
**Diagnostic microbiology**
Identification of the organism causing an infectious process is frequently essential for effective antimicrobial and supportive therapy.

Microbiologic diagnosis of an infectious disease usually involves one or more basic laboratory techniques: direct microscopic visualization, cultivation and identification of the organism, detection of microbial antigens, detection of microbial DNA or RNA and detection of an inflammatory or host immune response to the microorganism.

**Direct visualization of the organism**
Pathogenic organism can be directly visualized by microscopic examination of patient specimens, such as sputum, urine or cerebrospinal fluid.

**Methods of staining:** Gram stain, Acid-fast stain, methylene blue stain.

Gram-stain separates bacteria into two classification groups, according to the composition of their cell walls.

Acid-fast stain such as Ziehl-Neelsen is used to identify organisms that have waxy material (mycolic acids) in their cell walls. The most clinically important acid-fast bacteria involves *Mycobacterium tuberculosis*.

**Collection of specimens**
Two general strategies are used to isolate pathogenic bacteria, depending on the nature of the clinical sample.

**Media:** enriched media, selective media

**Immunological detection of microorganisms:**
In the diagnosis of infectious disease, immunologic methods take advantage of the specificity of antigen-antibody binding. Most methods for determining whether antibodies or antigens are present in patient’s sera or other body fluids require some type of immunoassay procedure.

**Detection of microbial DNA or RNA**
A highly specific method of pathogen detection involves identification of its DNA or RNA in a patient sample. The basic strategy is to detect a relatively short sequence of nucleotide bases of DNA or RNA.

**Sterilization and disinfection**
**Sterilization** is defined as the process where all the living microorganisms, including bacterial spores, are killed. Sterilization can be achieved by physical, chemical and physicochemical means. Chemicals used as sterilizing agents are called chemisterilants.
**Disinfection** is the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects. Disinfection can be achieved by physical or chemical methods. Chemicals used in disinfection are called disinfectants. Different disinfectants have different target ranges, not all disinfectants can kill all microorganisms. Some methods of disinfection such as filtration do not kill bacteria, they separate them out.

**Asepsis** is the employment of techniques (such as usage of gloves, air filters, uv rays, etc.) to achieve microbe-free environment.

**Antisepsis** is the use of chemicals (antiseptics) to make skin or mucus membranes devoid of pathogenic microorganisms.


**Chemotherapeutic agents**

Chemotherapeutic agents can be classified into groups:
- broad spectrum chemotherapeutics
- narrow spectrum chemotherapeutics
- those with bacteriostatic effect
- those with bactericidal effect

Chemotherapeutic agents may have different mechanisms of action on bacterial cell:

1. agents that inhibit cell wall synthesis – penicillins, cephalosporins, vancomycin, bacitracin
2. agents that inhibit protein synthesis - streptomycin, neomycin, erythromycin, doxycycline, gentamycin, chloramphenicol, clindamycin, lincomycin
3. agents that inhibit the cell membrane function : nystatin, polymixin, amphotericin
4. agents that inhibit nucleic acid synthesis : nalidixic acid, quinolones
5. agents that act as antimetabolites: sulfonamides, isoniazid, trimethoprim, nitrofurans

Proper selection of an antimicrobial agent for the therapy should be based on many criteria, including both microbial and host factors.

Microorganism may manifest different mechanisms of the resistance to antibiotics.

Some mechanisms of antibiotic resistance will be discussed during the course.

In order choose the most effective antibiotic, susceptibility tests must be performed. There are 2 types of the tests: qualitative tests(e.g., disc diffusion method) and quantitative tests (E-test, MIC, MBC).

### 3. Genera: *Staphylococcus*, *Streptococcus* and *Enterococcus*.

**Staphylococcus**

Gram-positive cocci, arranged in grape-like clusters.

The main pathogenic species is *Staphylococcus aureus*. The pathogenicity of *S. aureus* is connected with production of enzymes and toxins: plasmocoagulase, protein A, leukocidins, haemolysins, lipase, hyaluronidase, TSST-1 toxin, exfoliatin, enterotoxins.

*S. aureus* may be responsible for many local skin infections (folliculitis, furuncle, “scalded skin syndrome”, impetigo), but also for serious invasive diseases (pneumoniae, gastroenteritis, osteomyelitis, sepsis).
The diagnosis is based on culturing a sample on blood agar or Chapman medium. Also coagulase-negative \textit{Staphylococci} (\textit{S. saprophiticus} and \textit{S. epidermidis}) may be pathogenic (biomaterial-associated infections, urinary tract infections in women). In antibiotic therapy of infection caused by \textit{Staphylococcus}, the presence of MRSA and MRCNS strains must be taken into account.

**The most important species include:** \textit{Streptococcus pyogenes}, \textit{S. pneumoniae}, \textit{S. agalactiae}, \textit{Enterococcus faecalis} and \textit{E. faecium}.

The streptococci are facultatively aerobic, catalase-negative, Gram-positive cocci that grow in pairs or chains. Virtually all pathogenic species can colonize the host without causing an infection. The upper respiratory, gastrointestinal and the female genitourinary tracts are the sites customarily colonized. During periods of immune system dysfunction, streptococci may quickly transform from harmless commensals to life-threatening pathogens. In the microbiological laboratory, streptococci are initially categorized by their manner of growth on 5\% sheep blood agar and type of haemolysis. Laboratory tests used for diagnosis of streptococci are, e.g., direct antigen detection (Lancefield grouping), optochin susceptibility test (for \textit{S. pneumoniae}) or bacitracin susceptibility test, and ASO reaction (for \textit{S. pyogenes}). \textit{S. pyogenes} is an important cause of upper respiratory tract (pharyngitis, tonsillitis) and cutaneous infections (e.g. impetigo, erysipelas, cellulitis, myositis). In complications, the influence of protein M is very important. \textit{S. pneumoniae} is the leading cause of community-acquired bacterial pneumonia and, in children, of bacteraemia, otitis media, sinusitis and meningitis. \textit{S. agalactiae} is an important cause of neonatal sepsis and meningitis. \textit{S. mutans} plays an important role in dental caries formation and endocarditis.

The enterococci are members of the normal gastrointestinal tract flora of humans. They are important aetiologic agents of urinary and biliary tracts infections, wound infections, intra-abdominal abscesses, endocarditis, bacteraemia and a variety of nosocomial infections.

4. Genera: \textit{Neisseria, Moraxella} and \textit{Haemophilus}.

\textit{Neisseria}  

The genus \textit{Neisseria} consists of Gram-negative, aerobic diplococci. Two \textit{Neisseria} species are pathogenic for humans – \textit{Neisseria gonorrhoeae} and \textit{Neisseria meningitides}. \textit{Neisseria gonorrhoeae} is frequently observed inside polymorphonuclear leukocytes of clinical samples obtained from infected patients. \textit{N. gonorrhoeae} is usually transmitted during sexual contact, or during the passage of a foetus through an infected birth canal. Structure: Pili, LOS (lipooligosaccharides), OMPs (outer membrane proteins, IgA protease. Auxotype AHU (arginine, hypoxanthine, and uracil). Pili and OMP II facilitate the adhesion of the gonococcus to epithelial cells of the urethra, rectum, cervix, pharynx or conjunctiva.  

Clinical significance:
Genitourinary tract infections. The patient presents with yellow, purulent discharge. In females, infection occurs in the endocervix and extends to the urethra and vagina. The disease may progress to uterus, causing salpingitis, pelvic inflammatory disease (PID). Rectal infection, pharyngitis, ophtalmia neonatorum, disseminated infection.

Laboratory identification:
Smear of the urethral exudates – Gram-stain
Selective media – Thayer-Martin medium (chocolate agar supplemented with several antibiotics)
PCR

*Neisseria meningitidis* is one of the most frequent causes of meningitis, it can also take the form of a fulminant meningococcaemia, with intravascular coagulation, circulatory collapse with a potentially fatal shock, but without meningitis.

Structure.
Pili, IgA protease, polysaccharide capsule
Serogroups:
The lipooligosaccharide capsule is antigenically diverse, forming 14 capsular polysaccharide types called serogroups A,B,C, W and Y.

Epidemiology:
Transmission occurs through inhalation of respiratory droplets from a carrier or a patient in the early stage of the disease.

Pathogenesis
The antiphagocytic properties of the meningococcal capsule aid in maintenance of infection. LOS also is released during autolysis and bacterial cell division, and is for many toxic effects can be found in disseminated meningococcal disease.

Clinical significance:
Meningitis, septicaemia

Laboratory identification;
Direct microscoping – Gram-stained CSF smears.
Culture: Thayer-Martin agar in increased CO₂
Latex agglutination test

Treatment and prevention
Cephalosporin III generation

Vaccines - a capsular vaccine for serogroups A, C, W, and Y
Vaccine for group B
Rifampin is usually used to treat family members of an infected individual, the drug is effective in eliminating the carrier state.

*Moraxella*

The genus involves nonmotile, Gram-negative diplococci. The most important pathogen involves *Moraxella catarrhalis*. This organism provides the cause of infections of the respiratory system, middle ear, eye, CNS and joints.
**Haemophilus**

*Haemophilus influenzae* is responsible for producing a variety of infections including meningitis and respiratory infections. Six serological types (a,b,c,d,e,f) are recognized, based on the antigenic structure of the capsular polysaccharides. Non-encapsulated strains are nontypable. Other species of *Haemophilus* include: *H. parainfluenzae* (pneumonia, endocarditis), *H. ducreyi* (venereal chancre) and *H. aegyptius* (conjunctivitis). The genus *Haemophilus* is composed of Gram-negative coccobacilli. These organisms are fastidious and require factors X (haemin) and/or V (NAD). *Haemophilus* contains LPS in the cell wall but produces no apparent extracellular toxins. *Haemophilus* is transmitted from an infected human being to other humans. The organisms colonize the nasopharynx and are spread by direct contact. *Haemophilus* are capable of penetrating the epithelium to produce a bacteraemia that may lead to spread of the organisms to many organs. Its capsule is the major determinant of virulence yet unencapsulated strains produce ear, sinus and respiratory infections. *H. influenzae* type b is the most common cause of bacterial meningitis in children aged 6 months-2 years. It is uncommon in adults because of protective antibody. Cellulitis, conjunctivitis, epiglottitis and arthritis may also result from *Haemophilus* infection. For pneumonia in adult men, the unencapsulated *H. influenzae* may provide the cause.

5. **Gram-negative rods; genera: Bordetella, Legionella and Pseudomonas.**

**Bordetella**

*Bordetella pertussis* is the aetiologic agent of pertussis, more commonly known as whooping cough. *Bordetella* are Gram-negative coccobacilli. They produce a capsule and are strict aerobes. *Bordetella pertussis* produce several exotoxins. These include: pertussigen: A 120 kD protein, exhibiting the A-B model for toxin activity. Pertussigen is an ADP-ribosyltransferase that interferes with the transfer of signals from cell surface receptors. Pertussigen is also involved in mediating attachment to respiratory epithelia. Adenylate cyclase toxin: this toxin increases cAMP levels, inhibiting immune effector cell functions. Tracheal cytotoxin: this toxin causes ciliostasis and extrusion of ciliated epithelia. Dermonecrotic toxin: this heat labile substance causes tissue destruction. Filamentous haemagglutinin is involved in attachment to host cells. Whooping cough results from colonization and multiplication of *Bordetella pertussis* on the mucous membranes of the respiratory tract, in particular, the ciliated epithelial cells. Production of toxins irritates cells, causing ciliostasis and leukocytosis. The hallmark of pertussis is the spasmatic cough that may last 6 weeks. Occasional secondary complications include encephalopathy, seizures and pneumonia. The disease is highly contagious. Whooping cough requires a 7-14 day asymptomatic incubation period. This is followed by the catarrhal stage, which lasts 1-2 weeks. Symptoms include fever, rhinorrhea and a highly infectious cough. The next 2-4 weeks define the paroxysmal phase, during which the spasmatic (“whooping”) cough is observed. Vomiting and leukocytosis (> 100,000 lymphocytes/mm³) are also evident. Finally, the convalescent phase marks the end of disease and may last 3-4 weeks or longer. During this period, secondary complications may occur.
**Legionella**

*Legionellaceae* are facultative Gram-negative rods, and intracellular parasites. The *Legionellaceae* family includes 34 species, but the most important for human diseases is *Legionella pneumophila*. The organism gains entry to the upper respiratory tract by aspiration of water containing the organism, or by inhalation of a contaminated aerosol. *Legionellaceae* primarily cause respiratory tract infections: Legionnaires’ disease (LD) and Pontiac fever. Legionnaires’ disease (LD) is an atypical, acute lobar pneumonia with multisystem symptoms. Predisposing factors include, for example, immunocompromise, pulmonary compromise. Pontiac fever is an influenza-like illness that characteristically infects otherwise healthy individuals.

**Pseudomonas aeruginosa**

*Pseudomonas* are Gram-negative rods. They are motile, non-fermentative aerobes that can utilize acetate for carbon and ammonium sulphate for nitrogen. Many species are resistant to high salt, dyes, weak antiseptics and most antibiotics. *P. aeruginosa* can grow at 42°C and it produces many exoenzymes including haemolysins, leukocidins and proteases. In addition, a toxin, called toxin A, is the most toxic product produced by *Pseudomonas*. This product causes the ADP-ribosylation of translation factor EF-2, producing ADP-ribosyl-EF-2. The effect of this enzymatic activity is the loss of host cell protein synthesis capability. This mechanism is identical to that produced by diphtheria toxin. *Pseudomonas* can be found in the soil, in water, or on vegetation.

On average, 3% of persons entering the hospital have *Pseudomonas* in their stools. After a hospital stay of just 72 hrs, 20% patients have *Pseudomonas*. The organisms are spread from patient to patient via staff, contaminated reservoirs, respiratory equipment, food, sinks, taps, mops; most moist environments. *Pseudomonas* produces localized infections following surgery or burns. Localized infections can lead to generalized, and occasionally fatal, bacteraemia.

*Pseudomonas* is also responsible for a number of nosocomial infections including urinary tract infections following catheterization, pneumonia resulting from contaminated respirators, and eye and ear infections.

6. Genera: *Corynebacterium* and *Mycobacterium*.

**Corynebacterium**

*Corynebacterium diphtheriae* are Gram-positive rods. They occur in characteristic clumps – Chinese characters or picket fence patterns. *C. diphtheriae* cause life-threatening illness – diphtheria. Diphtheria is caused by the local and systemic effects of an exotoxin that inhibits eukaryotic protein synthesis. The structural gene for diphtheria toxin (tox) is encoded in the genome of a corynebacterial bacteriophage (β-phage). Only *C. diphtheriae* strains that are lysogenic for a β-phage can produce toxin and are therefore virulent. Diphtheria consists of a strictly local infection (usually of the throat) with a pseudomembrane (thick, grayish, adherent exudate) production. As the disease progresses, generalized symptoms occur due to production and absorption of toxin. The major clinical effects involve the heart and peripheral nerves. Treatment of diphtheria requires prompt neutralization of toxin (antitoxin), followed
by eradication of the organism (antibiotic therapy). Prevention of diphtheria – immunization with toxoid (DTP, DTaP vaccine).

Other corynebacterium species morphologically resemble the type species, C. diphtheriae, and they are commensals of e.g. nose, throat, nasopharynx, skin, urinary tract. They are called diphteroids, and are generally unable to produce exotoxin. E.g. of the other corynebacteria species: C. jeikeium, C. urealyticum, C. ulcerans.

**Mycobacterium**

*Mycobacteria* are long, slender rods. Mycobacterial cell walls have a β-hydroxylated fatty acids called mycolic acid. *Mycobacteria* are strictly aerobic. Most species grow slowly. The most important species are: *Mycobacterium tuberculosis* and *Mycobacterium leprae*. The *Mycobacterium tuberculosis* (MTB) complex includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *Mycobacterium tuberculosis*, developing tuberculosis.

Tuberculosis bacilli are inhaled into the alveoli of the lung, and are ingested by macrophages, but are not killed by them.

- Obligate aerobes, growing most successfully in tissues with a high oxygen content, such as the lungs.
- Facultative intracellular pathogens, usually infecting mononuclear phagocytes (e.g. macrophages).
- Hydrophobic with a high lipid content in the cell wall.
- Acid-fast bacilli

*M. tuberculosis* stimulates both a humoral and a cell mediated immune response.

**Clinical significance**

- Primary tuberculosis – initial phase
- Primary disease – tubercle formation

Miliary tuberculosis – acute infection

**Multidrug-resistant tuberculosis (MDR)**

Extensively drug-resistant tuberculosis (XDR)

Extremely drug-resistant tuberculosis (XXDR-TB)

**Laboratory diagnosis**

Ziehl-Neelsen acid-fast staining

Media used to grow MTB: Middlebrook's medium and Lowenstein-Jensen medium.

PCR

Quantiferon-TB test (QFT)

T-SPOT Tb tests

Tuberculin skin tests

**Treatment and Prevention**

Multiple drug therapy is recommended, eg. isoniazid, rifampicin, ethambutol, streptomycin, pyrazinamide

X-ray patterns
Vaccines – attenuated BCG - Bacillus Calmette-Guérin (attenuated strain of Mycobacterium bovis)

MOTT Mycobacterium other than tuberculosis
Mycobacterium avium – intracellulare complex
Mycobacterium kansassii
Mycobacterium marinum

Mycobacterium leprae is an acid-fast bacillus, causing leprosy (or Hansen’s disease). The incubation period is protracted, so that clinical disease may develop years after initial contact with the organism. M. leprae is generally transmitted from human to human e.g. with exudates of a leprosy patient’s skin lesions. Leprosy is a chronic granulomatous condition of peripheral nerves and mucocutaneous tissues (particularly the nasal mucosa). The general leprosy classification includes tuberculoid and lepromatous forms. In tuberculoid leprosy lesions occur as large macules, neuritis leads to patches of anaesthesia in the skin, the patient mounts a strong cell-mediated immune response (positive skin test with lepromin). The course of lepromatous leprosy is slow but progressive. Large numbers of organisms are present in the lesions and immunity is severely depressed.


Escherichia coli
Members of the genus Escherichia are common bacteria that colonize the human large intestine. Most are opportunistic, normal flora but some are potent pathogens. Transmission of diarrheal disease is generally person to person, usually related to hygiene, food processing and sanitation. Escherichia are Gram-negative bacilli that ferment lactose. Most are motile by peritrichious flagella, cell wall containing LPS. Approximately 170 different O antigens have been delineated and some of these are cross-reactive with Shigella, Salmonella and Klebsiella. Motile strains possess H (flagellar) antigens that can be used for epidemiologic purposes. Escherichia also possess K (capsular) antigens similar to the Vi antigen of Salmonella. Enterotoxigenic strains may also display colonization factor antigens (CFA/I, CFA/II). Enterotoxigenic (ETEC) strains induce small intestine traveler’s diarrhea: watery stools, cramps, nausea, low fever enterotoxins ST and LT. Enteroinvasive (EIEC) strains induce large intestine diarrhea, fever, cramps, watery diarrhea, followed by scant, bloody stools resulting from tissue invasion and destruction of epithelial cells (plasmid-mediated). Enteropathogenic (EPEC) strains induce small intestine infantile diarrhea with fever, nausea, vomiting resulting from adherence and destruction of epithelial cells (plasmid-mediated) Enterohemorrhagic (EHEC, O157:H7) strains induce large intestine haemorrhagic coliti with severe abdominal pain, watery diarrhea, followed by grossly bloody stool SLT-I, SLT-II cytotoxins (“verotoxins”).
8. Enteric bacteria: *Salmonella*, *Shigella* and *Yersinia*.

**Salmonella**

*Salmonellae* possess 3 major antigens; the "H" or flagellar antigen (phase 1 & 2), the "O" or somatic antigen (part of the LPS moiety) and the "Vi" or capsular antigen (referred to as "K" in other *Enterobacteriaceae*. *Salmonellae* also possess the LPS endotoxin characteristic of Gram-negative bacteria. This LPS is composed of an "O" polysaccharide ("O" antigen) an "R" core and the endotoxic inner "Lipid A". Endotoxins evoke fever and can activate complement, kinin and clotting factors. *Salmonella typhi* the most common species isolated from this typhoid fever, has a 10-14 day incubation period and may last for several weeks. *Salmonella gastroenteritidis* is the most common form of salmonellosis and generally requires an 8-48 hour incubation period and may last from 2-5 days. Symptoms include nausea, vomiting and diarrhea (*Salmonella enteritidis, cholerae suis*).

**Shigella**

*Shigella dysenteriae* is responsible for bacillary dysentery, a disease most often associated with crowded, unsanitary conditions. Other species of *Shigella* may produce milder forms of diarrheal disease. *Shigellae* are facultative, non-motile, Gram-negative bacilli. They possess the heat stable endotoxin (LPS) characteristic of Gram-negative bacteria. *Shigellae* are pathogenic primarily due to their ability to invade intestinal epithelial cells. Dysentery is an oral infection transmitted via fecal contamination of water or food. During the 1-4 day incubation period, penetration of bacteria into the mucosal epithelial cells of the intestine causes an intense irritation of the intestinal wall, producing cramps and a watery, bloody diarrhea.

**Yersinia**

The genus *Yersinia* is composed of Gram-negative, bipolar staining coccobacilli. Like other *Enterobacteriaceae*, their metabolism is fermentative. The organism *Yersinia pestis* is responsible for the plague, other species of *Yersinia* generally produce a self-limiting gastroenteritis. Pathogenic *Y. pestis* produce two anti-phagocytic components; F1 antigen and the VW antigens. Both are required for virulence and, interestingly, are only produced when the organism grows at 37°, not at lower temperatures. This might explain why the bacteria are not virulent in their alternate host, the flea, which has a body temperature near 25°. Moreover, the bacteria are capable of surviving and multiplying within monocytes, but not PMNs, and upon emerging from the monocytic host, the bacteria possess their F1 and VW antigens. Most diseases produced by *Y. enterocolitica, Y. pseudotuberculosis* involve a typical gastroenteritis, characterized by fever, abdominal pain, and diarrhea. The illness generally lasts from 1 to 2 weeks but chronic cases may persist for up to a year. A rapid diagnosis must be made because of the speed at which the disease progresses and the high mortality rate. Bubonic plague can result in 75% mortality within a few days; the pneumonic form can result in greater than 90% mortality within 24 hours. Laboratory: examination of sputum or a lymph node biopsy will reveal Gram-negative, bipolar staining coccobacilli. However, for the safety of workers and the community, P-3 level containment is required for *Y. pestis*. *Y. enterocolitica, Y. pseudotuberculosis* may be grown on a variety of standard bacteriologic medias.

Lactobacillus
Various species of Lactobacillus are part of the commensal flora mucous membranes (e.g. vagina, GI tract). They are facultative anaerobic or microaerophilic, Gram- positive rods. Lactobacilli produce quantities of lactic acid during fermentation, and have been thought to assist in maintaining the acid pH of normal mucous epithelia (e.g. vagina). The acid production by oral lactobacilli may play a role in the production of dental caries. Lactobacilli are used as probiotics with potential therapeutic properties (e.g. during/after antibiotic therapy). The most important species of Lactobacillus: L. acidophilus, L. rhamnosus, L. casei, L. crispatus, L. fermentum.

Listeria
Listeria species are short, Gram- positive rods, which are intracellular parasites. They displays a distinctive tumbling motility (at 25°C). Listeria infections are usually foodborne and are most common in pregnant women, their fetuses or newborns, and in immunocompromised individuals. Listeria monocytogenes is a facultative, intracellular parasite, that attaches to and enters a variety of mammalian cells. The microorganism once internalized, escapes from the phagocytic vacuole by elaborating a toxin (listeriolysin O). Septicemia and meningitis are the most common forms of listeriosis. Pregnant women may have milder “flu-like” illness and the microorganism can be transmitted to a newborn (newborn meningitis) or to the fetus (abortion).

Actinomyces
Gram-positive rods or filaments, non-motile, anaerobic. The major species appears as normal saprophytes in the oral cavity of human. Sometimes they can be responsible for the disease called „actinomycosis”. There are 3 forms of the actinomycosis: the cervicofacial, the thoracic and the abdominal one. Laboratory diagnosis of the disease is based mainly on culturing of the sample in anaerobic conditions and, then, antibiotic therapy is performed.

Nocardia
Gram-positive rods, aerobic, most strains acid-fast. May produce yellow, orange, red or white colonies. The main representative is N. asteroides. Nocardia spp. are soil and dust saprophytes. In immunosuppressed patients they may be responsible for skin infections, broncho-pulmonary infections and brain abscesses. Laboratory diagnosis is based on culturing a specimen on Sabouraud medium or brain-heart infusion agar, then, antibiotic therapy is performed.
10. Spore-forming bacilli: *Bacillus* and *Clostridium*.

**Bacillus**

They are Gram-positive, form endospores, are either strictly or facultatively aerobic. Bacillus are found in soil and water and usually encountered in the medical laboratory as airborne contaminants.

Most important bacillus are: *Bacillus anthracis*, *Bacillus cereus*, *Bacillus subtilis*

*Bacillus anthracis* can cause anthrax. Anthrax is an enzootic disease of worldwide occurrence.

Two major virulence factors of *Bacillus anthracis*, a poly-c-D-glutamic acid capsule and two binary exotoxins, lethal toxin and edema toxin are considered to be the major pathogenic factors of anthrax.

*Bacillus anthracis* has 3 components: oedema factor, lethal factor and protective antigen.

Clinical significance
- Cutaneous anthrax
- Pulmonary anthrax (woolsorter’s disease)
- Gastrointestinal form

Treatment and prevention:
- Penicillin, Doxycycline, ciprofloxacin
- Prevention: vaccine

**Bacillus cereus**

*Bacillus cereus* is an endemic, soil-dwelling, Gram-positive, rod-shaped strain. Some strains are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for animals. It is the cause of diarrhea after rice food.

*B. cereus* bacteria are aerobes, and like other members of the genus *Bacillus* can produce protective endospores.

**Clostridia and other anaerobic rods**

*Clostridia* are obligate anaerobes microorganisms, consists of Gram-positive, spore-forming rods that are associated with soft tissue and skin infection, and antibiotic-associated colitis and diarrhea. These organisms also synthesize some of the most potent exotoxins, which can cause botulism, tetanus, gas gangrene, and pseudomembrane colitis.

**Clostridium tetani** – forms terminal spores.

Produces tetanotoxin, called tetanospasmin, an extremely potent toxin. It is transported from an infected locus by a retrograde neuronal flow or by blood.

Tetanus toxin blocks release of inhibitory neurotransmitters (such as γ-amino-butyric acid, glycine).

Tetanus presents as a specific type of paralysis, the spasmatic paralysis. In the early stages of the disease, the jaw muscle are affected, causing trismus or lockjaw with painful spasm, convulsion and opisthotonus.

Treatment and prevention:
Prompt administration anti-toxin to neutralize the toxin
Penicillin – dirty wounds
Spasmolytic drugs
Prevention - Active immunization with tetanus toxoid (inactivated toxin) is usually administered to children as a triple vaccine with diphtheria toxoid and pertussis antigens, called DPT

*Clostridium botulinum*, forming subterminal spores, causes botulism, which occurs in several clinical forms.

Botulism is caused by the action of a neurotoxin. A nerve stimulus prompts an influx of calcium that causes the release of acetylcholine. Botulinum toxin passes from the gut into the circulation. Botulinum toxin is taken up by an axon, and is cleaved into a light and a heavy chain. Botulinum toxin causes flaccid paralysis.

*C. botulinum* is found worldwide in soil and aquatic sediments, and the spores frequently contaminate vegetables, and meat or fish. There are a number of types of botulinum toxin, designated A through G, but human disease is almost always caused by types A, B, or E.

Clinical significance:
Classical botulism - is a food poisoning.
Infant botulism
Wound botulism

Treatment and prevention:
Antitoxin, which neutralizes unbound botulinum toxin, should be administered as soon as possible in persons suspected of botulinum intoxication.

*Clostrium perfringens*
Can cause anaerobic cellulitis, myonecrosis (gas gangrene), also food poisoning.
C. perfringens secretes a variety of exotoxins, enterotoxins and hydrolytic enzymes.
Exotoxins include at least 12 exotoxins. The most important is alpha toxin called lecithinase (phospholipase C^3^) that degrades lecithin in mammalian cell membranes, causing lysis of endothelial cells as well as erythrocytes, leukocytes and platelets.
Enterotoxin, a heat labile protein, acts in the lower portion of the small intenstine.

Clinical significance:
Myonecrosis (gas gangrene)
Anaerobic cellulitis
Food poisoning
Enteritis necroticans
Clostridial endometritis.

Treatment and prevention:
Hyperbaric oxygen chamber
Antibiotics

*Clostridium difficile*
Such a type of diarrhea is a common complication of anti-microbial and anti-neoplastic drug treatment and can cause a pseudomembrane colitis.
C. difficile is a component of the normal flora of the large intestine. Pathogenic strains produce two toxic polypeptides, A and B. Toxin A is an enterotoxin that causes fluid secretion. Toxin B is a cytotoxin.

Clinical significance:
AAD – antibiotic-associated diarrhea
AAC - antibiotic-associated colitis
PMC – pseudomembranous colitis

Laboratory identification
Cultured from stools. ELISA for developed exotoxins A and B.

Treatment:
Stop antibiotic treatment
Fluid replacement
Metronidazol or vancomycin.


Brucella
Brucella abortus (cow), Brucella melitensis (goat), Brucella suis (pig), Brucella canis (dog).
The Brucellae are generally associated with animal infections but most are also pathogenic for humans. All human infections come from animals; there is no human to human transmission. Such diseases are called "zoonoses". Brucellae are intracellular parasites. The genus Brucella is composed of Gram- negative coccobacilli. Most are aerobic but grow best in a 5-10% CO2-enriched environment. Their metabolism is oxidative. Brucellae possess a typical Gram-negative LPS endotoxin, as well as two major serological determinants; A and M. Symptoms of brucellosis are variable and diagnosis is, therefore, very difficult. Flu-like symptoms with limb and back pain, an intermittent fever with malaise may last up to 3 months for acute disease (a year or more for subacute or chronic disease). Laboratory: isolation of Brucella from the blood is possible. Cultures must be incubated 3-4 weeks with added CO2.

Campylobacter
Gram-negative, curved or S-shaped, motile, microaerophilic rods.
The majority of species there are pathogens in animals. Two species, C. jejuni and C. foetus, also can be causative agents of human disease.
C. jejuni may cause enteritis and pseudoappendicitis. C. foetus (occasionally) may be responsible for bacteraemia and central nerves system infection in immunocompromised patients.
The main diagnostic method it is a culture of microorganisms in microaerophilic conditions for 3 to 5 days.

Francisella tularensis
Francisella tularensis is a Gram-negative bacteria (Gram-negative bacteria contain an outer membrane outside the peptidoglycan cell wall, unlike Gram-positive bacteria that have a thicker layer of cell wall and no outer membrane; many of the Gram-negative bacteria are
pathogenic), with pili on the surface. They are nonmotile, aerobic, and non-spore forming bacteria. In nature, they can survive up to several weeks at low temperatures in water, soil, and animal carcasses. In laboratory settings, *Francisella tularensis* appears as small rods and is grown best at 35-37°C. Bacteria that causes tularemia, or "rabbit fever" (it is called rabbit fever because rabbits are vectors for the disease) that is contagious to humans. There are four known subspecies of *Francisella tularensis*. There are two strains of *Francisella tularensis* that are studied the most: the more virulent Type A strain (found in North America), and the less virulent Type B (subspecies holarctica, also referred to as palearctica) strain (found in Europe). Two other subspecies are the non-virulent mediiasiatica, found in central Asia, and novicida, about which not much is known. *Francisella tularensis* is a facultative intracellular bacterium that is capable of infecting most cell types but which primarily infects macrophages in the host organism. *Francisella tularensis* entry into the macrophage occurs via phagocytosis and the bacterium, which is sequestered from the interior of the infected cell by a phagosome.

**Pasteurella multocida**

*Pasteurella multocida*, a Gram-negative coccobacillus found in the normal flora of many wild and domestic animals, especially dogs and cats, is a common cause of human infections subsequent to animal bites. A recent survey in the United States has found that approximately 1/3 of the households have domestic cats or dogs. The risk of exposure to *P. multocida* in the home environment is therefore considerable. Infection in man is well recognized to occur through animal bites. A wide range of infections have been reported including meningitis, cellulitis, septicaemia, septic arthritis, osteomyelitis, and pulmnoary infections. Peritonitis due to this organism has occurred predominantly in patients with hepatic cirrhosis or high ethanol intake.

**12. Spiral bacteria; genera: Borrelia, Leptospira and Treponema.**

**Borrelia**

Motile, microaerophilic spirochetes, transmitted by arthropods.

There are 2 species responsible for human diseases: *B. burgdorferi* and *B. recurrentis*. *B. burgdorferi* is a causative agent of Lyme disease. Lyme disease is a tick-borne disease. The course of disease may be divided into 3 clinical stages: erythema migrans, neurological stage and chronic arthritis.

In laboratory diagnosis of Lyme disease different methods are used: culture, serological tests and PCR.

*B. recurrentis* is responsible for relapsing fever (tick-borne endemic relapsing fever and louse-borne epidemic relapsing fever)

Clinical diagnosis is confirmed by serological tests or by microscopic visualization of the microorganisms in blood of relapsing fever patients.

Treatment of *Borrelia spp.* is based on antibiotic therapy, no vaccine is available as yet.
**Leptospira**

Thin, flexible, helically shaped microorganisms with internal flagella, aerobic.  
The main species – *L. interrogans*, with most commonly occurring serovars:  
*L. icterohaemorrhagiae*, *L. canicola*, *L. pomona*, *L. grippotyphosa*.  
The microorganisms are responsible for the disease called leptospirosis, which is transmitted from animals to human (zoonosis).  
Leptospirosis may be associated with jaundice (Weil's disease) or without jaundice.  
Laboratory diagnosis based on serological tests, PCR, culture on enrichment media or direct microscopic identification under a dark field microscope.  
Treatment includes antibiotic therapy and sometimes dialysis of the patient.

**Treponema pallidum**

The primary stage involves multiplication of the bacteria at the site of entry to produce a localized infection. The secondary stage occurs following an asymptomatic period and involves dissemination of the bacteria to other tissues. The tertiary stage may occur after 20-30 years.

The *Treponema pallidum* are motile, helically coiled organisms having a corkscrew-like shape. They stain very poorly because their thickness approaches the resolution of the light microscope. The structure of these organisms is somewhat different: the cells have a coating of glycosaminoglycans, which may be host-derived, and the outer membrane covers the three flagella that provide motility. The organisms cannot be grown in vitro. *Treponema pallidum* is capable of infecting all body tissues. The disease caused by *T. pallidum* is syphilis. This is a relatively painless, slowly evolving disease. The host-parasite relationship leads to short symptomatic periods when the organism multiplies, followed by prolonged asymptomatic periods when host responses produce healing.

Syphilis is strictly a person-to-person transmitted disease.

An incubation period of from 10 to 90 days precedes the clinical presentation, despite the fact that the organisms disseminate immediately. The prominent feature of the disease is vascular involvement, particularly of arterioles and capillaries.

The primary stage occurs weeks to months following infection. The principal sign of infection is the hard chancre, generally found on the genitals. This lesion is essentially painless. The secondary stage occurs following an asymptomatic period of 2-24 weeks. In the secondary stage, a skin rash spreads from the palms and soles towards the trunk. This rash may last 2-6 weeks and is followed by recovery. On average, about 25% of patients experience relapses of the secondary stage. Following the secondary stage is a period of latency which may last many years and during which there are essentially no clinical symptoms. The tertiary stage may erupt following the period of latency and can affect all areas of the body and be fatal. Cardiovascular and neurological involvement are the most frequent causes of death. Typically, however, the appearance of lesions called "gummas" mark the tertiary stage. These lesions are, in fact, large granulomas resulting from hypersensitivity reactions and they can be extremely disfiguring.

Laboratory: Darkfield examination of material from a chancre can show the presence of spirochetes. Immunological techniques including VDRL, RPR, FTA, TPHA, TPPA, ELISA.

**Helicobacter**
The main species: *Helicobacter pylori*
Gram-negative, spiral rods, or may form coccoid forms, microaerophilic. Microorganism able to survive in a low pH of the stomach.
Pathogenicity is connected with production of urease enzyme, cytotoxin VacA, CagA protein, presence of flagella.
The pathogen is responsible for duodenal and gastric ulcers, acute and chronic gastritis, gastric carcinoma and lymphoma.
Methods for detection of H. pylori infection are classified into 2 categories: invasive (culture, staining, rapid urease test and PCR) and noninvasive ones (urea breath test, serological tests, detection of antigens in the stools.
Treatment includes a triple therapy (PPI and 2 antibiotics).

**Vibrio**
Gram-negative, short, curved, motile rods.
Vibrios may be divided into 2 groups: *Vibrio cholerae* serogroup 01 and non 01 *Vibrio cholerae* plus related strains.
*Vibrio cholerae* 01- the aetiologic agent of the cholera disease is classified into 3 serotypes (Inaba, Ogawa, Hikojima) and 2 biotypes (classic and El Tor).
Cholera is an internationally reportable disease, responsible for many pandemics (mortality rate even up to 50%).
Pathogenicity is connected with the presence of an adhesion factor and ability to produce enterotoxin (choleragen).
Laboratory diagnosis is based on isolation of the microorganisms, biochemical identification and serological typing.
Other *Vibrios (V. parahaemolyticus, V. vulnificus, V. alginoliticus)* are responsible for gastrointestinal illnesses and soft tissue infections.

14. Obligate intracellular bacteria; the genera of *Chlamydia, Rickettsia and Coxiella;*
Cell wall-less bacteria: the order *Mycoplasmales.*

**Chlamydia pneumoniae**

**Chlamydia trachomatis**

The *Chlamydia* are obligate intracellular parasites. *Chlamydia trachomatis* is responsible for the diseases such as trachoma, inclusion conjunctivitis, lymphogranuloma venereum (LGV) and nongonococcal urethritis (NGU). In other words, oculourogenital infections. *C. pneumoniae* produces atypical pneumonia.
The *Chlamydia* have an unusual developmental cycle that involves two distinct forms: infectious elementary bodies and intracellular reticulate bodies. Elementary bodies attach and are internalized by susceptible host cells. Once inside, they reorganize into a replicative form (the reticulate body). Over a 24 hour period, these reticulate bodies divide and begin to
reorganize back into elementary bodies. About 48-72 hours after infection, the cell is lysed and numerous infectious elementary bodies are released. Trachoma (A,B,C) is an infection of the epithelial cells of the conjunctiva, producing inclusion bodies. Vascularization and clouding of cornea along with trichiasis (inward growth of eyelashes) can produce scarring that may lead to blindness. Inclusion conjunctivitis is a milder form that occurs in both children and adults. This form generally heals without scarring or blindness. Sexually (D-K) transmitted nongonococcal urethritis (NGU) Lymphogranuloma venereum (LGV) involving inguinal lymphadenopathy (“buboes”) can occur. Psittacosis is a respiratory disease ranging from influenza-like to pneumonia-like and is generally acquired from infected birds.

Rickettsia, Bartonella, Coxiella

The *Rickettsia* are Gram-negative, obligate intracellular bacteria that infect mammals and arthropods. These organisms are small, pleomorphic coccobacilli about 2 µm in length. Their structure is typical of Gram-negative bacteria. *Rickettsia* replicate in the cytoplasm and nucleus of their host cell; *Coxiella* replicate only in the phagolysosome. Typhus, spotted fever and trench fever are transmitted via arthropod vectors; Q fever is acquired via inhalation or ingestion of contaminated milk or food. Within minutes, the bacteria enter host endothelial cells via an induced phagocytosis. The enzyme phospholipase A may help penetration. Replication of the bacteria causes lysis of the host cell and consequent spread to other cells. Initial replication occurs at the site of entry, producing a local lesion. This is followed by dissemination via the vascular system producing vasculitis and a skin rash. These lesions may become necrotic. Virulence is probably due to many factors including release of endotoxin, the production of immune complexes and hypersensitivity reactions. A characteristic triad of symptoms include fever, headache and rash (no rash with Q fever). Epidemic typhus and trench fever are transmitted from human to human via the louse. Endemic (murine) typhus is primarily maintained in rodent populations and is transmitted via the flea. Humans are an accidental host. Spotted fever is found predominantly in animals and is transmitted by the tick. Humans are accidental hosts. Most cases of Rocky Mountain spotted fever in the US occur during the summer months in North and South Carolina, Kansas and Oklahoma. Q fever is found mostly in animals. Humans acquire disease primarily by inhalation of contaminated aerosols. Laboratory use of immunofluorescent antibodies to examine a biopsy can be diagnostic. The organism can be inoculated into tissue culture and grown over 4-7 days but this is very hazardous to personnel.

Mycoplasma

*Mycoplasma* is a genus of bacteria that lacks a cell wall. Without a cell wall, they are unaffected by many common antibiotics, such as penicillin or other beta-lactam antibiotics that target cell wall synthesis. Several species are pathogenic in humans, including *M. pneumoniae*, which is an important cause of atypical pneumonia and other respiratory disorders, and *M. homonis* and *U. urealyticum*, characterized by urethritis in males and genital tract infections in females, which is believed to be involved in PID (pelvic inflammatory disease). *Mycoplasma pneumoniae*: person-to-person transmission by contact with respiratory
secretions. Incubation period is 1 to 4 weeks. Risk groups: persons of all ages are at risk but rarely children less than 5 years old. It is the leading cause of pneumonia in school-age children and young adults. Outbreaks can occur especially in crowded military and institutional (e.g., college) settings. Outbreaks in these settings can last several months. Basic diagnostic strategy in clinical practice includes serology and standard polymerase chain reaction (PCR). New diagnostic techniques (PCR-related methods) may enable a more rapid diagnosis.


Viruses depend on the host cells that they infect to reproduce. When found outside of host cells, viruses exist as a protein coat or capsid, sometimes enclosed within a membrane. The capsid encloses either DNA or RNA which codes for the virus elements. Some viruses may remain dormant inside host cells for long periods, causing no obvious change in their host cells (a stage known as the lysogenic phase). But when a dormant virus is stimulated, it enters the lytic phase: new viruses are formed, self-assemble, and burst out of the host cell, killing the cell and going on to infect other cells. The diagram below at right shows a virus that attacks bacteria, known as the lambda bacteriophage, which measures roughly 200 nanometers.

Viruses cause a number of diseases in eukaryotes. In humans, smallpox, the common cold, chickenpox, influenza, shingles, herpes, polio, rabies, Ebola, hanta fever, and AIDS are examples of viral diseases. Even some types of cancer - though definitely not all - have been linked to viruses.

The Herpesviridae are a large family of DNA viruses that cause diseases in animals, including humans. The members of this family are also known as herpesviruses. Herpesviridae can cause latent or lytic infections. Herpesviruses all share a common structure—all herpesviruses are composed of relatively large double-stranded, linear DNA genomes encoding 100-200 genes encased within an icosahedral protein cage called the capsid, which is itself wrapped in a protein layer called the tegument, containing both viral proteins and viral mRNAs and a lipid bilayer membrane called the envelope. This whole particle is known as a virion.


Human Papilloma Virus (HPV). Papillomaviruses are widespread and warts are common in young adults. Humans are the only host for HPV and infections are generally transmitted by direct contact. However, the virus can survive for extended periods (months) outside the host, and this may provide another means of transmission. While there is a strong correlation between HPV infection and certain forms of cancer (e.g. cervical cancer), infection alone does not result in malignancy; rather, additional factors such as radiation, immunosuppression, or tobacco use are involved. Diagnosis: clinical: warts of the skin, oral cavity and genital area are generally diagnosed by appearance. Laboratory: Microscopy of wart scrapings shows a characteristic histologic appearance.
**Herpesviruses:** Herpes Simplex (HSV-1, HSV-2) HSV-1 is responsible for a variety of infections. Most commonly, HSV-1 produces the condition known as gingivostomatitis in which oral cavity vesicles or ulcers form. These lesions may recur frequently as "cold sores" (herpes labialis). Another condition produced by HSV-1 is herpetic keratitis, which may be serious if accompanied by conjunctivitis because this can lead to corneal scarring and blindness. Another condition known as "whitlows" appears as lesions on the fingers.

HSV-2 is commonly referred to as genital herpes. This virus produces lesions on the genitals, urethra and bladder. Recurrence may be frequent. In neonates, infection may be local or disseminated and has about 50% mortality if untreated. HSV-2 may also cause meningitis or encephalitis

- **Polioviruses:** Poliovirus types 1, 2 and 3 are recognized. Their genome contains a 7000 base positive strand of RNA. These viruses adsorb only to intestinal epithelial cells and motor neuron cells of the central nervous system.
- **Coxsackie:** These viruses are divided into two groups; A and B. There are 23 serotypes of A, 6 serotypes of B. In humans, Coxsackieviruses produce respiratory disease, herpangitis, "hand, foot and mouth" disease, febrile rashes, pleurodynia, pericarditis, myocarditis, aseptic meningitis and paralytic disease.
- **Echoviruses:** An acronym for "Enteric Cytopathogenic Human Orphan" viruses, the Echoviruses contain 31 serotypes and produce respiratory disease, febrile illness (with or without a rash), aseptic meningitis and paralytic disease.
- **Rhinoviruses:** This group of viruses are sensitive to acid pH and their optimal growth occurs at 33°. There are over 100 serotypes of Rhinoviruses and they produce the common cold.
- **Clinical:** Diagnosis of enteroviral infections is usually not possible based on clinical presentation. However, some symptoms (pleurodynia, myocarditis) or conditions (aseptic meningitis) are suggestive. Diagnosis of rhinoviral infections, in contrast, is usually based on clinical presentation.
- **Laboratory:** Recovery of Enterovirus from the throat or feces is diagnostic. Recovery of Rhinoviruses is simply not practical.
- **Paramyxoviruses:** parainfluenza, mumps, measles, respiratory syncytial virus (RSV).
  - **Parainfluenza:** These viruses generally produce local infections in the upper and lower respiratory tract. The viruses implant in ciliated epithelia of respiratory tract (nose and throat). The virus can be shed over 3-16 days and the main pathologic response is inflammation. The most important (i.e. serious) diseases are croup, bronchiolitis and pneumonia. The severe diseases occur most often with types 1 and 2.
  - **RSV:** The RS virus initiates a local infection in the upper or lower respiratory tract but illness varies with age and previous experience. The virus infects ciliated epithelia of the nose, eye and mouth and remains generally confined. Virus spreads extracellularly and by fusion. Severe disease may present as bronchiolitis, pneumonia or croup, particularly in infants. Some evidence suggests that there are possible immunopathologic mechanisms involved. Orthomyxoviruses: Influenza. The Orthomyxoviruses are composed of one genus and 3 types; A, B and C.
• The disease caused by these viruses, influenza, is an acute respiratory disease with prominent systemic symptoms despite the fact that the infection rarely extends beyond the respiratory tract mucosa.
• Type A is responsible for periodic worldwide epidemics; types A and B cause regional epidemics during the winter.
• The recurring pattern of the influenza viruses is due to their ability to exhibit variation in surface antigens. Two phenomena account for this variability:
  1. **Antigenic drift** is due to mutations in the RNA that leads to changes in the antigenic character of the H and N molecules. Antigenic drift involves subtle changes that may cause epidemics but not pandemics.
  2. **Antigenic shift** is due to rearrangement of different segments of the viral genome that produces major changes in the antigenic character of the H and N molecules. Antigenic shift usually occurs in animal hosts and is responsible for producing both epidemics and pandemics.
• Orthomyxoviruses contain a single stranded, negative RNA genome divided into 8 segments.
• The viruses have a lipid bilayer envelope with surface glycoproteins (haemagglutinin and neuraminidase)
• There are 3 viral antigens of importance: the nucleoprotein antigen that determines the virus type (A, B or C), the haemagglutinin (H) antigen, and the neuraminidase (N) antigen. The H and N antigens are variable. There are about 13 different H antigens and 9 different N antigens found in birds. This provides a total of 117 (13 x 9) possible combinations, 71 of which have been observed. There are only about 3 combinations that affect humans, however.
• Viral attachment is mediated by the haemagglutinin. The virus enters host cells by pinocytosis and uncoating occurs by fusion of the viral envelope with the membrane of the vacuole. The RNA is capped and replication proceeds in the nucleus. The progeny are released by budding and cell death ensues.
• The segmented genome of the influenza virus allows rearrangements to occur in simultaneously infected cells. This accounts for the periodic appearance of new variants. The new variants are responsible for the process of antigenic shift.

**Cytomegalovirus**
Several species of cytomegalovirus have been identified and classified for different mammals. The most studied is human CMV (HCMV), which is also known as human herpesvirus-5 (HHV-5).
CMV is transmitted from person to person via a close contact
Symptomatic CMV disease in immunocompromised individuals can affect almost every organ of the body, resulting in fever of unknown origin, pneumonia, hepatitis, encephalitis, myelitis, colitis, uveitis, retinitis, and neuropathy.
Individuals at an increased risk for CMV infection include individuals who attend or work at daycare centers, patients who undergo blood transfusions, persons who have multiple sex partners, and recipients of CMV mismatched organ or bone marrow transplants.
**Epstein-Barr virus (EBV)**
The Epstein–Barr virus, also called human herpesvirus 4 (HHV-4), is a virus of the herpes family, which includes herpes simplex virus 1 and 2, and is one of the most common viruses in humans. It is best known as the cause of infectious mononucleosis. It is also associated with particular forms of cancer, particularly Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, and central nervous system.

Transmission of this virus through the air or blood does not normally occur. The incubation period, or the time from infection to appearance of symptoms, ranges from 4 to 6 weeks. Symptoms of infectious mononucleosis are fever, sore throat, and swollen lymph glands. Sometimes, a swollen spleen or liver involvement may develop. Heart problems or involvement of the central nervous system occurs only rarely, and infectious mononucleosis is almost never fatal.

**Cell cultures**
Cell culture is the complex process by which cells are grown under controlled conditions. In practice, the term "cell culture" has come to refer to the culturing of cells derived from multicellular eukaryotes, especially animal cells. However, there are also cultures of plants, fungi and microbes, including viruses, bacteria and protists. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture.

Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37°C, 5% CO₂ for mammalian cells) in a cell incubator. Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed. Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrients. The growth factors used to supplement media are often derived from animal blood, such as calf serum.

Mass culture of animal cell lines is fundamental to the manufacture of viral vaccines and other products of biotechnology. Biological products produced by recombinant DNA technology in animal cell cultures include enzymes, synthetic hormones, immunobiologicals. Vaccines for polio, measles, mumps, rubella, and chickenpox are currently made in cell cultures. Cell cultures are used in viral diagnosis, also.

The examples of human cell lines: HeLa (cervical cancer), AGS (gastric cancer), Lnca (prostate cancer), MCF-7 (breast cancer).

**Rubella virus**
Belongs to family of Togaviridae, it is a RNA-virus with spherical capsid, enveloped.

Responsible for the disease of rubella, which may develop as a postnatal rubella or congenital rubella.

Postnatal form is a mild disease, observed mainly in children (more severe in adults).

Rubella is very serious infection for pregnant women, the virus may penetrate through the placenta and infect the foetus (congenital form of the disease). Congenital rubella may result in severe abnormalities of the foetus, premature birth or foetal death.

Laboratory diagnosis of Rubella infection is based on serological tests or isolation of the virus.
The prophylaxis consists in vaccination. Rubella vaccine contains the live, attenuated viruses (MMR vaccine).

**Family of Flaviviridae**

**Genus: Flavivirus**

RNA-virus, with spherical capsid, enveloped, transmitted to human by mosquito. Viruses of medical importance are: Dengue fever virus, West Nile virus, Yellow fever virus and Japanese encephalitis virus.

**Dengue fever virus** is responsible for dengue- the disease which each year attacks 100 million people in tropics. The disease is caused by any one of four viruses: DEN-1, DEN-2, DEN-3 or DEN-4. It may develop in 3 clinical manifestations: classical dengue fever, dengue haemorrhagic fever or dengue shock syndrome.

There is no specific medication or vaccination for treatment of the infection. Infection caused by **West Nile virus** may course as an asymptomatic infection, mild febrile syndrome (West Nile fever) or neuroinvasive disease (West Nile meningitis or West Nile encephalitis).

There is no vaccine or specific treatment against the disease.

**Yellow Fever virus** is responsible for the serious infections in tropical and subtropical areas of South America and Africa (90% of all infections). The disease course is an acute haemorrhagic disease. Because there is no specific treatment, the prevention plays an important role (recommendations of WHO: a mass vaccination). Prevention based on vaccination with vaccine containing attenuated, live viruses.

**Japanese encephalitis virus** is responsible for the disease prevalent in Southeast Asia and Far East. May course as asymptomatic infection or acute encephalitis. There is no specific treatment. Prevention based on vaccination with vaccine containing inactivated viruses.

Diagnosis of infections caused by Flavivirus is based on serological tests, PCR or isolation of viruses (rare).

**HIV**

The human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS). HIV belongs to the family Retroviridae and subfamily Lentivirinae. This virus was first reported, as a lymphadenopathy-associated virus (LAV), in France in 1983 by Luc Montagnier and associates and in the United States (as HTLV-III) by Robert Gallo and colleagues. The core of HIV contains two molecules of positive-stranded RNA and the associated enzyme systems. There are three major structural genes (gag, pol and env) and numerous regulatory genes.

Infection with HIV may, in some cases, result in an early, acute phase of disease, with influenza-like symptoms. In general, antibodies to HIV develop within a few months of infection. This is followed by a lengthy asymptomatic period of several to many years. During
this phase the number of CD4-positive T lymphocytes declines and eventually the onset of AIDS is signalled by the appearance of one or more of the characteristic infections (e.g. *Pneumocystis carinii* pneumonia, CMV disease, toxoplasmosis, cryptosporidiosis), neoplasms (e.g. Kaposi’s sarcoma) or symptoms (e.g. fever, night sweats, weight loss, generalized lymphadenopathy).

Specific diagnosis of HIV infection is usually based upon the demonstration of antibodies to HIV or identification of genetic sequences by an appropriate amplification technology.

**Hepatitis viruses**

Several diseases of the liver, collectively known as hepatitis, are caused by viruses. The viruses involved, five of which have been reasonably well characterized, come from a wide range of virus families. Hepatitis A virus is a picornavirus, a small single strand RNA virus; hepatitis B virus belongs to the hepadnavirus family of double stranded DNA viruses; hepatitis C virus is a flavivirus, a single stand RNA virus; hepatitis E, also an RNA virus, is similar to a calcivirus. Hepatitis D which is also known as Delta agent is a circular RNA that is more similar to a plantal viroid than a complete virus.

**17. Pathogenic fungi: yeasts and dermatophytes.**

Fungi are diverse group of organism sufficiently different from other living matter to be considered as a separate kingdom. The structure of a fungus may be unicellular – as in the yeast or multicellular where cells form filaments or hyphae. The filaments create a network known as a mycelium. The classification of fungi is largely determined by morphology. Fungal infections are known as mycoses. Diseases which follow invasion of tissues are known as: superficial, subcutaneous and systemic mycoses. Entry of the fungus can be: directly onto the skin, by implantation via a superficial injury, by inhalation or from a previous deep focus of infection – these are exogenous infection. Examples are dermatophytosis or aspergillosis. Commensal fungi such as Malassezia species on the skin or Candida species in the alimentary tract can take on a pathogenic role when conditions are altered in the host allowing the organism to multiply and invade tissues so causing development of endogenous infection.

The superficial mycoses are the most frequently occurring human fungal infections. They include dermatophytosis, superficial candidosis, Malassezia infection as well as such rarer conditions like tineanigra and piedra. Dermatophytosis – dermatophytes are the group of closely related fungi which have the ability to invade keratinized tissues. They comprise three genera: Trichophyton, Epidermophyton and Microsporum. Dermatophyte infection are normally called tinea followed by Latin name of the appropriate part of the body involved: t. capitis, t. corporis, t. pedis, t. manuum, t. unguium – onychomycosis.

Superficial candidosis – the infection caused by the yeasts of the genus Candida, which frequently affect the mucous membranes, skin or nails.

The subcutaneous mycoses are infections of implantations. In most cases they develop following traumatic injury and the inoculation of environmental organisms into the host
where they affect the subcutaneous tissue, skin and other adjacent structures. The most frequently encountered are mycetoma, sporotrichosis, chromoblastomycosis and phaeohyphomycosis.

The systemic mycoses are infections which predominantly affect internal systems or organs, such as the lungs or blood. There are two main groups of fungi which cause systemic disease: the primary respiratory pathogens (Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis, Paracoccidioides brasiliensis) and the systemic opportunistic fungal pathogens (Candida species, Aspergillus species, Cryptococcus neoformans and Rhizopus, Rhizomucor and Absidia.

Laboratory diagnosis – the laboratory methods used in the diagnosis of fungal infections involve the detection of organism in the tissues, isolation of the pathogen in culture and recognition of specific responses in the host using immunological techniques (collection of specimens, direct microscopic examination, culture methods – Sabouraud’s agar, identification of fungi and serology).

Antifungal agents and management rules of the most common fungal infections.