are then amplified and identified by PCR.

b. Branched DNA (bDNA) - is based on "capture" oligonucleotides, synthesized thus so that they have complementary sequences with regions of HIV-RNA and that will thus hybridize to these regions fixing HIV-RNA to solid support.

c. The nucleic acid sequence-based amplification assay (NASBA) is a technique based on amplification of genetic material at constant temperature (41 ° C) under the action of 3 enzymes (o RNA -ase, a reverse-transcriptase and an RNA-polymerase).

Given the need to screen HIV infected people as early as possible, yet since the discovery of this virus in 1985, there has been interest in performing rapid tests, made from saliva. These tests are based on the fact that anti-HIV antibodies are also detectable in saliva, although their concentration in this biological product is much lower than in blood, and again levels are fluctuating, which affects sensitivity. However, research has continued with a view to optimizing saliva tests that have the advantages of harvesting more easy to sample and reduced invasiveness.

At present, any positive salivary test requires confirmation by the techniques mentioned above. According to the CDC, early detection is the key to interrupting HIV transmission, early antiretroviral therapy reduces the rate of transmission and decreases the risk of complications of HIV infection such as pneumonia with opportunistic germs and tuberculosis.

Researchers at Stanford University recently reported preliminary studies for a new HIV test in saliva that can detect low levels of anti-HIV antibodies using viral antigens attached to DNA which is then amplified and detected with the help standard equipment (PCR). The authors state that the new test will be 1000 times or even 10,000 times more sensitive than current saliva tests.

Parasitic infections

Stool Examination

Point of examination: is to place a diagnostic with certainty in case if an intestinal parasite.

There are 3 principals to take in to account before examination:

1. There are methods adapted to the biologic cycle of the parasite. We will see eggs, larva, cysts or adults
2. A negative result from an analysis doesn't eliminate the diagnostic of parasitosis. For exclusion, there are 4-5 successive exams throughout the week and then after the 5th yielding a negative result, we can say that the patient does not have parasitosis.
3. The sample is examined in a short period of time (4-6 hours)

Technique:

The patent is prepared 2-3 days in advance: avoiding the consumption of medications that contain carbon, bismuth, opaque substances (Ba), sulfate of Mg, green vegetables (cabbage, lettuce), fruit with hard seeds (strawberries, wild strawberries, raspberries) which can determine a false positive result.

Macroscopic Examination: Shows the following:

- Color of faeces (brown = normal; black= melena - can be an ankilostomiasis)
- Constituents (pus, blood or mucus -pathologic)
- Consistency (hard, soft, liquid, pasty, gelatinous)
- Smelly

Through the macroscopic examination we can see: proglottids of tenia, of botriocefal (fragments of strobila), adult Limbric or Oxiur.

Microscopic Examination: Involves the direct examination, concentration method and special methods

Direct Examination: Used to determine *cysts* (of protozoans), rarely for determining eggs of parasites

1) Dilution of the feces in physiologic serum.

2) Dilution of the feces in human serum diluted (1/10).

3) Coloration with Lugol's Iodine (iodine solution): on a glass slide we pour 1-2 drops of solution, on which we add a small quantity of fecal matter and mix it. Another glass slide is placed on top and we observe parasitic M.O. elements which appear yellow-brown on the colorless background of the preparation; the contracts is observable. We can see: cysts of *Entamoeba Histolytica*, of *Entamoeba Coli*, of *Giardia Lambria*, of *Balantidum Coil*, yeast cells, *Blastocistis Hominis* (type of yeast) and *Trichomonas Intestinalis*.

Concentration Method: Used for determining *eggs* of helminths

- Hydrostatic Method – WILLIS-HUNG Method
- Biphasic Method – TELEMANN-LANGERSON Method

WILLIS-HUNG Method: Is based in the difference of densities which is artificially created between the parasitic element (egg) and the solution. A hyper saturated solution of NaCl is utilized and settles at the bottom of the cap BOREL. A small quantity of feces (size of a cherry) is taken with a glass rod then mixed with the NaCl solution. After the solution homogenizes more NaCl is added until ¾ of the recipient is filled. 2 lamellas are placed over the homogenized solution. The first one is removed after 20 minutes, the second one is removed after 40 minutes. The eggs of the parasite, if they exist, arise at the surface of the solution, adhered to the inferior surface of the lamella and tend to be at the margins. Lamella are placed on a glass slide and is looked through M.O.

TELEMANN-LANGERSON Method: HCl and sulfuric ether are used. Technique: at the bottom of a jar with a molded lid, with glass pearls, small quantity of feces is placed with the help of a glass rod. 5 cm^3 of HCl is added. The jar is covered with the lid or, a gauze filter for approx. 5 min., after that 5cm^3 of ether sulfate is added. The jar is covered by a lid imprinted with circular movement for approx. 5 min. The obtained solution is transferred into a test tube for centrifugation then is centrifuged for 3-4 min. at a reduced rotation (500-1000 rotation/min.). Inside the test tube 4 layers are formed. The first 3 are separated by tipping over the test tube; the 4^{th} layer remains (the sediment), which contains the eggs of the parasite. It will be harvested with the help of a PASTEUR or pipette. It is the placed on a lamella to be looked at through M.O.

By the concertation methods we can highlight the eggs of: *Fasciola hepatica, Tenia, Bothriocefal, Ascaris lumbricoides, Trichiuris trichiura, Enterobius vermicularis, Himenolepis* etc.

Special Methods

N.I.H. Method – National Institute of Health: (Scotch or cellophane test): the method special for highlighting pinworm eggs. It is a very fast, useful and cheap method.

Carbon Culture Method: is used for the detection of *Strongiloides stercoralis* larvae or *duodenal Ancylostoma* and is based on the hydrotropism of its larvae their tendency to migrate to water. Used

for: animal carbon powder, feces and water, all placed in a transparent plastic container (PETRI dish). We form a paste in the form of pyramid with the tip extending beyond the edges of the container. The lid will flatten the tip. We'll keep it hermetically sealed for at least 72 hours. After 24 hours the lower face of the lid will have a few drops of water in which we can or can't find larvae of parasites highlighted or not by the stereomicroscope (ancylostoma larvae - 4-5 days; strongiloid larvae - 2-3 days).

PROTOZOANS PARASITES

Protozoans encompass the following classes of parasites

1. Sarcodina Class
2. Flagella Class
3. Ciliophoran class
4. Sporozoite Class

1 **Rhizopoda Class** encompass the parasites with the following characteristics

- Present pseudopodia (cytoplasmic expansions for movement)
- Ectoplasm in the cytoplasmic structures (the external layer of the pseudopod) and endoplasm (which encapsulates the nucleus and organelles)
- Localized in the digestive tract
- Are transmittable by cysts

Genus ENTAMOEBA encompasses the following species

a. Entamoeba Histolytica

b. Entamoeba Coli

c. Entamoeba Gingivalis

a. Determination of the amibian dysentery or intestinal amebiasis manifested by bloody diarrhea

By morphologic point of view, ectoplasm is undifferentiated from endoplasm. The parasite has two forms:

- forma magna (pathogenic form, Red blood cells in vacuoles)
- forma minuta (nonpathogenic form, but transforms itself into magna in specific conditions).

Diagnosis: coproparasitological examination, highlights double membrane cysts and 4 nuclei.

b. It is a saprophyte, at the level of the colon which is considered a pathogenic condition, the ectoplasm is poorly differentiated from the endoplasm; at the co-parasitological examination, cysts with 8 nuclei are highlighted.

c. It is found in the oral cavity. It has been isolated in cases of gingivitis, alveolar warts and untreated tooth decay. Ectoplasm is distinctly differentiated from endoplasm.

2. **Flagellate Class** encompass parasites with flagellum

    - Monoflagellate (*genus LEISHMANIA, genus TRIPANOSOMA*)

    - Polyflagelate (*genus TRICHOMONAS, genus GIARDIA LAMBRIA*)

Genus *LEISHMANIA* encompasses the following species:

a. *Leishmania DONOVANI* causes visceral leishmaniasis

b. *Leishmania TROPICA* causes cutaneous leishmaniasis

c. *Leishmania BRAZILIENSIS* causes mucocutaneous leishmaniasis

These illnesses are transmitted by a vector (PHLEBOTOMUS – a type of mosquito)

Genus *TRIPANOSMOMA* encompasses the following species

a. *Trypanosoma GAMBIENSAE* – causes sleeping sickness or trypanosomiasis

b. *Trypanosmoma RHODENSIENSE* – African transmission by vector a fly

Tse-Tse / *GLOSSINA PALPALIS* for *Gambiensae* and *GLOSSINA MORSITANS* for *Rohdesiense*

c. *Trypanosoma CRUZI* – causes american trypanosomiasis (Chagans disease)

Transmitted by a bug .

Trypanosoma is isolated from blood and LCR.

Genus *TRICHOMONAS* encompasses the following species:

a. Trichomonas INTESTINALIS

b. *Trichomonas TENAX* (bucalis)

c. *Trichomonas VAGINALIS*: It is often encountered causing urogenital trichomoniasis in women and urethral trichomoniasis in men, it has 4-5 flagella at the anterior extremity which is round. A flagellum is recurrent and upon reaching the cytoplasm it forms an undulating membrane. It presents a median axostyle, the result of cytoplasmic densification – a true backbone for a parasite.

Genus *GIARDIA LAMBRIA*:

Lambria INTESTINALIS - duodenal location but can be withdrawn into the gallbladder. Polyflagellate in pear-shaped, 2 nuclei in the anterior part, rounded, 4 pairs of flagella. Disease, lambliasis has nonspecific symptoms: abdominal pain, diarrhea, nausea, sometimes rash. It is transmitted through water, dirty hands, unwashed food (indirect contact) but also through direct contact (transmission homosexual).

3. **Ciliophora Class:**

BALANTIDIUM COLI – causes balantidiasis, has cilia on the surface for movement: is found in the colon of pigs and humans

4.**Sporoziote Class:** no cilia or flagella, are intercellular and don't move

Genus *TOXOPLASMA GONDII* causes Toxoplasmosis

Genus *PLASMODIUM* causes malaria .

MALARIA, TREMATODES

Malaria = illness caused by a class of sporozoan, genus *Plasmodium*.

Species from genus *Plasmodium*:

- *Plasmodium VIVAX*
- *Plasmodium MALARIE*
- *Plasmodium FALCIPARUM*
- *Plasmodium OVALE*

All four transmit malaria; the most severe – *P. Falciparum* (fatal)

Malaria is transmitted to the human by the female *Anopheles* mosquito

Evolution Cycle:

- ASEXUAL CYCLE (SCHIZOIT), found in man
- SEXUAL CYCLE (SPOROCYST) in the mosquito

A. ASEXUAL CYCLE includes two more cycles

- the exoerythrocytic cycle

- the red blood cell cycle

The EXOERITROCITAR CYCLE begins when the *Anophel* mosquito introduces the parasite to man in the form of sporozoites. Sporozoites migrate shortly from the peripheral blood to the Kupfer cells of the liver. This will create plasmodial spherical masses from which merozoites will result. A single plasmodial mass allows the formation of approx. 600 merozoites. They will be released after cell destruction and will be able to parasitize new Kupfer cells by resuming the exoerythrocytic cycle or they will enter the red blood cells where the erythrocyte cycle will begin.

ERITROCITAR CYCLE - comprises 6 phases:

1) The RING phase in which the parasite has barely penetrated into the bloodstream has the form of a thin ring, occupies 1/3 of the blood, nucleus peripheral and a central vacuole.

2) DEFORMED RING phase - the parasite grows in size, sketching future cytoplasmic expansions.

3) The phase of AMOEBA in which the parasite occupies 3/4 of the hematopoietic dimensions, has numerous cytoplasmic extensions; in the cytoplasm - numerous granulations of malarial pigment due to the breakdown of hemoglobin.

4) SCHIZONT (ADULT) phase - the phase in which the parasite occupies almost all the red blood cell.

5) PREROSETTA phase - the first nuclear fragments appear.

6) ROSETTA phase - around each nuclear fragment a little cytoplasm is individualized. In this phase it is it forms the future merozoites. Their number differs depending on the species, being 16-24 at *P. VIVAX* (clustered appearance) and 8-12 at *P. MALARIAE* (daisy flower appearance); they are thus a species indicator. Merozoites once formed will be released by bursting of the red blood cell. Shortly after the quantity of hemoglobin has been depleted, the released merozoites will be able to parasitize new blood cells resuming the red blood cell cycle. After several repeated erythrocyte cycles, the first sexual elements appear in the parasitic human blood (MACROGAMETOCYTE - female gametocyte and MICROGAMETOCYTE - male gametocyte).

B. Sexual Cycle: if an anopheles mosquito inhales blood filled with gametocytes from a malaria patient in the body the sexual cycle takes place. After a few divisions, the microgametocyte forms the male gamete that follows fertilization with macrogametocyte gives rise to egg cell (zygote). It undergoes the following transformations in the body of the mosquito: oocyst - sporoblast - sporocyst - sporozoites. They will be carried from the lymph to the salivary glands where with saliva they will be introduced by a mosquito into a healthy human resuming the cycle.

Establishment of the *Plasmodium* species (differentiating characters between the stages of the erythrocyte cycle):

- *P. Vivax* has an increased volume of red blood cell; in *P. Malariae* normal sized red blood cell
- Ring phase:

  For *P. Vivax* and *P. Malariae*: "SIGNET RING"

  P. Falciparum: 2-3 rings in a cell in a headphone shape
- Amoeba phase:

  - in *P. Vivax* the cytoplasm spreads in several directions; inside the hematoma some – granulations appear characteristic (Schuffner's granules)

  - in *P. Malariae* the cytoplasm spreads in one direction: axis or equatorial band
- Rosette Phase:

  In *P. Vivax*: 16-24 nuclei (cluster aspect)

  In *P. Malariae*: 8-12 nuclei (flower aspect)
- gametocytes are round in *P. Vivax* and *P. Malariae* and rod in *P. Falciparum*
- if in *P. Vivax* and *P. Malariae* all the stages of the erythrocyte cycle in *P. Falciparum* we will only find the ring phase and gametocytes.

HELMINTHS

Encompasses

1. *FLATWORMS Class*
2. *NEMATHELMINTHES Class (cylindrical worms)*
3. *ANELIZ* (important for veterinary medicine)

1. *FLATWORM:*

- ***TREMATODE*** (unsegmented body)
- ***CESTODE*** (segmented body)

TREMATODE: There are many pathogenic species for humans but most are found in tropical areas. In temperate areas there are important for 3 species:

- *Fasciola Hepatica*
- *Dicrocoelium Dendtriticum (lanceolatum);*
- *Opistorchis Felineus*

General Characteristics

- Flat worm, body is flat and unsegmented
- Form of body: foliage (leaf) and spear head
- Has 2 suckers for fixation, ventral and mouth
- Hermaphroditic (male and female genitalia)
- Within their evolutionary cycle requires at least one intermediate host

***Fasciola Hepatica*:** yellow-white color, L = 1-2 cm, H = 1 cm. Parasite of bile ducts in humans, sheep and cattle. The intermediate host in the evolutionary cycle is a snail of the genus *Limneea* (in water).

Evolutionary cycle: eggs (120-140 / 70 µ) are released with bile and feces in the external environment. Once in contact with water and at a favorable temperature (25-30ºC) will turn into a ciliated larva (*MIRACIDIUM*). This one it will be taken over by the *Lymnaea* snail in the body which will turn into a sporocyst.

***Dicrocoelium Lanceolatum*:** L = 10-15 mm, H= 1-3 mm: parasite of the bile ducts in humans and animals (sheep).

***Opistorchis Felineus* :** dimensions similar to *Dicrocelium*; it is found in humans, dogs, cats; it parasites the biliary ways. Illness produced by the last two: *Opisthorchiasis*.

CESTODE

They are parasites with segmented bodies that are found in the intestinal tract of humans or animals. By point of view. the structure presents:

- A head (SCOLEX), provided with tools for attaching can be: hooks or suckers.
- A neck, unsegmented, short, from the place which the segmented body regenerates.
- A body (STROBILA), composed of many segments – PROGLOTTDS; which become larger in size towards the terminal end of the parasite.

These parasites are hermaphroditic (belong to the *Plathelminth Class*)

The following species are:

- *TAENIA SOLIUM*

- *TAENIA SAGINATA*

- *TAENIA ECHINOCOCCUS (ECHINOCOCCUS GRANULOSUS)*

- *HYMENOLEPIS NANA*

- *DIPYLIDIUM CANINUM*

- BOTHRIOCEPHALUS LATUS (DIPHYLLOBOTHRIUM LATUM)

Internal Morphology:

- Absence of: digestive system (feed by diffusion/imbibition); circulatory system; respiratory system
- Exterior cuticle
- Muscular layer: external tunic (longitudinal fibers) and internal tunic (circular fibers)
- Nervous system: 2 lateral nervous cords which cross the entire strobila and stops at the level of the scolex, where 2 nervous ganglia are found.
- Excitatory system: 4 longitudinal canals (2 dorsal, 2 ventral); they are anastomosed across in between themselves. The longitudinal canals open in the last proglottid through a common orifice (CAUDUAL FORMEN).
- Genital apparatus:
    - Male: numerous spherical formations (testicles) – sperm ducts- common deferent canal – The circle is enveloped in a sac and opens laterally in the masculine genital pore.
    - Female: 2 branched ovaries – oviducts – a vitelline gland (produces vitelline needed for the egg); a Mehlis gland; a vaginal canal that comes from the orifice of the feminine genitalia and have the role of transporting the spermatozoa to the junction point of the oviduct with the viteloduct and the gland. There is also an uterine tube that leads into the axle of the proglottid near the edge where it ends in a glove finger. Through time, the uterus will grow lateral diverticula, which will develop under the pressure of the eggs.
    - The old proglottid of the tenia: the reproductive organs will atrophy and only the uterus remains (heavily branched, transformed into a sac of eggs) and a remnant of vagina and a remnant of deferent.

TENIA SOLIUM: between 2-8 m long and lives as the adult form in the jejunum and ilium (popular: ribbon). The transition of this parasite in the adult form to the human is possible by consumption of insufficiently cooked pork meat parasitized with cysts.

Pig = intermediate host in life cycle; human = definitive host. By point of view. the structure presents a scolex armed with 4 suckers, 1 rostrum and two crowns of cartilage with which it attaches itself to the walls of the intestine.

TENIA SAGINATA: measure 4-12 m long, found in the small intestine (jejune-ilium); intermediate host is in the life cycle = cow (bovine) transmission to the human is possible by consumption of insufficiently cooked beef parasitized with cysts. Structure- scolex is the same as that of T. Solium but is missing the rostrum and cartilage having just 4 suckers.

The disease is the same – TENIASIS: digestive disorders with nausea, bloating, abdominal pain, irritation caused by the scolex, weight loss, sometimes nervous disorders caused by the toxins released by the parasite.

TENIA ECHINOCOCCUS: measure 3-6 mm long, in the adult form – in the dog's intestines. The body presents 3-4 proglottids and the last one represents more than half the of the body's dimension. The dog is the definitive host in the life cycle, in the human it is found in the form of a HYDATID CYST similar to the sheep and pig.
The human, the sheep and the pig are the intermediate hosts. The disease = HYDATIDOSIS is characterized by having 1 or more cysts localized in: liver, lung, spleen, kidney, cerebral fluid, heart or bone.

BOTHRIOCHEPHALUS LATUS: Intestinal 2-10 m long sometimes even 16 m long according to many it is the longest. It's transmitted through the consumption of fish meat.

Disease =DIPHYLLOBOTHRIASIS: digestive disorders (diarrhea, bloating), nervous disorders (headaches, paresthesia), and pernicious anemia.

NEMATODES

There are intestinal parasites of different sexes and cylindrical in shape (they are round in section). The following belong to this class:

- *Ascarididae Fam.: ASCARIS LUMBRICOIDES*
- *Oxiuridae Fam.: ENTEROBIUS VERMICULARIS*
- *Trichinelidae Fam.: TRICOCEPHALUS DISPAR, TRICHINELLA SPIRALIS;*
- *Ankylostomidae Fam.: ANKYLOSTOMA DUODENALE;*
- *Rhabditidae Fam.: STRONGYLOIDES STERCORALIS.*

ASCARIDIDAE Fam.

Ascaris lumbricoides - intestinal parasite, the female is 20-25 cm long and the male 15 cm long (NOTE: The female nematode is always larger).

They number, 4-5 to 1000 in a single human. They determine disease (Ascaryza - in humans; Ascaridiosis - in animals). It is characterized by digestive disorders (nausea, weightlessness, weight loss, softer stools, abdominal pain inconsistencies doubled by minor nervous disorders).

Paraclinically, the patient has leukocytosis (over 10,000); eosinophilia (40-50% - eosinophilia is a predictive sign for parasitosis).

TRICHINELIDAE Fam.

1. *Trichocephalus dispar* - intestinal parasite, has 2 parts, a thicker extremity which houses internal organs and a longer and thinner one like a thread that serves to anchor the parasite to the intestinal wall. The disease (trichocephaloosis) is characterized through digestive disorders, allergies and moderate eosinophilia.
2. *Trichinella spiralis* - more in the muscles, but initially also intestinal. It is transmitted through

the consumption of pig meat infested with encysted larvae. Disease (trichinellosis or trichinellosis) manifests with headache, fever, accentuated palpebral edema, eosinophilia higher (60-80%), muscle pain (diaphragm, respiratory muscles, masticatory muscles). The natural carrier is the rat.

ANKYLOSTOMIDAE Fam.

Ankylostoma Duodenale - a parasite that is located in the duodenum (mucosa). Is more commonly found in in tropical areas (Egypt, India, China). The disease (ankylostomyosis) manifests through digestive disorders, epigastric pain, melena, anemia (simulates the duodenal ulcer), inattention, nausea, nervous disorders, seem as a result of toxins secreted by the parasite, eosinophilia, leukocytosis. The diagnosis is made using the method with charcoal.

ARACHNIDA. PEDICULIDE

Are a part of the *ARTHROPOD* (artros = articulations; podos = foot). The subclasses are:

1. *ARACHNIDA Class;*
2. *INSECTA Class;*
3. *MIRIAPODA Class;*
4. *CRUSTACEA Class*.

The first two classes present medical importance. There is an increased number of species (approx. 2 million); some have medical importance because:

- They have and venomous bite
- They can cause disease
- They can be vectors for infectious agents of microbial, viral or parasitic etiology

1*. ARACHNIDA Class*

Includes arthropods with 4 pairs of limbs being octopod arthropods. They have a cephalothorax and an abdomen. The abdomen is usually bulky and occupies more than half the size of the body. Of this class belongs the order ACARIENI. The ACARIENI order is divided into two families

- *Scarcoptidae Family*
- *Ixodidae Family*

Scarcoptidae Family: are of two types:

- *Scarcoptidae Psorice* (live in the skin)

1. *Sarcoptes scabiae* that transmit scabies

- *Scarcoptidae detriticole* (live in food scraps)

1. *Tirogliphus farina* (flour)
2. *Gliciphagus cursor* (jams)

Ixodidae Family: have two subfamilies *Ixodine* and *Argasine*

Ixodine (the ticks) has a mouthpiece fitted with a rostrum and a hypostome adapted for stinging and sucking. Its face is located terminal. The main species:

1. *Ixodes ricinus;*
2. *Ripicephalus sanguineus;*
3. *Dermacentor andersoni*.

Argasine: with the rostrum situated sub terminally, on the ventral face of the parasite. Main species:

1. *Argas persicus;*
2. *Ornithodorus*.

Ticks may transmit meningoencephalitis which sometimes have evolutions unfavorable for the human.

2.*INSECTA Class*

It has head, chest and abdomen. From the chest level they have 3 pairs of limbs (hexapod). They are also hematophagous.

They are classified by 4 orders:

1. Anoplura;

2. Aphaniptera;

3. Hemiptera;

4. Diptera.

The order *ANOPLURA* includes the *PEDICULIDAE family* (lice) that are a part of:

1. *Pediculus Corporis* – body lice
2. *Pediculus capitis* – head lice
3. *Phtirius Pubis* – wide lice

INSECTA CLASS

1. *Order ANOPLURA*

2. *Order DIPTERA:* includes insects that have 2 well developed wings. They are classified by type or by sub order.

A. Sub Order Nematocera:

- Has long antennae (12-14 segments)

3 families present medical importance :

a. *Family Culicidae (mosquitos*) are divided into 3 types – *culicini* (common mosquito)

- *anophelini* (malaria vector)

- *aeini* (tropical rainforest mosquito)

b. *Family Psychodidae: genus Phlebotomus*. Vector for some diseases: leishmania and 3 day fever (papatasi fever), a viral effect.

c. *Family Simulidae: genus Simulium*. Vector for some diseases: (Onchocerciasis).

B. *Sub Order Brachiera*:

- includes *dipteria* with short antennae (3-4 segments)

Only one family presents medical importance:

Family Muscidae: there are two types:

- Biting muscide : have a mouth apparatus adapted for stinging and sucking; they are hematophagous (ex: *Glossina*, which can transmit Trypanosoma);
- Non-biting muscide: have a mouth apparatus adapted for lucking and sucking (ex: Common fly – normal one). They are *mechanical vectors*, which means infectious agents (microbes, viruses or parasites) which don't suffer any modifications inside the vector.

The larva of muscidae causes the disease called MAYIASIS (ex: larva of *Hypoderma Bovis*). The most common type is cutaneous myiasis, but wound myiasis, eye myiasis, myiasis of the external auditive canal, nostrils etc.

3. *Order APHANIPTERIA:* includes insects with no wings, a big abdomen, wide laterally and has the third pair of legs more developed than the first two pairs, which allows for leaps of great distance (from one place to another, from one host to another).

The two species of family *Puricidae* (fleas) that present medical importance are:

Pulex irritans (human fleas).

Ctenocephalus canis (dog fleas).

Fleas, alongside the fact that the cause discomfort, they are also vectors for the plague.

4. *Order HEMIPTERA*: encompasses insects that have remnants of wings and have a very bulky abdomen when compared to with the head and thorax. *Family Cimicidae* presents medical importance with the representative *Cimex Lectularius* (bed bug), meaning that they are a vector for the plague, relapsing fever, Koch bacillus or viral hepatitis B.