Encyclopedia Infectious Disease Serology

Source: Encyclopedia of Medical Laboratory Diagnostics. Axel M. Gressner and Torsten Arndt (eds.). Springer-Verlag Heidelberg, 3rd edition, 2019

Adenoviruses

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Description of the pathogen. Family: *Adenoviridae*, Genus: Mastadenovirus, Species: Human adenovirus species A to G (53 serotypes). This is a global, environmentally resistant non-enveloped human pathogen and a double-stranded DNA virus with a diameter of 70-90 nm.

Diseases. Pharyngoconjunctival fever, epidemic keratoconjunctivitis, gastroenteritis, acute respiratory distress syndrome (ARDS)
Transmission: Direct contact, droplet or smear infection, organ transplant (cornea, liver)
Symptoms: This virus primarily causes respiratory disorders (flu, bronchitis, pneumonia: serotypes 1-39). Persons with a weakened immune system are susceptible to complications, such as ARDS, life-threatening pneumonia, hepatitis, urinary tract infection. Some serotypes cause gastroenteritis, especially in children: serotypes 40, 41; epidemic conjunctivitis: serotypes 8, 19, 37; haemorrhagic cystitis: serotypes 11, 21; rhinitis or pharyngitis. The following are possible late complications: persistent bronchiolitis, dilatative cardiomyopathy, type I diabetes. Some virus types can persist in the regional lymph nodes and tonsils for years.
Prevention by disinfection measures (chlorination of pool water, disinfection of hands and instruments, especially in ophthalmic practices); no vaccine and no specific therapeutics exist, only the symptoms are treated.

Analytics. Direct detection of the virus in bodily fluids or stool by cell culture cultivation, electron microscopy, direct immunofluorescence and polymerase chain reaction (PCR). Moreover, rapid antigen tests are also used for the immunological detection of adenoviruses in stool.
Serology: Detection of specific antibodies in serum through indirect immunofluorescence (immunofluorescence, indirect), enzyme immunoassay, complement fixation test and neutralisation assay.

Sample material and sample stability. **Direct detection**: Bodily fluids, stool, smears of nasal mucosa and throat, eyes and rectum are analysed. The patient samples must be stored at +4 °C to +8 °C for further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Due to the non-specific clinical symptoms, laboratory diagnosis is particularly important. Electron microscopy and direct immunofluorescence are less sensitive. By contrast, PCR methods are extremely sensitive and are also suitable for serotyping. Quantitative PCR allows the viral load to be determined. Rapid antigen tests generally only have a low sensitivity or specificity. The neutralisation assay can be used for serotyping. Indirect immunofluorescence and enzyme immunoassays enable a rapid and precise diagnosis. Due to the high general seroprevalence, a serological diagnosis can only be used based on a clear rise in titer in the IgG within 1-3 weeks. Cross reactions between the various adenovirus serotypes exist.

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Description of the pathogen. Family: *Togaviridiae*, Genus: *Alphavirus*, Species: Barmah forest virus. Positive-strand RNA genome, enveloped, 60-70 nm diameter; closely related to the Ross River virus.

Diseases. Distribution: Australia
Vectors: Mosquitoes (*Culex* ssp., *Aedes* ssp.)
Hosts: Humans; a natural virus reservoir has not been identified to date, potentially birds
Symptoms: Endemic polyarthritis; seasonal occurrence of the disease, especially in summer and autumn as well as spring in Western Australia; after an incubation time of 5-21 days, fever, arthralgia with arthritis, skin rash and lethargy may occur; in about 10% of cases, arthralgia and myalgia persist over several months.

Analytics. Direct detection: Virus isolation or detection of viral RNA by RT-PCR (polymerase chain reaction)
Serology: Detection of specific antibodies (IgM, IgG) in serum through indirect immunofluorescence (immunofluorescence, indirect), enzyme-linked immunosorbent assay, or neutralisation assay.

Sample material. Direct detection: Blood and blood components, tissue. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The anamnesis, especially information on extended stays in endemic areas, is important. Direct detection of the virus is essentially possible, but rarely occurs. Serological tests for the detection of antibodies are preferable. Specific IgM antibodies are present in the majority of patients when symptoms start. A significant rise in the anti-BFV IgG titer, compared to the first sample, measured in a second serum sample (interval between the blood collection of approx. 2 weeks) is a reliable verification of an infection. Cross-reactivities with antibodies against co-endemic alphaviruses are possible.

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Bartonella

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Description of the pathogens. *Bartonella henselae*, *Bartonella quintana* and *Bartonella spp.* are gram-negative, aerobic, facultative intracellular bacteria. Until 1993, *Bartonella bacilliformis* was the only known species of the genus Bartonella. All species of the genera Rochalimaea and Grahamella were subsequently integrated due to their molecular genetic relationship. The species *Bartonella henselae* (until 1993 *Rochalimaea henselae*) was only discovered in 1992, but is of great medical importance, especially for immunocompromised patients.

Diseases. *Bartonella henselae* takes on a particular importance as a significant pathogen of the global cat-scratch disease (CSD) (this disease was formerly ascribed to the pathogen *Afipia felis*, which plays a role in veterinary medicine). The prevalence of antibodies against bartonella amounts to between 9% and 28% (sufferers 81%) depending on the geographic location. Other pathogens, which are discussed in connection with CSD and which may display serological cross-reactions with *Bartonella henselae* are *Bartonella clarridgeiae*, *Bartonella quintana* and *Bartonella bacilliformis*.
The infection of cats is generally barely noticed. In humans, an infection is often associated with clinical manifestations; the disease particularly plays a role in paediatrics. No antibiotics are required if the disease presents a favourable course. Immunocompromised patients may develop vascular proliferative clinical pictures (bacillary angiomatosis, peliosis hepatis, e.g. in connection with an HIV infection), which require treatment with antibiotics.
Bartonella quintana is the pathogen of trench fever, an epidemic disease that was observed during the First World War. It is transmitted by the bite of the clothes louse (*Pediculus humanus corporis*). Immunocompromised persons often develop bacillary angiomatosis.
Poor hygienic conditions increase the probability of transmission: In a group of homeless persons, a seroprevalence of 54% was determined for *Bartonella quintana* (in healthy blood donors: 2%). Without treatment, patients with a weakened immunocompetence display a prolonged course of the disease, with recurring, but self-limiting relapses. *Bartonella quintana* is highly sensitive to antibiotics.

Analytics. Bartonella is detected in the tissue by light microscopy with a silver staining according to Warthin-Starry, while molecular biology methods are used to detect the pathogens (e.g. polymerase chain reaction). Cell-free special culture media are used for the aerobic cultivation of *B. henselae*.
In all stages, antibodies in the serum are predominantly detected by indirect immunofluorescence (immunofluorescence, indirect) with infected culture cells as antigen substrates, enzyme immunoassay or Western blots. Immunofluorescence currently delivers the most reliable results.

Sample material and sample stability. **Direct detection and culture**: Molecular biological detection and cultivation of the pathogen from tissue samples and blood. The samples are phosphate-buffered and stored in a cool sterile saline solution. They must be refrigerated for transport and analysed within 4 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Cat scratch disease must especially be taken into account for discriminatory tests with lymphadenitis in adults. For HIV patients, a bartonella infection must be considered for every fever with an unclear origin.
Bartonella henselae infections are diagnosed all too rarely or often only late in the clinical course. IgM and/or IgG antibodies can generally already be detected at the time of the swelling of the lymph nodes. However, low titers also occur in persons without clinical symptoms. A rise in titer within a few weeks confirms an infection.

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Bordetella pertussis and parapertussis

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Definition. The term pertussis (tussis, lat.: to cough), introduced by T. Sydenham in 1679, describes the whooping cough disease first mentioned in the 16th Century. O. Gengou and J. Bordet, who are considered to have discovered *Bordetella*, successfully cultivated the pathogenic bacteria in 1906. In 1937, W. L. Bradford and B. Slavin isolated the closely related *Bordetella parapertussis*.

i Description of the pathogen. The genus *Bordetella* belongs to the Alcaligenaceae family. Bordetella are a challenging, immobile, aerobic, coccoid, gram-negative rod-shaped bacteria (0.5–2 µm long, diameter of 0.2–0.5 µm), which are enclosed by a fimbriae-covered capsule. The reservoir for *Bordetella pertussis* and *parapertussis* are the ciliated epithelial cells of the human respiratory tract, but *B. parapertussis* has also been detected in sheep. It is spread by droplet infection. Bordetella occur worldwide. A range of toxins has a negative effect on the body's local defences and causes tissue damage.
Virulence factors are:

- Adhesins:
  - Filamentous haemagglutinin (FHA), a surface protein with an ability to bind to glycoproteins
  - Pertactin, outer membrane protein
  - Fimbriae as adhesive organelles
  - Subunit B of the pertussis toxin (PT)
- Exotoxins:
  - Subunit A of the pertussis toxin: effects an enzymatic (ADP ribosyl transferase) signal reduction and inhibits the host's immune cells (*Bordetella parapertussis* also contains the PT gene, but it is not expressed due to mutations in the promoter region).
  - Adenylate cyclase toxin (ACT): inhibits the effector function of the cells.
  - Tracheal cytotoxin (TCT): necrotises the ciliated epithelial cells and is therefore likely to be responsible for the convulsive coughing fits.
  - Dermonecrotic toxin (DNT): heat-labile toxin, presumed to have a necrotising effect.
- Endotoxins:
  - Lipooligosaccharides (LOS): correspond to the lipopolysaccharides (LPS) of other gram-negative bacteria

Diseases. The typical pertussis disease is divided into 3 stages:

- After a 14-day incubation period, non-specific catarrhal stage with slight flu-like symptoms,
- characteristic convulsive stage, with convulsive coughing attacks (hacking cough) followed by inspiratory drawing (wheezing),
- the decrementi stage with slow convalescence that can sometimes take months.

Complications include secondary bacterial infections with pneumonia or otitis media via *Haemophilus spp.* or pneumococci. *Bordetella parapertussis* causes milder forms of whooping cough.

Treatment and prophylaxis. The prompt administration of erythromycin or other macrolides is recommended for treatment. In the convulsive stage, antibiotics only have a minor influence on the course of the disease. The pertussis vaccine, now provided as a combination vaccine (incl. against diphtheria and tetanus) is recommended for babies and infants. The basic immunisations (4 injections) should be followed by regular booster shots every 10 years. Acellular vaccines are based on defined Bordetella antigens (FHA, PT, pertactin and, where applicable, fimbriae components) and have been available since 1995.

Analytics. The detection of the pertussis pathogen from deep nasopharyngeal smears is most significant for laboratory diagnostics. One area of focus is bacteria cultivation on selective culture media, which is primarily based on the potato extract, glycerol, blood agar described by Bordet and Gengou. The addition of selective supplement suppresses the accompanying flora. The cultivation of *Bordetella pertussis* takes at least 3 days, while *Bordetella parapertussis* grows in just 2 days. The cultural detection is 100% specific, but has a limited sensitivity and is time-consuming. As a result, DNA detection using PCR is becoming increasingly popular. Even just a small number of pathogens enable a positive direct detection. In addition, bacteria that are already dead are also detected. Microscopic pathogen detection using direct immunofluorescence can take place using FITC or rhodamine-labelled monoclonal antibodies against surface structures, e.g. LOS, and detects viable as well as dead bacteria.
Serology is not suitable for diagnosis in the early stages of an initial infection; specific antibodies can only be detected from the convulsive stage. Enzyme-linked immunosorbent assays (ELISA) based on the pertussis toxin (PT) are recommended for the detection of antibodies against *Bordetella pertussis*, as they enable Parapertussis infections to be excluded as well as the antibody titer to be quantified. The use of FHA as the antigen allows the sensitive detection of *B. pertussis* as well as *B. parapertussis* infections. FHA is present in all Bordetella species, but also in other bacteria, such as mycoplasma. The use of an antigen mixture (FHA and PT) in the ELISA is not recommended. The concentrations of the Bordetella antibodies should be indicated in international units (IU/ml); a WHO reference preparation is available.
A general reporting obligation exists for acute infections with *Bordetella pertussis* or *parapertussis* under the German Infection Protection Act.

Sample material and sample stability. Direct detection and culture: Nasopharyngeal smears are analysed; primary cultures should be prepared immediately where possible.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

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Borrelia burgdorferi

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Description of the pathogens. *Borrelia burgdorferi sensu lato* is the collective term for the human pathogen genospecies *Borrelia afzelii*, *Borrelia burgdorferi sensu stricto*, *Borrelia garinii* and *Borrelia spielmanii*. The bacteria were named after the Swiss bacteriologist Willy Burgdorfer.
Biological classification: *Borrelia burgdorferi sensu lato* belong to the family *Spirochaetaceae* in the genus *Borrelia*.
Morphology: Borrelia are corkscrew-like, wound, 20-30 µm-long and 0.2-0.3 µm-thick, extremely mobile bacteria. Flagella in the periplasmic area allow them to rotate, enabling them to penetrate into the surrounding tissue.

Diseases. *Borrelia burgdorferi sensu stricto*, *Borrelia afzelii*, *Borrelia garinii* and *Borrelia spielmanii* are the pathogens of the tick-borne Lyme disease (practically only *Borrelia burgdorferi sensu stricto* occurs in the USA). Vectors are primarily Ixodidae in every stage of development (larvae, nymphs, adults), which are found at the edge of the forest or in long grass and which are shed by passing people or animals. After a tick bite, the arachnids transmit the borrelia during their blood meal. The natural reservoir are small mammals and birds; however, red deer are responsible for the explosive spread of the bacteria in this part of the world over the past few years: A mighty sixteen-point deer with its much larger radius of movement compared to a field mouse, can be infested with hundreds of ticks; an ideal multiplier for a rapid expansion of borrelia.
New infections occur seasonally in the 3rd and 4th quarter of the year and must be reported in some federal states: Bavaria, Berlin, Brandenburg, Mecklenburg-Vorpommern, Rhineland-Palatinate, Saarland, Saxony, Saxony-Anhalt and Thuringia. The highest incidence is registered in children and older adults (6-16 per 100,000 residents/year).
Borreliosis can manifest in dermatological, neurological, ophthalmological, rheumatological and internal symptoms:

- [5] Stage I: The typical pathognomonic, but not compulsory, primary manifestation is erythema migrans, a reddening of the skin, which occurs around the bite site days or sometimes weeks after the tick bite and which spreads in a ring shape. The erythema can be accompanied by general flu-like symptoms. Lymphadenosis cutis benigna results in a small number of cases.
- [5] Stage II: Various symptoms may occur several weeks to a number of months after the tick bite. These are primarily neurological manifestations (neuroborreliosis): Meningitis, encephalitis, asymmetric polyneuritis, cranial nerve palsy, lymphocytic meningoradiculitis (Bannwarth's syndrome, Garin Bujadoux Bannwarth syndrome). Extensive itchy dermatosis as well as arthritides, especially of the knee joints, and bone, joint and muscle pains are also commonly observed. Cardiological manifestations, such as myocarditis or pericarditis, are also described.
- [5] Stage III: Typical late-stage manifestations are chronic recurrent erosive arthritides, acrodermatitis chronica atrophicans as well as progressive encephalomyelitis, which can have a similar course to multiple sclerosis.

Treatment and prophylaxis. The administration of antibiotics, incl. tetracycline, is recommended for treatment. The only preventive measure is the avoidance of tick bites.

Analytics. Culture: Borrelia can be cultivated in special media, but the culture is time-consuming due to the long generation time (7-20 h) and only rarely successful.
Direct detection: Borrelia can only be identified in exceptional cases in dark-field microscopy. A PCR from whole blood or serum only shows a low sensitivity, while higher detection rates (70%) are delivered from biopsy material (skin) and synovial fluid. Standardised methods for sample preparation and the implementation of borrelia PCR have not yet been found.
Serology: The German Society for Hygiene and Microbiology (DGHM) guidelines for diagnosing Lyme disease (MiQ 12/2000) recommend a step-by-step diagnosis: In the first step, a screening test (immunofluorescence, indirect or ELISA) with high sensitivity and reasonable specificity is used. Positive and marginal results are verified in a second step using a more specific technique, such as immunoblot, whose antigen spectrum displays strongly borrelia-correlated antigens as well as those without borrelia exclusivity.
However, in exceptional cases, the "two-stage strategy" can lead you down the wrong path, as, in some cases, a specific immune response in the stage of erythema migrans can be better detected with a differentiated immunoblot with separately defined antigen bands than, for instance, with an ELISA, in which the reactive solid phase is coated with an antigen mixture. Yet, it is precisely in the early phase of borreliosis that every individual patient should be able to be identified, as this provides the best opportunity of using antibiotics to nip the borreliosis in the bud.
After the start of the infection, class IgM antibodies against the surface antigen OspC are the first to appear. The borrelia target antigen VIsE has proven to be a highly sensitive and specific antigen when detecting class IgG antibodies. It is a system of exchangeable and variable cassettes to conceal the bacteria surface, which is only expressed in vivo, where the immune system of the host organism creates a high evolutionary pressure, in contrast to a bacteria culture.
The detection of an intrathecal synthesis of borrelia-specific antibodies by determining the specific cerebrospinal fluid/serum ratios is appropriate for diagnosing neuroborreliosis.

Sample material and sample stability. Direct detection and culture: Skin or synovial biopsies are analysed. The material must be stored at +4 to +8 °C in a sterile solution until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. According to the DGHM guidelines, Lyme disease is a clinical diagnosis and thus only clinical criteria are decisive for the need for treatment. In particular, a "reactive" serology alone is no reason to use antibiotics, especially if no titer rise can be detected with specific IgG. The seroprevalence in the population differs by region and is also influenced by various factors, e.g. by the borrelia infection of the ticks in various areas. Forestry workers display seroprevalences of around 20% depending on the region, while this drops to below 5% for city office workers.
Post-infection reactive arthritides (causative agents: salmonella, shigella, yersinia, chlamydia, campylobacter, mycoplasma), autoimmune diseases (autoimmunity) (rheumatoid arthritis, lupus erythematosus) and inflammatory diseases of the central nervous system are relevant for differentiation purposes.

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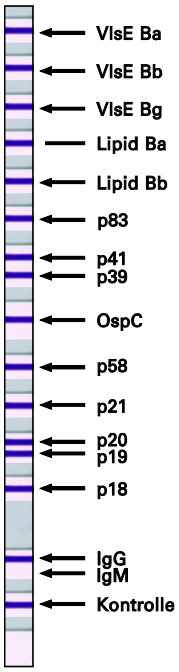

Borrelia burgdorferi. Fig. 1. Comprehensive combination of diagnostically relevant borrelia antigens in native or recombinant form on a line blot (IgG).

Brucella

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Description of the pathogen. Brucella are gram-negative, immobile, obligate aerobically growing, coccoid rod-shaped bacteria, which do not form spores. Their outer shell membrane contains a lipopolysaccharide, whose toxic effect is similar to that of the endotoxin of the enterobacteria. Brucella defend against intracellular bactericides using antileukocytic protection factors, which allows them to survive and propagate in leukocytes and macrophages. The genus *Brucella* is currently classified in the alpha-2 subgroup of the proteobacteria in the *Brucellaceae* family. Human pathogens are *B. abortus* (main host: cow), *B. suis* (pig), *B. melitensis* (sheep, goat) and *B. canis* (dog).

Diseases. Brucellosis is an anthropozoonosis, which only rarely occurs in Central Europe thanks to veterinary control measures (Germany: 24-37 reports/year). Endemic areas include the Mediterranean region, the Arabian Peninsula, Africa, Asia and parts of Central and South America. The pathogen is especially transmitted to humans in veterinarians, farmers, butchers and laboratory personnel via direct contact with infected animals and their excretions or via aerosols. Infection can also take place by consuming contaminated, unpasteurised milk or from products manufactured from this milk as well as meat products. Human-to-human infections are rare. Brucellosis (*B. abortus*: Bang's disease) has numerous manifestations. Up to 90% of all infections are subclinical. Acute forms start after an incubation time of one to several weeks with fever, nausea, fatigue, headaches and joint pains, night sweats and weight loss. Depending on the phases of the transient proliferation of *brucella* from the typical granulomas, this is followed by a characteristic undulating fever, which lasts for 7 to 21 days and which can be interrupted by intervals of several fever-free days. Swelling of the lymph nodes, spleen and liver occur to varying degrees. In the event of chronic brucellosis, non-specific general symptoms (depression and sleeplessness) are joined by various organ manifestations: hepatosplenomegaly, orchitis, pyelonephritis, endocarditis, osteomyelitis, arthritis and meningoencephalitis.

Treatment and prophylaxis. Several weeks (6-12 weeks) of combined antibiotic treatment (antibiotics) with, for example, doxycycline and gentamicin, which takes account of the intracellular growth of brucella. If the joints are affected or in case of neurological manifestations or pronounced organ involvement, especially if neurobrucellosis is present, other combinations of drugs as well as an extension of the treatment period are necessary. Surgical intervention is occasionally indicated. Prevention: Monitoring stables, extermination of infected animals, carcass destruction, import supervision, pasteurisation of produced milk. Persons who are exposed due to their professions should take adequate safety and hygiene measures to protect themselves. A vaccine for humans is not approved in Germany, but a vaccine for animals can be used if necessary. According to the German Infection Protection Act, the direct or indirect detection of brucella must be reported, if it points to an acute infection.

Analytics. The direct detection of pathogens takes place by a polymerase chain reaction (PCR). For cultural detection, brucella are enriched in complex liquid culture media (e.g. brain-heart infusion, tryptose or Albimi broth or blood culture bottles) for up to 4 weeks and regularly streaked on solid culture media (tryptose-blood, tryptose-soy, Albimi agar) and incubated for up to 8 days. Incubation takes place under aerobic conditions at 35 °C. Morphology, such as soft or rough colony form, negative gram behaviour, biochemical characteristics (oxidase positive, catalase positive, urease positive, glucose and lactose fermentation negative) as well as positive agglutination with *brucella* antiserum, are assessed to identify the slow-growing colonies. Specific antibodies against brucella can be detected by slow agglutination (Widal reaction), complement fixation test (CFT) or ELISA.

Sample material/sampling conditions and sample stability. Direct detection and culture: Blood (whole blood), cerebrospinal fluid, urine, puncture fluid (bone marrow, joint puncture fluid) and biopsies (liver, spleen, lymph nodes) are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. As the clinical diagnosis of brucellosis is complicated by the manifold symptoms, the information in the medical history as well as its detection in the laboratory are decisive. The PCR is considered a specific and sensitive method for identifying the various *Brucella* species in a short space of time. The cultural detection of pathogens takes a long time due to the slow in-vitro growth of the brucella and is often unsuccessful in the chronic stage of the disease.
In serology, agglutination tests are being increasingly replaced by specific enzyme immunoassays. A distinction between acute, subacute and chronic brucellosis is possible with the help of antibody titers of *brucella*-specific IgA, IgG and IgM. Moreover, a significant rise in the antibody titer within 2-3 weeks is considered evidence of an acute infection. Attention must be paid to cross reactions with the O antigens of, for example, *Yersinia enterocolitica*, *Francisella tularensis*, *Vibrio cholerae*, *Escherichia coli* and salmonella.

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Campylobacter

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Description of the pathogen. Family: *Campylobacteriaceae*; Genus: *Campylobacter*; *more than 30 different species, the most important are C. jejuni, C. coli* and *C. lari.*

Spiral- or S-shaped gram-negative rods (bacteria), 0.2–0.5 µm thick, 0.5–5 µm long, each with 1 flagellum on both poles. Microaerophilic.

Diseases. Clinical pictures: *Campylobacter* is the most common bacterial enterocolitis pathogen (70,190 reported cases in 2015). Prodromal stage with headaches, myalgia, arthralgia, followed by fever, abdominal cramps, diluted, slimy or bloody diarrhoea, vomiting. Remission after 3–7 days. Possible secondary diseases: Guillain-Barré syndrome, reactive arthritis.
C. coli is closely related to *C. jejuni* (distinguished from the latter by the hippurate test) and is an important food-transmitted pathogen for enterocolitis.
Transmission: Food that has not been fully cooked (poultry, milk), contaminated drinking water, occasionally faecal-oral, infection dose: >500
Incubation time: 2–10 days.
Treatment: In the event of a straightforward course, only symptomatic treatment (electrolyte substitution) is required, otherwise with 7 antibiotics (macrolides or fluoroquinolones). Resistances are being observed.

Analytics. Direct detection/culture: (Microscopy). Culturing on blood plates or selective media (48 h, 37 °C, optimum growth 42 °C, microaerobic, CO_2-enriched environment). Biochemical differentiation by detecting oxidase and catalase, H_2S formation, DNAse, hippurate hydrolysis, indoxyl acetate test, nitrate reduction, lack of glucose splitting. Where applicable, gas chromatographic detection of fatty acids and PCR (polymerase chain reaction).
Molecular biological typing using pulsed field gel electrophoresis.
Serology: The detection of pathogen-specific antibodies, especially of class IgA by enzyme-linked immunosorbent assay or Western blot is only indicated for secondary diseases: Guillain-Barré syndrome, reactive arthritis.

Sample material and sample stability. Direct detection and culture: Stool, food remnants or samples of suspected livestock. Blood components, cerebrospinal fluid or biopsy material are also analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The detection of pathogens using culture is of particular importance when diagnosing acute *Campylobacter* infections. Biochemical differentiation and serotyping enables further pathogen characterisation. A reporting obligation exists in the event of a direct detection of enteropathogenic *Campylobacter* species.
The antibody diagnosis is of secondary importance for diagnosing intestinal infections, persisting pathogen-specific IgA antibodies are observed in secondary diseases.

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Candida

W. Stöcker

Description of the pathogen. Polymorphic yeast fungus, which forms hyphae, mycelia, pseudomycelia, blastoconidia and, in some cases, chlamydospores, depending on the culture and ambient conditions. Omnipresent in nature (hygrophilous), including in the digestive tract of warm-blooded animals. Reproduction takes place both intracellular and extracellular.
Family: Endomycetaceae
Genus: *Candida*
Clinically relevant species: *Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. dubliniensis*, *C. krusei*, *C. guilliermondii, C. lusitaniae, C. kefyr, C. famata, C. inconspicua, C. rugosa, C. norvegensis.*

Diseases. Clinical pictures: *C. albicans* (approx. 90% of all human *Candida* infections) and the remaining *Candida* species cause opportunistic infections of the skin (intertriginous, perianal, perineal, genital, interdigital dermatitis) and the nails as well as the mucous membrane (thrush, oesophagitis, vulvitis, colpitis, balanitis, urinary tract infections).
Besides superficial colonisation, a weakened immunocompetence may lead to the occurrence of systemic candidiasis (endophthalmitis, basal meningitis, osteomyelitis, interstitial nephritis, pericarditis, peritonitis, etc.). Risk groups are newborns and infants (nappy area), persons with extensive skin injuries, organ transplantees, persons undergoing intensive treatment, diabetics.
Transmission: Endogenous infections, e.g. in the event of disruptions of the barrier function of the skin and mucous membrane (commensal reservoir). Exogenous infections, e.g. by contaminated respiratory systems or venous catheters.
Treatment: Eliminate favourable conditions, in the event of candidiasis, local treatment of the skin with nystatin, clotrimazole and other azoles, systemic fluconazole, itraconazole, in the event of disseminated systemic candidiasis, parenteral administration of amphotericin B/flucytosine or caspofungin, fluconazole or itraconazole. In case of resistances, use of new glucan synthesis inhibitors.

Analytics. Direct detection/culture: Tissue analysis and fungal cultivation. Biochemical, microscopic and antigen features enable differentiation.
Serology: Detection of specific antibodies using the haemagglutination test, indirect immunofluorescence (immunofluorescence, indirect) and ELISA.

Sample material and sample stability. Direct detection and culture: Removal of material from the relevant infected area, non-sterile samples must receive antibacterial treatment (addition of antibiotics to the culture medium). The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The detection of pathogens using microscopy and culture is important when diagnosing superficial, systemic disseminated and invasive *Candida* infections. Biochemical differentiation and morphological analysis enable further pathogen characterisation.
The use of serological methods for the detection of antigens and antibodies is diagnostically sensible for screening at-risk patients and for monitoring life-threatening candidiasis in immunocompromised patients.

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Chikungunya virus

W. STÖCKER

Description of the pathogen: Family: *Togaviridae*, Genus: *Alphavirus*, Species: *Chikungunya virus*. Positive-strand RNA genome, enveloped, 50–70 nm diameter.

Diseases. Chikungunya fever
Distribution: Africa, Indian subcontinent, South-East Asia, southern Europe, South America, Caribbean
Vector: Mosquitoes (*Aedes aegypti*, *Ae. albopictus* and *Culex* sp.)
Host: Primates, rodents, humans
Transmission: By insects and transplacental transmission, as well as blood transfusion or organ transplantation.
Symptoms: Sudden high fever and flu-like syndrome, polyarthritis in 80% of sufferers, which can persist for months or years, exanthema, petechia; very occasionally haemorrhaging, meningoencephalitis, hepatitis
Treatment and prophylaxis: No specific treatment exists, treatment is only symptomatic. No approved vaccine is currently available; prevention consists of avoiding mosquito bites and combatting the vectors.

Analytics. Working with the pathogen requires the use of class 3 safety laboratories.
Direct detection: RT-PCR (polymerase chain reaction), direct immunofluorescence or virus cultivation in culture cells.
Serology: Detection of specific antibodies (IgG, IgM) in serum through indirect immunofluorescence (immunofluorescence, indirect), ELISA, neutralisation assay or haemagglutination inhibition assay.

Sample material and sample stability. Direct detection: Blood and blood components are analysed. The patient samples must be stored at +4 °C to +8 °C for further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Screening of blood reserves for viral RNA or specific antibodies. Direct detection is only possible during the first 3-5 days of the disease, as the pathogens are eliminated from the bloodstream, partially by virus-neutralising antibodies that are established.
Serology: Specific antibodies (IgG, IgM) can be found in the serum from about the 8th day of the disease. Class IgM antibodies indicate an acute infection, which also holds true for a rise in the IgG titer within 2 weeks.
Differential diagnoses: dengue fever (dengue virus), Zika fever (Zika virus), Mayaro fever, O'nyong-nyong fever in Africa, Ross River fever (Ross River virus) in Australia.
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya viruses, dengue viruses, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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Chlamydia pneumoniae

W. STÖCKER

Description of the pathogen. The pathogen *Chlamydia pneumoniae* belongs to the human-pathogenic Chlamydia species in addition to *Chlamydia trachomatis* and *Chlamydia psittaci*. They are gram-negative and are amongst the smallest obligate living intracellular bacteria. Their metabolism primarily uses the adenosine triphosphate of the host cells. Their unique development cycle plays an important role in diagnosis, treatment and pathogenesis. The generic name *Chlamydophila* has been used for *Chlamydia pneumoniae* for many years, but is not universal.

Diseases. *Chlamydia pneumoniae* causes infections in the upper respiratory tract and pneumonia. Its role in the pathogenesis of coronary heart disease is being discussed. The pathogen was detected in 1986. It is present worldwide, is exclusively human pathogenic and is transmitted by aerosols. The seroconversion is predominantly observed between 5 and 15 years of age. Over 50% of all adults have experienced an infection and are seropositive to the pathogen. Currently, there is no vaccination against chlamydia. Detected infections are effectively treated with specific antibiotics administered over 7-14 days.

Analytics. **Direct detection** by nucleic acid amplification tests (e.g. PCR) has not yet been standardised for *Chlamydia pneumoniae*. The culture requires a high level of technical expertise. Serology: Detection of antibodies against *Chlamydia pneumoniae* using enzyme immunoassays or indirect immunofluorescence (immunofluorescence, indirect) (species-specific microimmunofluorescence test: purified elementary bodies as the substrate, inactivation of the lipopolysaccharide (LPS) antigen in order to minimise cross-reactivities, serological “gold standard”).

Sample material and sample stability. Direct detection and culture: Swab material (cell-containing secretion from the lower respiratory passages) is injected into special transport media. It must be refrigerated for transport and analysed within 4 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Laboratory diagnosis plays an important role, as *Chlamydia pneumoniae* infections cannot be clearly detected clinically or radiologically. Pathogen detection often fails, especially if the infection occurred a while ago, in which case the diagnosis of specific Chlamydia antibodies becomes extremely important.
Cross-reactions with other Chlamydia species must be ruled out, e.g. by a parallel analysis of antibodies against *Chlamydia psittaci* and *Chlamydia trachomatis*, with BIOCHIP mosaics where applicable.

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Chlamydia psittaci

W. STÖCKER

Description of the pathogen. *Chlamydia psittaci* belongs to the human-pathogenic Chlamydia species in addition to *Chlamydia trachomatis* and *Chlamydia pneumoniae*. They are amongst the smallest living intracellular, gram-negative bacteria and their metabolism primarily uses the adenosine triphosphate of the host cells. The generic name *Chlamydophila* has been used for *Chlamydia psittaci* for many years, but is not universal.

Diseases. *Chlamydia psittaci* is the pathogen for psittacosis (parrot fever or ornithosis), a zooanthroponosis, which is generally transmitted to humans aerogenically by infected exotic or domesticated birds, especially parrots, via pathogen-containing secretion and excrement, occasionally also by bites. Human-to-human infection also occurs in rare cases. Besides keepers of decorative and domesticated birds, animal traders and employees in the poultry processing industry are at risk of infection. The clinical picture includes subacute or chronic pneumonia, while mild courses as well as acute, fulminant infections are observed. The disease is rare in Germany, only 40 cases were reported in 2002. Currently, there is no vaccination against chlamydia. Detected infections are treated with antibiotics over 7-14 days.

Analytics. PCR, cultivation in cell cultures or direct immunofluorescence using fluorescein-labelled monoclonal antibodies against the outer membrane proteins of *Chlamydia psittaci* are available for direct detection. Due to the contagious nature of the pathogen and the associated risks, direct pathogen detection in the cell culture can only take place in facilities that have a class L3 safety laboratory.
Serology: Detection of antibodies against *Chlamydia psittaci* using enzyme immunoassays or indirect immunofluorescence (immunofluorescence, indirect) (species-specific microimmunofluorescence test: purified elementary bodies as the antigen, inactivation of the lipopolysaccharide (LPS) antigen in order to minimise cross-reactivities, serological “gold standard”)

Sample material and sample stability. Direct detection and culture: Sputum, tracheal secretion and bronchoalveolar lavage fluid are used for cultivation and gene detection (risk of infection!). The material must be refrigerated for transport and analysed within 4 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The clinical picture and anamnesis (contact with birds) play an important role in diagnosing *Chlamydia psittaci* infections. The laboratory diagnosis opportunities for detecting a *Chlamydia psittaci* infection are limited. PCR methods have proven to be useful for detecting acute infections, but have not been evaluated with large case numbers to date. Infections with *Chlamydia psittaci* are therefore generally diagnosed serologically. Cross-reactions with other Chlamydia species must be ruled out, e.g. by a parallel analysis of antibodies against *Chlamydia peumoniae* and *Chlamydia trachomatis*, with BIOCHIP mosaics where applicable.

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Chlamydia trachomatis

W. STÖCKER

Description of the pathogen. *Chlamydia trachomatis* belongs to the human-pathogenic Chlamydia species in addition to *Chlamydia pneumoniae* and *Chlamydia psittaci*. They are amongst the smallest living intracellular, gram-negative bacteria and their metabolism primarily uses the adenosine triphosphate of the host cells.

Diseases. The pathogen causes the following diseases:

- Trachoma, chronic, follicular keratoconjunctivitis (serotypes A-B1, B2-C),
- infections of the urogenital tract in men and women (urethritis, cervicitis, salpingitis), in some cases with reactive arthritis (serotypes D-K),
- lymphogranuloma venereum, a sexually transmitted disease that predominantly occurs in warm countries (serotypes L1-L3).

Serotypes A-C are transmitted by infectious secretion of the eyes, while serotypes D-K and L1-L3 are transmitted by sexual contact or perinatally. Humans are the only natural reservoir.
Currently, there is no vaccination against chlamydia. Chlamydia infections can be well treated with antibiotics over 7-14 days. A protracted, differentiated treatment, both locally and systemically, in case of reactive arthritis.

Analytics. Direct detection by nucleic acid amplification tests (e.g. PCR) or by direct immunofluorescence. The culture requires a high level of technical expertise.
Serology: Detection of antibodies against *Chlamydia trachomatis* using enzyme immunoassays (the reactive surfaces are primarily treated with membrane proteins) or indirect immunofluorescence (immunofluorescence, indirect) (species-specific microimmunofluorescence test: purified elementary bodies as the substrate, inactivation of the lipopolysaccharide (LPS) antigen in order to minimise cross-reactivities, serological “gold standard”)

Sample material and sample stability. Direct detection and culture: In the case of genital infections, first-void urine or cervix, vaginal and urethral smears are obtained (use Dacron or Rayon swabs!). Laparoscopically obtained tube smears or puncture specimens of Douglas' pouch are ideal for detecting specific cervicitis. For the cell culture, the patient material is injected into special transport media, it is refrigerated for transport and must be prepared within 4 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. For acute urogenital infections, the aim is to identify the pathogens using direct detection methods. Due to its specificity of almost 100%, the cultural detection of *Chlamydia trachomatis* was considered the gold standard in diagnosing urogenital Chlamydia infections for some time, but was replaced by PCR due to the excessive time and effort and insufficient sensitivity.
Direct pathogen detection is often no longer possible for *Chlamydia trachomatis*-associated secondary diseases, such as tubal sterility and reactive arthritis. In these cases, the detection of specific antibodies can help confirm the diagnosis. Cross-reactions with other Chlamydia species must be ruled out, e.g. by a parallel analysis of antibodies against *Chlamydia psittaci* and *Chlamydia pneumoniae*, with BIOCHIP mosaics where applicable.
Chlamydia trachomatis screening of both parents prior to a planned pregnancy is recommended. The direct detection of *Chlamydia trachomatis* on the cervix uteri at the start of a pregnancy is now considered a standard part of screening, as this infection is associated with the risk of premature births and stillbirths (prenatal care).

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Corynebacterium diphtheriae

W. STÖCKER

Synonym(s). Diphtheria

Description of the pathogen. *Corynebacterium diphtheriae* is a gram-positive, facultative anaerobic, club-shaped rod bacteria from the order Actinomycetales. It is immobile and does not form capsules or spores. *C. diphtheriae* is relatively resistant to environmental influences, but can be quickly killed off by heating and disinfectants.
The virulence of *C. diphtheriae* is caused by the extremely potent diphtheria toxin (LD_{50} 0,3 µg/kg), which consists of two polypeptide chains A and B. As the genetic information on the synthesis of this exotoxin is coded by the β prophage, only *C. diphtheriae* strains that are infected with this prophage are toxigenic. The diphtheria toxin deactivates the eukaryotic elongation factor EF2. This inhibits the protein biosynthesis and leads to the death of the infected cells.

Diseases. Diphtheria is a global infectious disease, which is still endemic in many developing countries and Russia and must therefore continue to be monitored, but only occurs in isolated cases in Central Europe. A risk of contracting the disease exists for persons with no or insufficient vaccine protection (in Germany about 50% of adults). The pathogen exclusively attacks humans and, in the case of a pharyngeal attack, is transmitted by droplet infection and, in the case of cutaneous diphtheria, is predominantly transmitted by smear infection.
The disease generally occurs after an incubation time of 2-5 days with general malaise, fever, throat, abdominal and joint pain, followed by pharyngitis, laryngitis and tonsillitis with pseudomembranes of fibrin, leukocytes, cell residues and germs. Characteristic symptoms are:
- sweetish halitosis,
- barking cough with stridor,
- hoarseness,
- breathlessness,
- velum paresis,
- swelling of the lymph nodes.

Additional local manifestations are nasal, wound, cutaneous and conjunctival diphtheria. All forms may lead to a systemic intoxication and life-threatening organ damage. Possible late effects are:
- myocarditis,
- liver and renal function disorders,
- paralyses in the region of the motor cranial nerves.

In the event of a suspected clinical diagnosis, persons with a lack of or uncertain vaccine protection are immediately administered diphtheria antitoxin. An antibiotic treatment with penicillin or a macrolide has a supporting effect. Courses with complications may require further interventions, such as intubation or removal of the pseudomembranes moving the airways. The prophylaxis consists of active immunisation with a toxoid. According to the German Infection Protection Act, suspicion of the disease, falling ill and death as a result of diphtheria as well as the direct or indirect detection of toxin-forming diphtheria bacteria are reportable.

Analytics. Microscopic preparations of *C. diphtheriae* display gram-positive, club-shaped rods with a characteristic V- or Y-shape. The terminal polar bodies are visible as black/blue granules in Neisser staining.
C. diphtheriae is cultivated on protein-containing culture media (blood agar, Löffler serum agar) and tellurite-containing selective media (Tindsdale agar, Clauberg III agar) under aerobic conditions with 5-10% CO_2 gas application at 37 °C. Greyish colonies, potentially with a weak haemolysis ring on blood agar as well as black colonies with a blue ring on tellurite substrates are suspicious. If they display the typical pattern in a microscopic preparation, subcultures are prepared (blood agar, Löffler's medium). The final identification is based on biochemical characteristics (catalase positive, urease negative, glucose fermentation positive, saccharose fermentation negative, nitrate reduction positive). The ability of isolated strains to form toxins is analysed using the Elek Ouchterlony immunodiffusion test or by detecting the toxin gene using PCR.
Indirect immunofluorescence, enzyme immunoassays, neutralisation assays or haemagglutination tests are used to detect specific antibodies against the diphtheria toxin.

Sample material and sample stability. Direct detection and culture: Smears (from below the pseudomembranes) of the throat, tonsils and nasal mucosa are analysed. The material must be stored at +4 °C to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. *C. diphtheriae* cannot be clearly differentiated from other, apathogenic corynebacteria microscopically. Instead, suspected colonies must be isolated in pure culture, identified and checked for toxin production. The positive detection of toxin alone is decisive for the detection of acute diphtheria. The quantitative detection of antibodies against the diphtheria toxin takes priority in epidemiological matters as well as when checking the immunity situation and vaccination status.
Differential diagnostics must include:
- Infectious mononucleosis,
- Plaut-Vincent angina,
- streptococcal pharyngitis,
- virus-induced pharyngitis,
- mumps.

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Coxsackie viruses

W. STÖCKER

Synonym(s) Human Coxsackie virus A/B

Description of the pathogen. Non-enveloped single-stranded RNA virus, icosahedral shape, diameter of approx. 30 nm, heat-labile, inactivation with 0.1 N HCL or 0.3% formaldehyde. High environmental resistance.
Coxsackie viruses represent a range of different serotypes of the genus human enterovirus, which do not display any cross-immunity. They belong to the *Picornaviridae* family.

Diseases. Infections are predominantly (90-95%) asymptomatic. Manifestation as summer flu, pneumonia, diarrhoea, encephalitis, aseptic meningitis, hepatitis, myocarditis, pericarditis, pleurodynia (Bornholm disease), gingivostomatitis, exanthema (hand, foot and mouth disease) conjunctivitis, foetal damage.

Transmission. Faecal-oral, aerogenic (droplets, mouth-to-mouth contact, contaminated drinking water, contaminated seafood) and diaplacentally. Men are affected twice as often as women, infants more frequently than adults. Significant spread by excretions of asymptomatic infected persons.

Incubation time. An average of 7-14 days, occurs worldwide.

Treatment and prophylaxis. Symptomatic; antiviral drugs and vaccines are not yet available.

Analytics. Culture: Some Coxsackie A viruses can only be cultivated in newborn mice, others in primary monkey kidney cells or in RD or SKCO-1 cells. Coxsackie B viruses can easily be cultivated in HEp-2 and HeLa cells as well as in primary monkey kidney cells.
Direct detection: Primarily using PCR (PCR, quantitative), also using cDNA probes in research. Antigen ELISA tests are too insensitive and require preliminary virus enrichment. Pathogen typing via defined 7antibodies.
Serology: Indirect immunofluorescence (immunofluorescence, indirect), neutralisation assay, ELISA and complement fixation test. The latter should no longer be used for the serodiagnosis of enteroviruses due to the significant cross-reactivity with other enteroviruses and the restricted sensitivity. When using antigen lysates, immunological cross-reactions can also be observed with the ELISA, which is why the focus is on detecting group-specific antibodies. Cross-reactions are sometimes also observed in the neutralisation assay (A3 with A8, A11 with A15, A13 with A18). The serological detection of a fresh infection requires a significant rise in the concentration of pathogen-specific IgG antibodies within 7-14 days or the detection of specific IgM antibodies.

Sample material/sampling conditions and sample stability. Direct detection and culture: Stool, throat, rectal and conjunctival smears as well as blood, cerebrospinal fluid and vesicle secretion or biopsies are analysed. The material must be stored at +4 °C to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Pathogen detection using PCR or culture plays an essential role. Importance is also ascribed to the detection of neutralising antibodies in serology. The benefits of serology are limited due to the high rates of infection and significant cross-reactivities between the various serotypes. A significant rise in the specific antibody titer within two weeks confirms a fresh infection.

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Cytomegaloviruses

W. STÖCKER

Description of the pathogen. Family *Herpesviridae*, *Human Herpes virus 5*

Disease. Cytomegaly (manifestation of a CMV infection) is transmitted horizontally via infectious bodily fluids (breast milk, saliva, urine, genital secretion), blood and blood products as well as transplanted organs, or vertically by the pregnant mother to the child. The incubation time amounts to 3-12 weeks depending on the transmission path. The infection is generally asymptomatic, occasionally similar to infectious mononucleosis with fever, pharyngitis and lymphadenopathy. By contrast, in immunosuppressed patients (transplant recipient, AIDS), cytomegaly often manifests in retinitis, interstitial pneumonia and enteropathy. Reactivations of a latent infection occur asymptomatically in immunocompetent persons, while this often occurs with the symptoms of an acute infection in persons with weakened immunity. In Germany, the seroprevalence amounts to 30–40% in adults depending on the standard of living and age.
Cytomegaloviruses are the most common cause of connatal infections with a rate of about 1% of all newborns. In the event of primary infections during pregnancy, the transmission rate amounts to 30-40%, or just 1.4% in case of reactivations. Damage to the foetus is primarily observed with infections in the first 20 weeks of pregnancy: Of those infected, 5% are born with haemorrhaging, hepatosplenomegaly, jaundice, microcephaly, chorioretinitis and other damage (mortality 10%). The remaining 95% of CMV-infected newborns initially appear to be clinically inconspicuous, however, one in ten suffer from hearing impairment and mental retardation. Infection via the breast milk is possible, but is generally inconsequential for fully developed newborns – however, premature babies may contract an acute CMV primary infection.
It is important to identify seronegative pregnant women at an early stage in order to suggest exposure prophylaxis and hygiene measures. The detection of the CMV antibody status is currently not included in the infection serological preventive diagnostics for pregnant women, which has been criticised by some experts. Transfusions for transplant recipients or other immunocompromised patients must exclusively take place with the blood of CMV-seronegative donors, if none are available, e.g. the filtration of leukocytes before the transfusion is advisable.
An appropriate vaccine is currently not available. Virostatic agents are used in case of connatal cytomegaly as well as with CMV diseases in immunosuppressed patients. These drugs are not permitted during pregnancy. Studies on the administration of CMV-specific hyperimmunoglobulins after contact with virus excretors are available. In some federal states, CMV-seronegative pregnant kindergarten teachers are prohibited from working.

Analytics. Detection of pathogens: Occasionally the virus is still cultivated in human fibroblasts. The specific immunocytological detection of the CMV antigen pp65 in leukocytes is used (good correlation with the disease activity). PCR diagnosis enables the detection of the viral genome. It is used to detect the CMV viral load (treatment monitoring).
Serology: Class IgG and IgM anti-CMV antibodies against the whole virus are detected by indirect immunofluorescence (immunofluorescence, indirect) or using various enzyme immunoassays (e.g. enzyme-linked immunosorbent assay, chemiluminescence immunoassays). The detection of IgM and IgG antibodies against recombinant CMV proteins p52 and glycoprotein B (gB) as well as avidity tests complement the classic serology to distinguish between acute and convalescent infection phases or in case of ambiguous findings. The detection of intrathecally synthesised antibodies in the cerebrospinal fluid, together with direct detection by PCR, is used to identify involvement of the CNS.

Sample material and sample stability. Direct detection and culture: Urine, saliva, bronchoalveolar lavage fluid, blood, serum, cerebrospinal fluid, amniotic fluid and breast milk are analysed. The material must be stored at +4 to +8 °C until further processing. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. A CMV infection cannot be clearly diagnosed based on the clinical picture. Symptomatic acute infections are identified by a combination of direct detection and serology. An acute infection can be assumed with IgM-reactive samples, which simultaneously display a low-avidity IgG, potentially in combination with a lack of IgG antibodies against gB. If the avidity of the IgG is high or IgG against gB can be detected, a reactivation is likely to have occurred. A significant rise in the specific anti-CMV IgG within 1-2 weeks indicates an acute infection. If class IgG antibodies with a high avidity are detected, immunocompetent persons are protected from a secondary infection.
In transfusion and transplant medicine, the donor's and recipient's CMV status must be noted in order to prevent primary infections and reinfections. A reinfection can also be triggered in seropositive recipients, especially if the immune system is weakened.
If an acute infection is suspected during pregnancy, specific IgM can be detected in the foetal blood or the viral genome can be detected in the amniotic fluid (amniocentesis) by PCR. At the same time, the pregnancy is closely monitored by ultrasound diagnostics.
Early analysis of the maternal serostatus as part of prenatal care is indicated. Moreover, initial baseline findings can subsequently contribute to a reliable diagnosis in suspected cases during pregnancy if they are observed together with a follow-up sample as this allows confirmation or exclusion of a rise in the antibody level.

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Dengue viruses

W. Stöcker

Description of the pathogen. Family: *Flaviviridae;* Genus: *Flavivirus;* Species: Dengue virus (serotypes 1–4). Positive-strand RNA genome, enveloped, 50 nm diameter.

Diseases. Dengue fever (in 1% of cases: dengue haemorrhagic fever, dengue shock syndrome).
Distribution: Worldwide in over 100 tropical and subtropical countries. Dengue fever is the most common vector-borne viral infection in humans.
Vector: Mosquitoes (*Aedes aegypti* and *albopictus*).
Hosts: Primates, humans (about 95% of those infected are children).
Transmission is possible transplacentally, via blood transfusion or organ transplantation.
Symptoms: Initial infections are often asymptomatic. The disease is characterised by sudden high fever and flu-like symptoms, muscle and joint pains, exanthema, petechiae, splenomegaly, bradycardia, hypotension, thrombocytopenia, lymphocytopenia.
In children under 15 years of age and in general after a second infection with a different serotype: Haemorrhaging and shock symptoms, mortality 6-30%.
Treatment and prophylaxis: Only symptomatic treatment; vaccine still in development. Prevention: protection against mosquito bites, combatting the vectors.

Analytics. Working with the pathogen requires the use of laboratories of safety class 3.
Direct detection: In the blood with RT-PCR (polymerase chain reaction), antigen-capture ELISA (NS1 protein), or virus cultivation in cell culture.
Serology: Specific class IgG and IgM antibodies can be detected using the haemagglutination inhibition assay, neutralisation assay, indirect immunofluorescence (immunofluorescence, indirect) and various enzyme immunoassays. Avidity tests supplement the serology in case of ambiguous findings.

Sample material and sample stability. Direct detection and culture: Blood or blood components (PCR). The samples must be transported at +4 °C and analysed within 6 h (PCR) and 24 h (culture, direct immunofluorescence).
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Direct detection: Possible during the first 2-7 days of the disease; serotyping using RT-PCR. For patients infected with dengue virus, the detection of the highly-specific dengue NS1 antigen in serum is possible from the start of the clinical symptoms in the event of both primary as well as secondary infections. As a result, this antigen detection is not just an important tool for detecting acute dengue virus infections, it can also replace the more time-intensive RT-PCR.
Serology: Dengue virus-specific IgM appears from the 2nd-4th day of the disease. It reaches its peak after 2 weeks and remains detectable for 2-3 months. In the event of a primary infection, specific IgG only appears from the 9th day of the disease. The detection of IgG at the start of the disease can therefore be considered evidence of a secondary infection. In the event of this kind of secondary infection with a heterologous dengue virus serotype (1-4), the IgG titer can be up to 10 times higher than after the initial infection, while IgM can often not be detected. IgG antibodies are retained over months and years as an immunological memory. Low dengue fever virus-specific IgM titers can persist for months and simulate an active dengue infection. By contrast, a significant rise in the specific IgG titer can be considered a clear indication of a fresh infection. Attention must be paid to cross-reactions, between the 4 serotypes (but which do not effect any reciprocal immune protection) as well as with antibodies against other flaviviruses (Zika, TBE, yellow fever, West Nile fever, Japanese encephalitis virus, etc.).
Differential diagnoses: Zika virus infection, yellow fever and other arbovirus infections, typhus, malaria, measles, rubella.
PCR tests or serological methods can be used for screening blood transfusions for viral RNA or for specific antibodies.
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya virus, dengue virus, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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Echinococcus granulosus and Echinococcus multilocularis

W. STÖCKER

Description of the pathogen. Family: *Taeniidae*; Genus: *Echinococcus* (*E.*); Species: *E. granulosus*, *E. multilocularis*, *E. vogeli*, *E. oligarthrus* and others.
Echinococcus granulosus ("dog tapeworm") and *Echinococcus multilocularis* ("fox tapeworm") are the most important representatives of the *Echinococcus* genus.
E. granulosus is a 3–7-mm-long tapeworm, *E. mulitlocularis* is slightly shorter at 1-4 mm. They consist of a hooked head (scolex), a budding zone and typically 3 or 5 joints (proglottids) in which the environmentally resistant eggs mature. The spread of echinococcus is connected with an obligate host change.

Diseases.
Echinococcosis is an infectious disease caused by an infection with parasites in the Echinococcus genus. The dog tapeworm (*E. granulosus*), which causes cystic echinococcosis (CE), as well as fox tapeworm (*E. multilocularis*), which causes alveolar echinococcosis (AE), are medically significant in Europe. The development of all types of echinococcosis requires an obligate host change: the final hosts are carnivores, while the intermediate hosts are generally herbivores. Humans are an accidental intermediate host for fox as well as dog tapeworms.
The sexually mature tapeworms live in the intestines of their final host (in Europe, primarily dogs and red foxes), which excrete the mature cestode eggs with the faeces. These eggs are highly resistant to environmental influences and can remain infectious for several months in the right conditions. The eggs are ingested by the intermediate hosts (rodents or ungulates) in their food. In their internal organs (especially the liver), the larval stages develop and propagate in fluid-filled blisters (hydatids). Human infection may occur by smear infections, handling contaminated soil or ingesting contaminated food.
The resulting clinical pictures of both echinococcosis (CE and AE) differ with respect to the growth behaviour of both parasites in the human body. The appearance of AE corresponds to that of a malignoma: The larva develops in the liver in the form of multiple connected vesicles (pseudocysts), which invasively proliferate into the surrounding tissue and destroy it. The clinical course of CE is characterised by the slow growth of individual cysts, which displace the surrounding tissue over time. The occurrence of echinococcosis-caused cysts can affect all organs. The courses of both diseases are asymptomatic in humans for many years before they become noticeable after 10-15 years due to cholestatic icterus, epigastric pains, fatigue, weight loss and hepatomegaly. Untreated echinococcosis can lead to the death of the patient. Cysts, malignant and benign tumours, abscesses, as well as the differentiation between AE and CE are important for differentiation purposes.
In Central Europe, the risk of infection is low. People in endemic areas, who live close to dogs, sheep, goats and other potential intermediate hosts, are at particular risk. The most important prevention measures are compliance with general hygiene rules and the regular deworming of free-roaming dogs and cats. Offal, especially of private origin, should only be fed to animals after adequate cooking.
Echinococcosis patients are most effectively treated in specialist facilities. The surgical sanitation of the source of infection and chemotherapy with mebendazole and albendazole are established practices.

Analytics. Direct antigen detection by microscopy and polymerase chain reaction (PCR). Immunological detection using genus- and species-specific antisera. The standard tests for the serological detection of antibodies are indirect immunofluorescence (IIFT; substrate: frozen section of echinococcus larvae), enzyme-linked immunosorbent assay (ELISA; suitable as a screening test, e.g. when using highly purified vesicle fluid of *E. multilocularis* as the substrate to detect antibodies against *E. multilocularis* and *E. granulosus*), indirect haemagglutination, immunoelectrophoresis and immunoblot (combination of native and species-specific recombinant antigens is possible). In the event of a positive serology and suspicion of a cerebral infection, the specific antibodies and the whole antibody are simultaneously detected in cerebrospinal fluid and serum and the specific cerebrospinal fluid-serum ratio is calculated. A value significantly > 1 indicates an intrathecal antibody synthesis.

Sample material and sample stability. Direct detection: From biopsy and surgical material (hydatid fluid).
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C.

Diagnostic value. Imaging methods, such as sonography, CT and MRT are initially used for diagnosis. The use of serological test systems to detect parasite-specific antibodies in serum or plasma serves to confirm the imaging methods. Echinococcus can be detected with a good sensitivity in ELISA and IIFT methods when using the echinococcus whole antigen (purified E. multilocularis vesicle fluid or frozen sections). In many cases, the serological differentiation of *E. granulosus* and *E. multilocularis* is possible using species-specific antigens in a line blot (immunoblot). A negative serological result does not exclude a disease.
Differential diagnosis: hepatocellular cancer of the liver, liver metastases of other tumours; hepatitis; cysticercosis; cirrhosis of the liver; round lesions of another genesis in the lungs. The disease is reportable in accordance with Section 7 (3) IfSG.

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ECHO virus

W. Stöcker

Synonym(s). Enteric cytopathic human orphan virus

Description of the pathogen. Family: *Picornaviridae*; Genus: *Enterovirus*; Species: Human ECHO viruses 1–7, 9, 11–21, 24–27, 29–33, 69, 73–78
Unenveloped single-stranded RNA viruses with cubic symmetry, a diameter of 24-30 nm, 4 non-glycosylated virus capsid proteins (VP1-VP4), apathogenic (except for ECHO 9) for newborn mice (as distinct from Coxsackie viruses); global presence.

Diseases. 90-95% of infections are asymptomatic. Lower neurotropism compared to poliomyelitis virus infections (but the involvement of the CNS is not ruled out, as is the case for infections with all enteroviruses), however, it has a broader disease spectrum: Infections of the upper respiratory tract, gastrointestinal diseases, exanthema, enanthema, myopericarditis, disseminated neonatal infections, meningitis, encephalitis and chronic meningoencephalitis in immunosuppressed patients.
Transmission: Faecal-oral, aerogenic (droplets, mouth-to-mouth contact, contaminated drinking water), nosocomial
Incubation time: 7-14 days on average
Treatment: Symptomatic, specific antiviral drugs are not yet available.

Analytics. Culture/direct detection: Virus reproduction occurs in monolayer cell cultures, e.g. of MRC 5, HeLa or Vero cells; virus identification by neutralisation assay using antisera with a known specificity. RT-PCR (polymerase chain reaction) is used to detect viral RNA, typing is possible by subsequent sequencing of the coding region VP1.
Serology: Indirect immunofluorescence, neutralisation assay and enzyme-linked immunosorbent assay (ELISA) are used. When using antigen lysates, immunological cross-reactions can be observed with the ELISA, which is why the focus is on detecting group-specific antibodies. Cross-reactions are sometimes also observed in the neutralisation assay (type 1 with 8, type 12 with 29, type 6 with 30). The best serological evidence of a fresh infection is a significant rise in the IgG titer within 1-3 weeks or the detection of pathogen-specific IgM antibodies.

Sample material and sample stability. Direct detection and culture: Throat smears, stool and, depending on the organ manifestation, rectal and conjunctival smears as well as blood, cerebrospinal fluid and vesicle secretion or biopsies are analysed. The transport medium should be neutral and have an antibacterial effect. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Pathogen detection using culture and subsequent typing using PCR play an important role. The benefits of serology are limited due to the high rates of infection and significant cross-reactivities between the various serotypes. This primarily involves the detection of the neutralising antibody. The significant rise in the specific antibody titer within 2 weeks confirms a fresh infection.

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W. STÖCKER

Synonym(s).
Human herpes virus 4; HHV-4

Description of the pathogen.
Family: *Herpesviridae*; Subfamily: *Gammaherpesvirinae*; Genus: Lymphocryptovirus
The Epstein-Barr virus belongs to the human herpes viruses. It is 150-180 nm in size and has a linear dsDNA genome (~172 kb). The DNA is wound around a core structure and enveloped by a capsid consisting of a protein matrix. This is, in turn, enveloped in a lipid shell with glycoproteins.

Diseases.
The Epstein-Barr virus (EBV) is the pathogen of infectious mononucleosis. The course of the infection is often not apparent in childhood, while an initial infection in young adults leads to infectious mononucleosis ("kissing disease", glandular fever) in 30-60% of cases after an incubation time of 30-50 days, which generally starting with pharyngitis and lymphadenopathy, which can be joined by exanthema and often also hepatosplenomegaly. It is characterised by a modified blood count (mononuclear Pfeiffer cells). In rare cases, the symptoms persist over an extended period of time (chronic form). EBV can cause various tumour diseases: Endemic Burkitt lymphoma in Africa, a percentage of Hodgkin's disease and nasopharyngeal cancer. In the event of congenital or acquired immunodeficiencies, an EBV infection can lead to serious complications, such as X-linked lymphoproliferative syndrome or post-transplant lymphoproliferation, both of which have high mortality rates.
The virus exclusively attacks humans. It is predominantly transmitted by saliva and initially infects B lymphocytes, which differ from lymphoblasts. Alternative transmission paths are blood transfusions and transplants. EBV persists throughout the lifetime (latency in "memory lymphocytes"). Recurring, clinically barely perceptible, reactivations with excretion of the virus arise in immunocompetent persons. The prevalence of EBV in Germany increases from 40% in 2-year-olds to almost 100% in adulthood.

Analytics.
Direct detection: Detection of EBV DNA using polymerase chain reaction (PCR).
Serology: The gold standard for detecting class IgA, IgG and IgM antibodies against the various EBV antigens, virus capsid antigen (VCA), early antigen (EA) and Epstein-Barr nuclear antigen (EBNA), is the indirect immunofluorescence assay (immunofluorescence, indirect). In addition, enzyme immunoassays (enzyme-linked immunosorbent assays, chemiluminescence immunoassays) and immunoblots with native as well as recombinant antigens are also popular. Ambiguous antibody constellations can be distinguished by analysing the avidity of the IgG against VCA (low avidity: fresh infection). The detection of heterophile antibodies (Paul-Bunnel reaction) is virtually no longer used.

Sample material and sample stability.
Direct detection: Using PCR. EDTA blood is analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h.
Serology: Serum, plasma or cerebrospinal fluid for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value.
Direct detection: The quantitative detection of EBV DNA by PCR is the method of choice in order to determine the reactivation of an EBV infection or uncontrolled virus replication with the risk of an EBV-associated lymphoproliferation process in immunosuppressed patients. The serology is not suitable in this case, as the antibody titers dot not display any correlation to the viral load.
Serology: Detection of class IgA, IgG and IgM antibodies against VCA, EA and EBNA to distinguish between acute and EBV infections that have subsided. Class IgM and IgG antibodies against VCA and EA are characteristic of an acute primary infection. During the course of an infection, VCA IgM occurs first, followed by VCA IgG and then EA IgG. While VCA IgM and EA IgG generally disappear again after a few months, VCA IgG persists for a lifetime. EBNA-1 IgG are formed about 6-8 weeks after an infection, they indicate that an infection has subsided. EBNA antigens 1–6 are synthesised earlier than EA and VCA during the infection. But, they are only represented in the immune system after the destruction of the B cells, which is the reason why antibodies against VCA and EA occur before the antibodies against EBNA. The heterophile antibodies (antibodies, heterophile) typical of an EBV infection also occur before the antibodies against EBNA.
Distinguishing between fresh infections and those that have existed for an extended period of time is one of the greatest challenges in serology. Disruptive factors include the persistence of the IgM response, insufficient or delayed IgM formation as well as non-specific IgM reactions due to polyclonal B cell simulation with acute infections or a lack of EBNA-1 IgG with infections that have subsided. No class IgM antibodies against VCA can be detected in up to 20% of all acute EBV infections, while the IgM is formed with a delay in 15% of cases and it persists in 4%. No EBNA-1 IgG is formed in about 5% of infected patients. Antibodies against EA are found in patients with fresh EBV infections as well as those that have overcome EBV infections. In the past few years, the analysis of the avidity of the formed IgG antibodies has established itself as a reliable method for identifying primary infections: The immune system initially reacts to an infection by forming low-avidity antibodies. As the disease progresses, IgG that is adapted to the antigens with increasing precision is secreted – the avidity increases. As long as no high-avidity IgG can be detected in the serum, it can be assumed that the infection is in its early stages.
A distinction must be made between infectious diseases that cause similar symptoms, such as HIV, CMV, rubella, fifth disease, HCV, streptococcal infection, toxoplasmosis and malaria.

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TBE virus

W. STÖCKER

Synonym(s). Tick-borne encephalitis, tick-borne meningoencephalitis, early summer meningoencephalitis virus

Description of the pathogen. The TBE virus belongs to the genus *Flavivirus* (family: *Flaviviridae*). The spherical, 50 nm virion consists of a 10.5 kDa positive-strand RNA in a capsid, which is enclosed by a virus envelope of lipids of the host cells and the protein E. Three subtypes are known; the Central European subtype that exists in Germany, as well as a Far East and a Siberian subtype.

Diseases. Tick-borne encephalitis (TBE) is a reportable disease, which tends to peak seasonally from early summer to autumn. Endemic areas are located in Bavaria, Baden-Württemberg, South Hesse, Thuringia and Rhineland-Palatinate as well as in regions of Saarland and Saxony.
Vectors are ticks (*ixodidae*) in every stage of development (larvae, nymphs, adults). They live at the edge of the forest and in high grass and transmit the virus during their blood meal. The natural reservoirs are predominantly small mammals and birds.
About 200-300 new infections are registered in Germany every year. The majority of the persons infected with TBE viruses do not notice any objectifiable signs of the disease – in this case the seroconversion provides the only indication. Only about 10-30% of those infected display flu-like symptoms with fever, headaches and joint pains, vomiting and nausea 7-14 days after infection. In about 10% of symptomatic patients, about one fever-free week is followed by meningoencephalitis with headaches, vomiting, meningeal irritations and, in isolated cases, stupor or coma.
Prevention is possible by active immunisation and exposure prophylaxis: These days you should remain safe by staying on the path during summer stays in the forest or field. Treatment is symptomatic.

Analytics. Direct detection: In the early phase of the disease, detection is possible using the culture method or polymerase chain reaction (PCR) from blood or cerebrospinal fluid.
Serology: Detection of TBE virus-specific class IgG and IgM antibodies in serum or cerebrospinal fluid by indirect immunofluorescence (immunofluorescence, indirect), enzyme immunoassays or immunoblot assays.

Sample material and sample stability. Direct detection and culture: Blood or cerebrospinal fluid are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. In laboratory diagnosis, the determination of a TBE virus infection is primarily based on the detection of IgG and IgM antibodies against the TBE
virus. A fresh infection is characterised by a seroconversion or a significant IgG titer rise. If involvement of the central nervous system is suspected, the specific antibodies and the whole antibody are simultaneously detected in cerebrospinal fluid and serum and the specific cerebrospinal fluid-serum ratio is calculated. A value > 1.5 indicates an intrathecal antibody synthesis.
Cross-reactions with other human-pathogenic flaviviruses (dengue, West Nile fever and yellow fever viruses) must be excluded. Poliomyelitis, Lyme disease and viral meningoencephalitis are relevant with regard to differential diagnosis.

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Yellow fever virus

W. STÖCKER

Description of the pathogen. Family: *Flaviviridae*, Genus: *Flavivirus*, Species: Yellow fever virus.
Positive-strand RNA genome, enveloped, 50 nm diameter.

Diseases. Yellow fever
Distribution: In tropical and subtropical regions of Africa (90% of cases), Central and South America.
Vector: Mosquitoes (*Aedes*, *Haemagogus*).
Hosts: Primates, birds, bats, snakes, humans (in the jungle as a secondary host, in the cities as the primary host via direct blood contact).
Symptoms: Viraemia with sudden high fever and flu-like symptoms, bradycardia, in 15% of sufferers a second, toxic phase with haemorrhaging, blood-clotting disorders, icterus, nephritis, CNS disorders. Mortality of 10-20%, peaks in people aged between 20 and 30 years.
Treatment and prophylaxis: Only symptomatic treatment is possible. A live vaccine is available, which, e.g. in Germany, may only be administered by yellow fever vaccination centres due to an increased risk of side effects. Prevention: protection against mosquito bites, combatting the vectors.

Analytics. Working with the pathogen requires the use of a laboratory of safety class 3.
Direct detection of the virus with RT-PCR (polymerase chain reaction) or virus cultivation in cell culture.
Serology: Detection of specific antibodies (IgG, IgM) in serum through indirect immunofluorescence (immunofluorescence, indirect), enzyme-linked immunosorbent assay (ELISA), neutralisation assay or haemagglutination inhibition assay.

Sample material and sample stability. **Direct detection and culture**: Blood or blood components (PCR). The samples must be transported at +4 °C to +8 °C and analysed within 6 h (PCR) and 24 h (culture, direct immunofluorescence).
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. **Direct detection**: Possible during the first few days of the disease.
Serology: Specific IgM antibodies can be detected shortly after the occurrence of the first symptoms, while IgG antibodies can be detected with a delay of two days. Attention must be paid to cross-reactions with other flaviviruses (TBE, dengue, West Nile fever, Japanese encephalitis virus, etc.). Attention must also be paid to vaccine-induced antibodies.
Differential diagnoses: Other viral haemorrhagic fevers (dengue, Crimean-Congo, Rift Valley, Ebola, Marburg fever), leptospirosis.
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya virus, dengue viruses, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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Haemophilus influenzae

W. Stöcker, C. Krüger

Description of the pathogen. The bacteria in the *haemophilus* genus are gram-negative bacteria in the Pasteurellaceae family;
Species: encapsulated (serotypes a to f) as well as unencapsulated strains of *Haemophilus influenzae* are known.

Diseases. *Haemophilus influenzae*, as a commensal organism, exclusively resides in the mucous membranes of humans, primarily in those of the upper respiratory system (nose, throat, windpipes) where it occasionally causes inflammatory diseases (epiglottitis, bronchitis, pneumonia). Over 95% of infections with *Haemophilus influenzae* are caused by the serotype b. The bacterium is transmitted by contact and droplet infection, fostered by cramped living conditions. There is a high risk of infection in children under two years of age, patients with viral infections of the airways and defective self-cleaning of the bronchi.
In newborns, infections with *Haemophilus influenzae* lead to conjunctivitis and otitis media and, especially in infants, this bacterium is also the pathogen that causes meningitis and other inflammatory diseases. In adults, infections with *Haemophilus influenzae* occur less frequently and primarily manifest as bronchitis.
Vaccination against *Haemophilus influenzae* type b is recommended as the prophylaxis for children from 2 months until 4 years of age. Treatment is primarily based on antibiotics.

Analytics. Culture: Pathogen diagnosis occurs by cultivation from bodily fluids and subsequent differentiation based on the growth factor requirement. *Haemophilus influenzae* requires specific active agents for cultivation (X factor: haemin, V factor: nicotinamide adenine dinucleotide). Cultivation under increased CO_2 concentration on boiled blood agar; additionally smeared *Staphylococcus aureus* ensures ideal growth conditions. Smooth, slightly transparent colonies can be detected after a one- to two-day incubation period at 37 °C. *Haemophilus influenzae* is distinguished from other species by testing metabolism properties (e.g. porphyrin production) or by direct detection.
Direct detection: Identification of the capsule serovar using immunological methods, such as latex agglutination, immunoprecipitation or indirect immunofluorescence.
Serology: Indirect immunofluorescence (immunofluorescence, indirect) and enzyme-linked immunosorbent assay test systems are used for serological analyses.

Sample material and sample stability. Culture and direct detection: Cerebrospinal fluid, blood, sputum, secretion and smears of conjunctiva or the throat. The samples must be refrigerated for transport and analysed within 4 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. *Haemophilus influenzae* b is primarily represented in three categories of antigenic determinants: Capsule polysaccharides, outer membrane proteins and lipooligosaccharides. Antibodies against capsule polysaccharides are only formed during the course of an acute infection, while antibodies against outer membrane proteins can also occur in healthy normal persons in relatively high titers. The surest sign of an acute infection is therefore not an existing high initial titer, but rather a significant titer rise within 2 weeks. The detection of the specific antibodies also takes place to check the success of vaccination.

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Hantaviruses

W. Stöcker

Description of the pathogen. The different hantavirus types belong to the hantavirus genus (family: *Bunyaviridae* with over 200 types). The virus particle is 80–110 nm in size and contains a single-stranded RNA, associated with a nucleocapsid, which is surrounded by a shell consisting of two glycoproteins (G1, G2). The virus types Sin-Nombre and Andes occur in the USA and South America, while the Hantaan and Seoul types are present in Asia and Puumala, Tula and Dobrava occur in Europe.

Diseases. Hantaviruses are the pathogens of hantavirus pulmonary syndrome (as interstitial pneumonia predominantly in America) and haemorrhagic fever with renal syndrome (acute renal failure). A moderate form of the disease, nephropathia epidemica, occurs in the Balkans. The viruses are aerogenically transmitted to humans, in dust or aerosols, via excretions from infected rodents (primarily mice). After an incubation time of 2-5 weeks, the disease starts with a high fever and general flu-like symptoms (headache, myalgia). This is followed by severe pains in the lumbar and abdominal region. The third phase is characterised by a restriction of the kidney function up to acute kidney failure. Treatment is symptomatic, prevention occurs by avoiding contact with rodents.

Analytics. Direct detection: In the acute phase of the disease, direct pathogen detection is possible by polymerase chain reaction (PCR) from blood or biopsy material, but is not successful with every patient.
Culture: Virus cultivation is generally unsuccessful.
Serology: Detection of hantavirus-specific class IgG and IgM antibodies by indirect immunofluorescence (immunofluorescence, indirect), enzyme immunoassays or immunoblot assays. The virus neutralisation assay remains reserved due to the safety classification of the hantavirus special laboratories.

Sample material and sample stability. Direct detection and culture: Blood and biopsies are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Virus detection from the urine or blood by PCR or virus cultivation during the acute phase of the disease. However the relatively short viraemia limits the prospect of success. Specific antibodies can generally be detected when the clinical symptoms start. The diagnosis is considered reliable if both specific IgM and IgG are present, or a significant rise in the IgG titer is detected in a serum pair. Due to the manifold and non-specific symptoms, a distinction must be made between other bacterial or viral diseases with kidney involvement as well as acute kidney diseases. Other viral haemorrhagic fevers must be considered if haemorrhaging occurs.
Positive results must be reported.

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Helicobacter pylori

W. STÖCKER

Synonym(s). *H. pylori*; *Campylobacter pylori*

Description of the pathogen. It was first isolated from stomach biopsy material by Marshall and Warren (1983). Initially named *Campylobacter pylori*, it was subsequently renamed *Helicobacter pylori*. Genus: *Helicobacter*; Family: Helicobacteriaceae, division of proteobacteria.
Helicobacter pylori is a 3-4 x 0.5-1.0 µm, gram-negative, curved, helical rod bacterium, which is characterised by its high mobility due to its 3-7 unipolar flagella (2.5 µm long, Ø 30 nm). A range of virulence factors are involved in the pathogenesis. It has a characteristically high urease activity, which ensures its survival in the gastric mucosa by neutralising the gastric acid by splitting urea into ammonia and carbon dioxide. Pathogenicity factors VacA (vacuolating cytotoxin A) and CagA protein (cytotoxin-associated gene A), whose presence is associated much more strongly with secondary diseases (e.g. stomach cancer), have been the subject of intense research. The exclusive reservoir for *Helicobacter pylori* is the human stomach.

Diseases. *Helicobacter pylori* has been proven to be involved in the pathogenesis of chronic-atrophic gastritis, stomach and duodenal ulcers, adenocarcinoma as well as MALT lymphoma. Therefore, it was classified as a "category 1 carcinogen" by the WHO (World Health Organisation) in 1994.
More than half of the world's adult population carries *H. pylori* in their gastrointestinal tract. In industrialised nations, the infection rate amounts to 20-50%, while this increases to over 95% in some developing countries. *H. pylori* infections are treated with triple therapy consisting of two antibiotics and a proton pump inhibitor.

Analytics. Invasive test methods of gastroendoscopic biopsy material:

- Helicobacter rapid urease test: Biopsy material is analysed for urease activity via a change in the pH value by a change of colour of the indicator.; ideal for rapid primary diagnosis (urea/urease test).
- Histology: Microscopic detection of *H. pylori* in the gastric mucosa using HE staining (haematoxylin and eosin). Detection of pathological changes (activity and chronicity). Applications: Primary diagnosis and treatment monitoring.
- Culture: Cultivation of the bacteria on special culture media over 5-7 days, from freshly extracted gastric mucosa biopsies.
- PCR (polymerase chain reaction) method: Pathogen detection by analysing the DNA; antibiotics resistance-associated point mutations can be identified at the same time. This means that the resistance can be determined, even if conventional cultivation is not possible.

Non-invasive methods.

- Stool antigen test: Pathogens are detected via monoclonal antibodies in the stool. Suitable for primary diagnosis and treatment monitoring. Particularly the method of choice for young children.
- ^{13}C-urea breath test: Orally administered, labelled urea is split by bacterial urease. The $^{13}CO_2$-containing expiration air is collected after 10 minutes (50 mg ^{13}C-urea, Diabact UBT tablets) or 30 minutes (75 mg ^{13}C-urea, dissolved in a drink) and the relative rise in $^{13}CO_2$ compared to $^{12}CO_2$ from a breath sample before ingesting the ^{13}C-urea is measured.

δ value (‰) with 75 mg ^{13}C-urea/30 minutes	Evaluation
< 2.5	Negative
> 4	Positive
δ value (‰) with Diabact UBT tablets/10 minutes	
< 1.3	Negative
> 1.7	Positive

Suitable for primary diagnosis and treatment monitoring.

- Serology: Indirect immunofluorescence (immunofluorescence, indirect) and enzyme immunoassays have a low significance for diagnosis as direct detection, but are helpful if there is no indication for a gastroscopy and in the event of reduced colony density in the gastric mucosa. Specific antibodies against virulence factors CagA and VacA can be detected by immunoblot.

Sample material and sample stability. Direct detection and culture: Gastroscopic biopsy material is analysed. It must be stored in special transport media at +4 °C to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. Do not freeze the material!
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.
In Germany, a national reference centre deals with *Helicobacter pylori* infections.

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Herpes simplex virus type 1 and 2

W. STÖCKER

Synonym(s). HHV-1; human herpes virus 1; HHV-2; human herpes virus 2

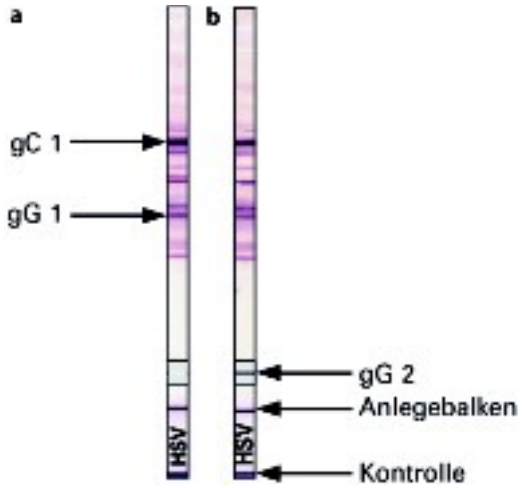
Description of the pathogen.

Family: *Herpesviridae* (enveloped viruses with a double-stranded, linear DNA as the genome); subfamily: *α-Herpesviridae*; Genus: *Herpes simplex virus;* Species: Herpes simplex virus 1 and 2 (HSV-1, HSV-2).
The term herpes (Greek: herpein = to creep) describes the spread of the herpes lesions on the skin.
The representatives of *Herpesviridae* are amongst the largest and most complex viruses with regard to their genome and their morphology. The herpes simplex virions (Ø 140–180 nm) contain an icosahedral capsid (Ø 100–110 nm). This is enclosed by about 20 tegument proteins and surrounded by an outer viral envelope. The exclusive reservoir for herpes simplex viruses are humans.

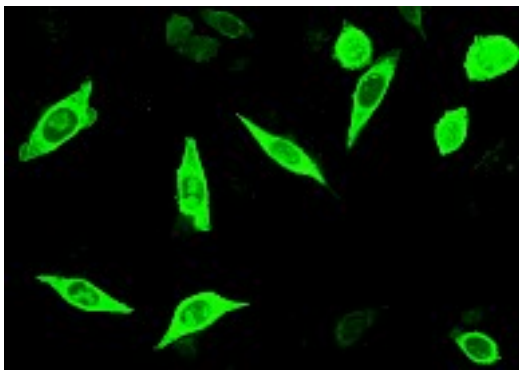
Diseases. Herpes simplex is characterised by blisters on the skin and mucous membrane. Herpes labialis is more commonly caused by HSV-1, while herpes genitalis is primarily caused by HSV-2. After an initial infection, which often passes without symptoms or as aphthous stomatitis, the virus always remains in the organism for its lifetime (persistent infection) in an inactive state (latency site: sensory nerve ganglia).
The disease is present worldwide and generally progresses without serious symptoms. Serious cases of herpes simplex encephalitis and herpes neonatorum very occasionally occur in newborns (infection in the birth canal). About 70-90% of the population is infected with HSV-1, while only 10-20% are infected with HSV-2. Several, primarily local virostatic agents are available to treat HSV, e.g. acyclovir and its derivatives.

Analytics. Direct detection: Classic virus isolation on cell cultures with subsequent typing via specific monoclonal antibodies is extremely time-consuming (cultivation over several days). Immunofluorescence tests for the direct detection of virus-specific antigens deliver a result within a short period of time, but only have a limited sensitivity and specificity. The detection of nucleic acid by polymerase chain reaction is the gold standard for diagnosing an acute HSV infection.
Serology: Detection of HSV-specific antibodies by complement fixation test (CFT), indirect immunofluorescence (immunofluorescence, indirect) as well as enzyme immunoassays. Virus type-specific serology with ELISA or immunoblots based on the glycoproteins C-1 and G-1 for HSV-1 as well as glycoprotein G-2 for HSV-2 are possible (Fig. 1; Fig. 2).

Sample material and sample stability. Direct detection and culture: Vesicle smears, suspected secretions (e.g. vaginal secretion), biopsy material, cerebrospinal fluid or EDTA blood are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. Material for virus isolation must be refrigerated for transport.



Herpes simplex viruses 1 and 2. Fig. 1. Combination lines (gG 2) with Western blot: Antibodies against herpes simplex viruses: a positive reaction for HSV-1, b positive reaction for HSV-2



Herpes simplex viruses 1 and 2. Fig. 2. Indirect immunofluorescence: Antibodies against herpes simplex viruses

Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The direct detection of the virus by PCR is an established method for diagnosing an acute HSV infection, especially in urgent cases such as herpes simplex encephalitis. Alternatively, a detected anti-HSV IgG seroconversion is considered evidence of an acute primary infection. Type-specific antibody detection by ELISA or immunoblot plays an important role in assessing the risk of herpes neonatorum during pregnancy as well as for epidemiological studies. A distinction between acute primary infections and a relapse is only possible by a combination of PCR and serology on a serum sample from the early stage of the infection.

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Description of the pathogen. Family: *Hepeviridae*, Genus: *Orthohepevirus*, Species: Hepatitis E virus (Orthohepevirus A), further differentiation into the human pathogenic genotypes 1-4 and into subgenotypes, positive-strand RNA genome

Diseases. Distribution: Global, differences in the epidemiology and symptoms depending on the genotype, HEV-3 is endemic in Germany and large parts of Europe.
Transmission: Zoonotic transmission by consuming insufficiently cooked meat of infected animals, especially domestic and wild pigs (main transmission path for HEV-3), faecal-oral via contaminated drinking water or contaminated food, infection via contaminated blood products is possible as well as person-to-person transmission by smear infection (main transmission path for HEV-1 and HEV-2).
Symptoms: Hepatitis E caused by HEV-3 often does not involve any symptoms, or mild and non-specific symptoms, such as fatigue, loss of appetite, nausea and vomiting or headaches, muscle and joint pains, hepatitides up to liver failure is possible; increased risk of a serious progression, especially in pregnant women and immunosuppressed persons; mortality with hepatitis E in the entire population: 0.5-4 %

Analytics. Culture: Virus cultivation, only has a low level of significance in laboratory diagnosis due to its time-consuming nature.
Direct detection: Detection of viral RNA by RT-PCR (polymerase chain reaction).
Serology: Detection of specific antibodies (IgA, IgG, IgM) in serum through indirect immunofluorescence or enzyme-linked immunosorbent assay.

Sample material. Direct detection: Blood, stool. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. As the clinical picture of hepatitis E is similar to hepatitis A as well as other hepatitides, laboratory diagnostic methods are extremely important for a diagnosis. Besides the direct detection of RNA in the blood or stool (confirms a fresh infection; method of choice for immunosuppressed persons), the serological detection of class IgA, IgG and IgM antibodies against HEV is the most important tool for confirming an infection. Pathogen-specific antibodies can generally be detected shortly after the commencement of clinical symptoms. A positive IgA and/or IgM test and a significant IgG titer rise in a serum pair, taken 8-14 days apart, indicate an acute infection. IgA and IgM anti-HEV titers generally decrease rapidly after an infection, while IgG anti-HEV titers can often persist for more than 10 years.

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HIV-1 and -2

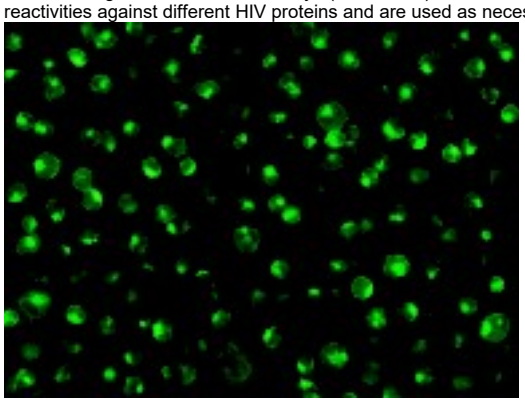
W. STÖCKER

Synonyms. Human immunodeficiency virus; AIDS virus

Description of the pathogen. HIV belongs to the family of retroviruses and to the lentivirus genus. The two known types are referred to as HIV-1 and HIV-2, their amino acid sequences are about 50% homologous. Both infections are similar with regard to their clinical picture, but the course of the disease is slower with HIV-2. HIV-1 and HIV-2 arose from different types of the simian immunodeficiency viruses (SIV) in certain monkey populations.
The virus particle has a diameter of 100–120 nm and is enclosed in a lipoprotein shell. Envelope glycoprotein complexes are embedded in the shell, which consist of an external part (gp120) and a transmembrane protein (gp41). Gp120 is crucial for the bonding of the virus to the CD4 receptors of the target cells. As the shell of the HIV consists of the membrane and the host cell, it contains, for example, molecules in HLA classes I and II as well as adhesion proteins. The capsid (core) with the viral genome, consisting of two copies of single-stranded RNA in a positive-strand orientation as well as reverse transcriptase (RT) enzymes and integrase, are located inside the virion.

Diseases. An infection with HIV may progress asymptomatically or lead to the currently still incurable AIDS (acquired immunodeficiency syndrome) after an incubation time of varying lengths, generally over years, which can manifest as an acute primary infection or as generalised lymphadenopathy. An infection with HIV is progressive, does not have any regression tendency without treatment and is generally fatal. The occurrence of opportunistic infections (thrush, mycobacteriosis, toxoplasmosis, etc.), malignant tumours and neurological diseases are characteristic of the stage of the immunodeficiency.
HIV is host-specific for humans. In chimpanzees, an infection with HIV leads to chronic viraemia, but not to the development of AIDS. Transmission of HIV is possible during homosexual and heterosexual contact, vertical transmission by the HIV-infected mother to the newborn, parenteral transmission via the blood or blood products, transplants, needle-stick injuries in the medical area as well as in drug addicts as a result of an infected injection tool. The infectivity of different bodily fluids varies depending on their virus concentration: The blood and sperm of HIV-infected persons contain large quantities of the virus, while other bodily secretions, such as saliva, tear fluid, urine or stool only contain low quantities. The virus concentration in the peripheral blood is particularly high immediately after an infection, before adequate antibodies have formed, and then decreases before rising once again in the late stages of the disease. Homosexual and heterosexual persons with frequently changing sexual partners, prostitutes and drug addicts are at particular risk and represent a potential risk of infection.
Towards 1980, an excessively promiscuous homosexual flight attendant contributed to the spread of HIV to over one hundred predominantly male persons within a year. The epidemic developed into a pandemic, which has led to the death of about 35 million people to date. Almost 37 million people are currently infected with the virus (2015). The prevalence differs considerably in the different regions of the world, 70% of those infected live in central and southern Africa.
Prophylaxis: A vaccine against HIV is not (yet) established. Prevention is therefore focussed on informing the population as well as the careful monitoring of blood reserves, plasma products and transplants. The antiretroviral therapeutic agents currently used, based on inhibiting virus-specific enzymes (reverse transcriptase and viral protease), can now extend the life expectancy of HIV-infected persons by years and even decades and also reduce the risk of transmitting the virus to other people. About 18 million HIV-positive people had access to antiretroviral therapies in 2016.

Analytics. Culture: Virus cultivation in stimulated lymphocyte cultures is only performed in exceptional cases, such as in the newborns of HIV-positive mothers, as, in this case, an infection cannot be diagnosed serologically due to the presence of maternal antibodies.
Direct detection: The detection of nucleic acid using reverse transcriptase PCR (polymerase chain reaction) is one of the most reliable analysis methods. If this is negative, either no HIV infection exists or the viral load is extremely low. The PCR is used for infected persons to monitor the progress and treatment of the disease and is also used in the blood donor service to analyse blood and plasma donations. A PCR is regularly also performed in patients when they are admitted to first-aid stations, if an acute HIV infection is suspected.
Serology: Third- or fourth- generation enzyme immunoassays are used as screening tests. All third generation enzyme-linked immunosorbent assays, luminescence immunoassays or microparticle enzyme immunoassays and their predecessors only detect antibodies against HIV-1 and -2, while fourth generation immunoassays (since 1999) also detect the capsid antigen p24 of HIV-1. Western blots (immunoblot) can indicate antibody reactivities against different HIV proteins and are used as necessary confirmatory tests (Fig. 1).



HIV-1 and HIV-2. Fig. 1. Indirect immunofluorescence: Antibodies against HIV

To also be able to detect HIV infections in countries with a poor laboratory infrastructure, home or rapid tests (home tests, point-of-care tests, bedside tests or rapid/simple test devices) have been developed, which are based on different immunodiagnostic principles, such as particle agglutination, immunodot (dipstick), immunofiltration or immunochromatography. They are also useful in situations in which an immediate result is necessary, such as when making a decision on post-exposure prophylaxis after needle-stick injuries (analysis of the source of the infection).

Sample material and sample stability. Culture: Whole blood, buffy coat, plasma, cerebrospinal fluid or tissue.
Direct detection: Whole blood, serum, plasma, tissue. Tissue and blood samples for the PCR must be sent to the laboratory within 6 h at 5–20 °C.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Screening tests and confirmatory tests have been developed for the HIV serology with the aim of identifying HIV antibodies, antigens or RNA. The aim of a screening test is to identify all infected persons with the highest possible sensitivity. Reactive samples are subsequently analysed in the (more time-consuming) confirmatory test with a higher specificity than the screening test.
In the event of a positive result in a screening test, it must be repeated and an antibody confirmatory test using Western blot or nucleic acid detection must be carried out. Based on a recommendation by the World Health Organisation (WHO), a “HIV-positive” diagnosis is made based on antibodies against at least two different virus proteins (e.g. gp160, p120, gp41, p55, p40, p24, p17, p66, p51, p32). The result must now be verified by a blood sample taken independently of the first test in order to rule out possible mix-ups.
“Third-generation immunoassays” were generally only able to reliably diagnose HIV infections after 12 weeks (“diagnostic gap”), as the organism requires several weeks to form specific antibodies as part of the immune response. Fourth-generation immunoassays generally enable the diagnostic gap to be shortened due to the simultaneous detection of the HIV-1 p24 antigen, which can be found in the blood even before the formation of the anti-HIV antibodies. However, it must be noted that the HIV-1 p24 antigen can only be detected in the body for ~4 weeks. This

normally does not represent a further disadvantage, as the period of detectability of the HIV-1 p24 antigen and the HIV antibodies overlap. However, in certain cases the p24 antigen can fall below the detection limit in infected persons while the HIV antibodies can still not be measured. A “second diagnostic gap” has therefore opened. When diagnosing HIV-2 infections using a fourth-generation immunoassay, the p26 antigen must be analysed rather than the p24 antigen.
A positive IgG antibody test cannot be evaluated to check for HIV infection in the newborns of seropositive mothers, as the IgG could stem from the mother and be diaplacentally transmitted to the child via the bloodstream. In this case, it may be possible to detect specific class IgA and IgM antibodies, as they cannot pass the placental barrier; but the established method of analysis for newborns and infants is virus detection using reverse transcriptase PCR.
The specificity of reverse transcriptase PCR amounts to almost 100% and, with a lower detection limit of 50 copies/mL, the method exceeds the specificity of all other HIV tests (95%). The method has the shortest possible diagnostic gap: Viral RNA can be detected after (on average) 11 days after a risk exposure.

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Human herpes virus type 6

W. STÖCKER

Description of the pathogen. The human herpes virus 6 (HHV-6), first described in 1986, was discovered during an attempt to isolate the HIV from the lymphocytes of an AIDS patient. It was therefore initially referred to as the human B-lymphocyte virus (HBLV). However, it was subsequently shown that the virus primarily reproduces in T-cells.
HHV-6 belongs to the *Herpesviridae* family (enveloped viruses with a double-stranded, linear DNA as the genome); subfamily: β-Herpesviridae; Genus: Roseoloviruses. The HHV-6 virions (Ø 200 nm) consist of an icosahedral capsid, which is enveloped by tegument proteins and an outer viral envelope. The exclusive reservoir for HHV-6 is the infected human. HHV-6 exists as species HHV-6A or HHV-6B, they have a homology of 90% at the nucleotide level, but cannot recombine with one another. The mode of transmission of HHV-6A remains unclear. HHV-6B is generally transmitted as a contact or droplet infection via the saliva. The rate of infection with HHV-6 in 2-year-old infants is approx. 95%.

Diseases. HHV-6A infections tend to be asymptomatic. HHV-6B is the aetiologic agent of exanthema subitum (roseola infantum or tertian fever), which is a classic childhood disease that almost exclusively affects children under 2 years of age. The disease is characterised by sudden high fever, while seizures can occur as a complication; either febrile convulsions, generally with a good prognosis, or encephalitis-mediated seizures due to the involvement of the CNS. Gastrointestinal and respiratory symptoms, swelling of the cervical lymph nodes and red spots on the gums and uvula (Nagayama spots) also occur but are less common. Once the fever recedes, after 3-4 days, a skin rash with fine spots or papules appears on the body and neck.
Treatment is symptomatic; in the event of serious neurological complications, treatment with ganciclovir, forscarnet or cidofovir is required.

Analytics.
Direct detection: Virus culture or detection of viral DNA by virus subtype-specific PCR (polymerase chain reaction).
Serology: Antibody detection by indirect immunofluorescence (immunofluorescence, indirect) or enzyme immunoassays.

Sample material and sample stability. Direct detection and culture: Cerebrospinal fluid and, occasionally, plasma are analysed. The material must be stored at +4 to +8 °C until further processing and analysed within 24 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Tertian fever generally does not require any laboratory diagnosis. In cases with unclear symptoms, tests for detecting IgG and IgM antibodies against HHV-6 can support the diagnosis. However, a distinction between HHV-6A and HHV-6B cannot yet be made. Attention must also be paid to cross-reactivities with HHV-7 and cytomegaloviruses. The virus culture is only prepared in special laboratories and is diagnostically insignificant. If encephalitis is suspected, the virus subtype-specific PCR with a cerebrospinal fluid sample has the highest significance.

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Human herpes virus type 7

W. Stöcker

Description of the pathogen. The human herpes virus 7, first described in 1990, reproduces in $CD4^+$-T lymphocytes and persists in the epithelial cells of the mouth's salivary glands. HHV-7 belongs to the *Herpesviridae* family (enveloped viruses with a double-stranded, linear DNA as the genome); subfamily: *β-Herpesviridae*. The HHV-7 virions (Ø 170 nm) consist of an icosahedral capsid, which is enveloped by tegument proteins and an outer viral envelope. The exclusive reservoir for HHV-7 is the infected human. The virus reproduces in salivary gland epithelia and is excreted in the saliva.

Diseases. HHV-7 is considered a possible cause of a range of different diseases. These include non-specific feverish viral diseases with exanthema; in rare cases also neurological symptoms, such as facial nerve paresis, meningitis and seizures. Moreover, a connection with pityriasis rosea has been described. In rare cases, exanthema subitum symptoms may also occur (human herpes virus 6).
The primary infection occurs in babies and infants (later than the HHV-6 infections); the prevalence in adults amounts to 95%. Symptomatic treatment of the primary infection. Ganciclovir displays a poor efficacy with HHV-7, in contrast to HHV-6, and is not recommended for treatment.

Analytics.
Direct detection: Virus culture or detection of viral DNA by virus subtype-specific PCR (polymerase chain reaction).
Serology: Antibody detection using enzyme immunoassays. ELISA methods are primarily described, whose antigens are extracted from virus cultures.

Sample material and sample stability. Direct detection: using PCR: Cerebrospinal fluid
Serology: Serum or cerebrospinal fluid. Patient samples for antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Laboratory diagnosis is generally not necessary for HHV-7 infections. The virus culture is only prepared in special laboratories and is diagnostically insignificant. If encephalitis is suspected, the virus subtype-specific PCR with a cerebrospinal fluid sample has the highest significance. Particular attention must be paid to the cross-reactivity with CMV and HHV-6 for antibody detection, as the β-herpes viruses share a large number of epitopes.

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Human herpes virus type 8

W. Stöcker

Description of the pathogen. The human herpes virus 8, first described in 1994, is also referred to as the Kaposi sarcoma-associated herpes virus (KSHV) due to its discovery in the tissue of Kaposi sarcoma. HHV-8 primarily infects B-lymphocytes, which are also the latency site.
HHV-8 belongs to the *Herpesviridae* family (enveloped viruses with a double-stranded, linear DNA as the genome); subfamily: γ-Herpesviridae; Genus: Rhadinovirus. The virions (Ø 140 nm) consist of an icosahedral capsid, which is enveloped by tegument proteins and an outer viral envelope. The exclusive reservoirs for HHV-8 are infected persons, who can also shed the virus asymptomatically.

Diseases. The primary infection of the oral cavity and pharynx with HHV-8 mostly progresses asymptomatically in people with a healthy immune system; fever or exanthema may occasionally arise. HHV-8 is aetiologically involved in the formation of Kaposi sarcoma, from which predominantly AIDS patients suffer. In addition, various rare lymphoproliferative diseases occur, such as primary effusion lymphoma (PEL) and a variant of the multicentric Castleman disease (MCD) appear to be associated with the virus. The virus is predominantly transmitted through saliva and by sexual contact. The rate of infection in Germany is low compared to other herpes viruses, at 1-8%, while the seroprevalence in Africa amounts to 50%.

Analytics. Direct pathogen detection using polymerase chain reaction (PCR). The immunohistochemical detection of HHV8-LNA (latent nuclear antigen) in formalin-fixed, paraffin-embedded material is also possible. Antibody diagnosis by indirect immunofluorescence (immunofluorescence, indirect) or enzyme-linked immunosorbent assay.

Sample material and sample stability. Direct detection and culture: Saliva, blood, as well as biopsies from Kaposi sarcoma are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or cerebrospinal fluid for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Saliva samples for direct pathogen detection display the highest sensitivity for detecting an infection with HHV-8. Biopsy material is required to diagnose HHV-8-associated diseases; a negative PCR from suspected tumour tissue provides evidence against a Kaposi sarcoma. A positive anti-HVV-8 IgG finding can confirm the suspicion of a HHV-8 infection or an associated disease, while a negative finding does not exclude an infection.

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Influenza viruses A, B and C

W. Stöcker, C. Krüger

Description of the pathogen. Family: *Orthomyxoviridae*, consists of the genera influenza virus A, B and C (species: influenza A virus, influenza B virus and influenza C virus) as well as isavirus, quaranjavirus and thogotovirus. Influenza A viruses occur in humans (serotypes H1N1, H2N2 and H3N2), in other mammals and a large variety exists in birds. Transmission between the different host species is possible and is significant for the creation of new virus variants. Influenza B and C viruses only occur in humans. Influenza viruses are characterised by a considerable genetic variability, which is based on a high mutation frequency and easy gene exchange. The resulting excellent antigen variability is a reason for the characteristic epidemiology of influenza.

Diseases. Influenza viruses are the pathogens of flu (influenza). The disease occurs in epidemics, whereby the severity of the individual epidemics can vary considerably. The influenza viruses and the resulting diseases are distributed worldwide. However, in contrast to the other virus types (especially A), influenza C viruses only rarely arise as pathogens of the viral flu, rather, they tend to cause bronchopneumonia. According to estimates by the World Health Organisation (WHO), 10-20% of the global population is affected annually.
Influenza viruses enter the body via the mucous membrane of the airways, mouth and eyes. They reach these access points through droplet, contact or smear infection, through hosts who catch the disease from faecal particles and vectors or through viruses on skin cells, hair, feathers and dust. Symptoms arise after an incubation time of 1-5 days, but the viruses can already be transmitted to other people 2 days prior to the occurrence of the first symptoms. As the signs of the disease, fever, chills, headaches and joint pain, coughing and nausea, are relatively non-specific, influenza can be confused with many other acute respiratory diseases. In any event, the rapid manifestation of the full extent of the disease is an important characteristic. The symptoms generally last for 1-2 weeks. However, symptoms, such as tracheitis, bronchitis and pneumonia, and complications, such as myocarditis, meningitis and encephalitis, as well as bacterial superinfections may arise. In its most serious form, influenza in a person who is already ill, who has a weakened immune system or in young people leads to primary pneumonia and death.
Seasonal influenza is an infectious disease with one of the highest levels of mortality. The Robert Koch Institute puts estimates of the annual number of influenza patients in Germany at between 1-7 million with about 20,000 deaths in years of severe flu outbreaks (e.g. winter 2012/2013). Global outbreaks occurred in 1889 (subtype A/H2N2), 1918 (Spanish flu, subtype A/H1N1), 1957 (Asian flu, subtype A/H2N2), 1968 (Hong Kong flu, subtype A/H3N2) and 1977 (Russian flu, subtype A/H1N1).
In principle, humans can be vaccinated against influenza and this is considered the most effective preventive measure. However, influenza A viruses are extremely adaptable, which is the reason why an annual immunisation with a current vaccine strain is necessary. Specific antiviral drugs are available to treat an infection with influenza viruses. If they are promptly administered, they can shorten the duration of the disease and mitigate critical complications in at-risk patient groups. Virostatic agents should only be administered in exceptional cases due to the possible development of resistance. Besides the specific treatment of influenza, patient conditions are generally only treated symptomatically.

Analytics. Culture: Influenza viruses are isolated from nasal, pharyngeal and bronchial secretion in the first few days after the start of the disease. Chicken eggs or dog kidney cells (MDCK cells) are used for cultivation. The isolate is identified using the haemadsorption assay (HADH), direct immunofluorescence or enzyme-linked immunosorbent assays.
Direct detection: Detection of the antigens in infected cells from nasal and pharyngeal secretion by direct immunofluorescence. The rapid test provides a result within 30 min. Influenza viruses can also be identified using reverse transcriptase PCR (polymerase chain reaction).
Serology: Serum antibodies are detected using enzyme-linked immunosorbent assays, indirect immunofluorescence (immunofluorescence, indirect), complement fixation tests, haemagglutination inhibition assays, neutralisation assays or complement fixation.

Sample material and sample stability. Direct detection and culture: Nasopharynx secretion, throat washings, throat smears and other human samples (PCR). The samples must be refrigerated for transport and analysed within 6 h (PCR) and 24 h (culture, direct immunofluorescence).
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The cultivation and typing of isolated viruses allows to confirm which influenza viruses (influenza A or B, serotypes) has led to the disease. As most people become infected with influenza viruses several times during their life, the detection of specific antibodies is not proof of a fresh infection. A retrospective serological diagnosis is possible via a significant (four-fold) titer rise within 1-3 weeks. The main applications of antibody measurements are vaccination titer controls as part of clinical tests.

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Japanese encephalitis viruses

W. STÖCKER

Description of the pathogen. Family: *Flaviviridae;* Genus: *Flavivirus*: Species: *Japanese encephalitis virus* (JEV); positive-strand RNA genome, enveloped, 50 nm diameter.

Diseases. Japanese encephalitis
Distribution: South-East Asia, China, Japan, Korea, Indian subcontinent, East Siberia, Australia (north-east).
Vectors: Arthropods: Various *Culex* species (esp. *Culex tritaeniorhynchus)*, *Aedes togoi*, *japonicus* and *vexans nipponii*, *Anopheles annularis* and *vagus*.
Hosts: Birds, livestock (esp. swine), reptiles, field mice, humans.
Transplacental transmission is also possible; infections via blood transfusion or organ transplants are conceivable, but have not been proven to date.
Symptoms: Sudden high fever and flu-like symptoms, meningoencephalitis; permanent neurological and psychological damage in 20-30% of patients, mostly in infants and the aged; mortality in case of meningitis of 10-30%.
Treatment and prophylaxis: Only symptomatic treatment is possible. An inactivated vaccine is available. Prevention: protection against mosquito bites, combatting the vectors.

Analytics. Working with the pathogen requires the use of a laboratory of safety class 3.
Direct detection: Virus detection from the blood or cerebrospinal fluid with RT-PCR (**7**polymerase chain reaction) or virus cultivation in cell culture.
Serology: Detection of specific antibodies (IgG, IgM) in serum or cerebrospinal fluid through indirect immunofluorescence, enzyme-linked immunosorbent assay, neutralisation assay or haemagglutination inhibition assay.

Sample material and sample stability. Direct detection and culture: Blood, blood components, cerebrospinal fluid or biopsy material are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C (cerebrospinal fluid, one week), or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Direct detection: Possible during the acute phase of the disease; generally only short viraemia (max. 1 week) due to the early occurrence of neutralising antibodies, isolation is difficult.
Serology: Detection of specific serum antibodies (IgG, IgM) shortly after the start of the disease. Anti-JEV IgM antibodies can be detected within a week in over 65% of patients. A significant titer rise in specific IgG also points to an acute infection. Attention must be paid to cross-reactions with other flaviviruses (7TBE, dengue, Zika, West Nile fever, yellow fever viruses, etc.). Vaccine-induced antibodies must also be considered.
Differential diagnoses: Other viral or bacterial meningitides and encephalitides.
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya virus, dengue viruses, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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Klebsiella pneumoniae

W. STÖCKER

Description of the pathogen. Family: Enterobacteriaceae; Genus: *Klebsiella* (*K*); Species: *K. oxytoca*, *K. planticola*, *K. terrigena*, *K. ornithinolytica*, *K. granulomatis*, *K. pneumoniae* (subspecies: *pneumoniae*, *ozaenae*, *rhinoscleromatis*).
Klebsiellae are gram-negative, immobile, short, plump and sporeless rod bacteria. They form a characteristic polysaccharide-containing capsule and grow aerobically or facultative anaerobically.

Diseases. Klebsiellae are ubiquitous opportunistic microorganisms. They occur in the nasopharyngeal cavity as well as in the intestine of humans and animals without causing any symptoms. In humans with a weakened immunity, such as with predisposing underlying diseases, especially in hospital patients, *K. pneumoniae* can cause serious diseases, including pneumonia (Friedlander's pneumonia), urinary tract infections, gastroenterocolitis, wound infections and septicaemia. These days, *K. pneumoniae* is one of the 6 most common pathogens of nosocomial infections in German intensive care and neonatal wards. It is primarily transmitted by contact with excretions (stool, urine, wound secretion) of hospitalised patients, predominantly as a result of inadequately disinfected staff hands or contaminated objects.
Outbreaks of multiresistant strains lead to particular therapeutic and hospital hygiene problems. *K. pneumoniae* is a β-lactamase producer and is therefore resistant against many broad-spectrum antibiotics, which are structurally based on a β-lactam ring (e.g. penicillins, cephalosporins). However, strains with an extended resistance spectrum (ESBL: extended spectrum β-lactamases) also exist. According to a study from 2001, they account for 8.2% of cases. Antibiotics to treat *Klebsiella* infections should therefore always be selected based on an antibiogram.

Analytics. *Klebsiella pneumoniae* can be detected directly in the blood by polymerase chain reaction or culture. They grow well on lactose-containing, selective and non-selective culture media (exception: *K. granulomatis*). Klebsiellae can be biochemically distinguished based on their metabolic products. The polysaccharide-containing capsule can be detected in the ink preparation and differentiated into about 77 capsule antigens with the capsule swelling reaction.
The increasing distribution of multi-resistant ESBL strains requires the use of sensitivity tests, especially for clinically relevant isolates. Appropriate methods include the modified agar diffusion test, the MIC differentiation assay and the microbouillon assay. The differentiated detection of the ESBL type is only possible using molecular biological methods (PCR).

Sample material and sample stability. Direct detection and culture: Sample material, such as urine, wound swabs, pus, airway secretion, puncture fluid, sputum, blood, cerebrospinal fluid, stool, intestinal biopsies or swab samples must be stored and sent in sterile tubes in an appropriate transport medium, potentially with the addition of sterile saline solution for protection against drying. It must be refrigerated for transport and processed within 4 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. An infection with *Klebsiella pneumoniae* must primarily be differentiated from other nosocomial infections. The most typical colony and capsule form in extraintestinal samples provides an initial indication of the pathogen. Direct detection (in blood or cerebrospinal fluid) is primarily relevant when diagnosing septicaemia. The analysis of intestinal and stool samples is less conclusive, as klebsiellae without any relevance to the disease can also be found in these areas. A diagnosis can be confirmed by serological and biochemical differentiation. Besides the established process for resistance testing, the detection of ESBL resistance types by PCR is constantly increasing in significance. The serological detection of antibodies only plays a secondary role in klebsiellae diagnosis (e.g. cystic fibrosis patients).

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Crimean-Congo fever virus

W. STÖCKER

Synonym(s). Crimean-Congo haemorrhagic fever virus (CCHFV)

Description of the pathogen. Family: *Bunyaviridae*; Genus: *Nairovirus*; Species: Crimean-Congo haemorrhagic fever virus. Single-stranded negative-strand RNA genome, three segments, enveloped, diameter of 80-120 nm.

Diseases. Crimean-Congo haemorrhagic fever.
Distribution: Eastern and southern Europe, Central Asia, Indian subcontinent, Africa, Middle East.
Vector: Ticks, especially hyalomma species.
Infection: Also possible as a result of contact with infectious bodily fluids and tissue or blood transfusion and organ transplants.
Hosts: Livestock and wild animals (ruminants, rabbits), humans. Risk groups: People working in agriculture or in slaughterhouses, campers, medical staff.
Symptoms: Sudden high fever and flu-like symptoms, abdominal pain, petechiae, haemorrhaging, hepatitis with liver failure, neuropsychiatric and cardiovascular changes, encephalitis. Mortality of 2-50%.
Treatment and prophylaxis: Only symptomatic treatment is possible; the oral or intravenous administration of ribavirin is effective in individual cases. No vaccine is available to date.
Prevention: Protection against tick bites, combatting the vectors, prevention of contact with infected individuals, strict isolation of patients.

Analytics. Working with the pathogen requires the use of a laboratory of biological safety class 4.
Direct detection: Virus detection from the blood with RT-PCR (polymerase chain reaction) or virus cultivation in cell culture.
Serology: Detection of specific antibodies (IgG, IgM) in serum: indirect immunofluorescence test (IIFT, immunofluorescence, indirect), enzyme-linked immunosorbent assay (ELISA), haemagglutination inhibition assay. In a newly developed IIFT, recombinant antigens are used to detect antibodies against the CCHFV glycoprotein (GPC) and the CCHFV nucleoprotein (N) of the virus membrane.

Sample material and sample stability. Blood or blood components, cerebrospinal fluid or biopsy material are analysed. The samples must be stored and transported at +4 °C to +8 °C. Direct detection: must beperformed within 24 h, cultures: must be prepared within 6 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The detection of both virus components as well as specific antibodies is part of a complete diagnosis – in certain phases of the disease, the presence of a specific infection can only be confirmed based on one of the two diagnostic principles. The reproduction in cell culture and the subsequent positive specific immune response as well as a reactive PCR confirm the presence of the virus. However, an infection can also not be ruled out in the negative case, especially as the human body forms specific antibodies, which neutralise the virus, within just a few days.
Direct detection: Possible during the first 5 days of the disease. Virus cultivation takes 4-7 days, has a low sensitivity and is only permitted in laboratories with the highest safety level.
Serology: Specific antibodies appear in the serum from the 6th day of the disease. Serum diagnosis also has epidemiological significance. Specific IgM antibodies can be detected 4 months after an infection, while specific IgG antibodies can continue to be detected for 5 years after infection. The use of recombinant proteins as target antigens increases the diagnostic competence for the detection of CCHFV-specific antibodies.
Differential diagnosis: Other viral haemorrhagic fever diseases [Rift Valley fever (Rift Valley viruses), dengue fever ([7] dengue viruses), Lassa fever, Ebola and Marburg fever], other infections, which can be associated with haemorrhaging (rickettsiosis, leptospirosis, louse-borne relapsing fever, malaria, meningococcal infections)
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya virus, dengue viruses, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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Description of the pathogen. Family: *Bunyaviridae;* Genus: *Bunyavirus;* Species: La Crosse virus. Negative-strand RNA genome, enveloped, 90-100 nm diameter.

Diseases. Distribution: USA
Vectors: Mosquitoes (*Ochlerotatus triseriatus*, Culex and Aedes spec.)
Host: Rodents (squirrels, chipmunks) are the natural reservoir, humans are the final host
Symptoms: La Crosse encephalitis (synonyms: Californian meningitis, Californian meningoencephalitis) is one of the most common viral encephalitides in children in the USA; symptoms are only displayed in a small percentage of cases (fever, headache, nausea, neck stiffness, lethargy, encephalitis, epilepsy, pareses), in which case long-term neurological consequences are possible (epilepsy, mental disorders, behavioural problems); the mortality rate is less than 1%.

Analytics. Direct detection: Detection of viral RNA by RT-PCR (polymerase chain reaction), virus cultivation
Serology: Detection of specific antibodies (IgM, IgG) in serum through indirect immunofluorescence (immunofluorescence, indirect), enzyme-linked immunosorbent assay (ELISA), or neutralisation assay.

Sample material. Direct detection: Blood and blood components, tissue or cerebrospinal fluid. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Direct detection of the virus is only possible during the viraemic phase, which is believed to be extremely short in humans (1-3 days). However, diagnosis is generally based on the detection of specific IgM or a significant rise in the IgG titer in an antibody detection system (indirect immunofluorescence, ELISA).
Differential diagnosis: Herpes simplex virus encephalitis

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Legionella

W. Stöcker

Classification. Family: *Legionellaceae*; Genus: *Legionella* (*L.*); Species: *L. micdadei*, *L. pneumophila* (*L. pneumophila pneumophila*, *L. pneumophila fraseri*, *L. pneumophila pascullei*), *L. longbeachae*, *L. jordanis*, *L. gormanii*, *L. dumoffii*, *L. bozemani*, etc.

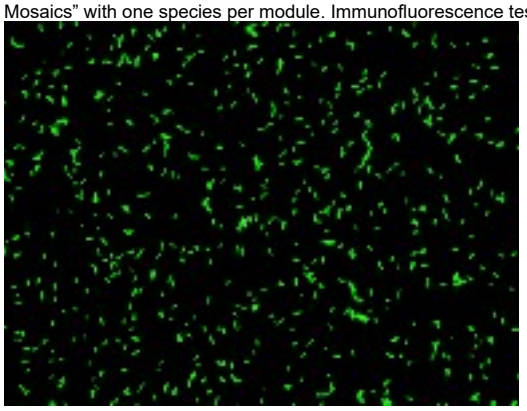
Description of the pathogen. Legionellae belong to the Legionellaceae family with only one genus: *Legionella*, which is currently comprised of about 57 species and 79 serogroups (SG). All legionellae are potentially human pathogens, but the majority of diseases are caused by *L. pneumophila* (90%), of which SG 1 is most frequently detected in environmental and patient samples. In addition to other known legionella species (*L. non-pneumophila*), *L. micdadei*, *L. longbeachae*, *L. jordanis*, *L. gormanii*, *L. dumoffii* and *L. bozemani* also exist.
Legionellae are gram-negative (they are more clearly displayed in Giemsa band staining) aerobically growing, capnophilic rod bacteria, which occur in fresh water, which form neither capsules nor spores. Most are mobile thanks to one or more polar or subpolar flagella (temperature-dependent). The inability to metabolise carbohydrates is characteristic of the *Legionella* genus; they are catalase-positive, some are oxidase-positive, and require cysteine for growth. The growth of legionellae is inhibited by physiological NaCl concentrations. They require water temperatures of 25-45 °C for reproduction, which predominantly occurs in amoeba and other phagocytic protozoa or, after infection, primarily in the macrophages. Temperatures ≥ 50 °C delay their development, while temperatures > 60 °C deactivate legionellae, which is important for hygiene measures.

Diseases. In their natural habitat, legionellae present virtually no health risk to humans. Only the spread of technical advancements, such as air-conditioning and hot water systems, in which the bacteria can easily reproduce, has led to their current significance as human pathogens. Legionellae are predominantly transmitted by inhaling aerosols of bacteria-containing water (shower), infection by aspiration is also possible. Sources of infection primarily include older and extended hot and cold water systems, cooling towers, humidifiers of air-conditioning systems, spas as well as respirators and inhalers. Two types of diseases dominate with legionellosis:

- Self-limiting Pontiac fever, which is similar to an influenza infection and primarily characterised by fever, coughing and muscle pains, and
- Legionnaire's disease, which is generally more serious and primarily associated with multi-focal necrotising pneumonia with additional symptoms, such as disorientation, drowsiness, diarrhoea or vomiting.

A distinction is made between nosocomial and out-patient Legionnaire's disease. Travel-related infections are special types of out-patient Legionnaire's disease.
Persons at increased risk primarily include older people, chronically ill people, persons with underlying cardiopulmonary diseases, defects in the cellular defence system, immunosuppressed patients after transplants, cytostatic treatment or permanent corticosteroid administration. Alcohol abuse and smoking are also considered risk factors. Men are affected more frequently than women. Only about 1% of exposed persons catch Legionnaire's disease. The infection dose, the virulence of the Legionella strain and the strength of the individual defence system are critical factors for the manifestation of the infection. In 2015, the Robert Koch Institute registered 880 cases of Legionnaire's disease in Germany. In particular, the contamination of hot water-conducting, aerosol-forming systems represent a significant health risk, which can be reduced by taking targeted measures (temperature > 60 °C, disinfection, renovation). The microbiological monitoring of water and air-conditioning systems is appropriate in facilities such as hospitals, while filters must be installed in particularly sensitive areas (intensive care wards).
There is no indication of a carrier stage, in which infected persons without symptoms can infect others, or in general for human-to-human transmission. Treatment is based on the administration of intracellular antibiotics, such as macrolide antibiotics (e.g. azithromycin) and quinolones (e.g. levofloxacin, ciprofloxacin, moxifloxacin)

Analytics. Different methods are used for direct pathogen detection: The direct immunofluorescence test with respiratory sample material based on monoclonal antibodies labelled with fluorescence dye. The polymerase chain reaction detects the pathogenic DNA in urine and serum. The ELISA antigen test and an immunochromatographic rapid test primarily identify *L. pneumophila* in SG 1 in the urine.
For cultural detection (gold standard) legionella must be cultivated on special culture media (BCYE, including supplemented, BMPA). However, cultivation is often unsuccessful and a positive result is only provided after 3–5 days. Specific serum antibodies are detected by indirect immunofluorescence (IIFT) (Fig. 1); enzyme-linked immunosorbent assay (ELISA) and microagglutination are other methods. Antibodies against *L. pneumophila* in SG 1-14 and another 6 *L.-non-pneumophila* species are generally analysed in parallel in one assay, e.g. with “BIOCHIP Mosaics” with one species per module. Immunofluorescence tests with antigen mixtures are suitable for screening.



Legionellae. Fig. 1. Indirect immunofluorescence: Antibodies against Legionella pneumophila

Sample material and sample stability. Direct detection and culture: Bronchial lavage fluid, lung tissue, tracheal secretion, pleural exudate, sputum, urine (especially in patients who do not produce enough sputum to yield results) and serum are analysed. The adsorption buffer must not contain any sodium chloride. The material must be stored at +4 °C to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The diagnosis of legionellosis (Legionnaires’ disease) is based on the clinical and radiological picture of pneumonia and the laboratory diagnostic pathogen detection. Since legionella are not part of the normal human bacterial flora, a positive finding indicates an infection. The direct immunofluorescence assay is inadequate as a stand-alone test due to the unsatisfactory sensitivity and possible specificity problems with the microscopic evaluation. The detection of legionella DNA using PCR is a quick and sensitive method, which also allows legionella species that are difficult to cultivate to be diagnosed. DNA detection is also possible in urine and serum.
The urine antigen test with an ELISA is dominant, accounting for more than 60% of tests, but is restricted to serogroup 1. The diagnostic gold standard remains the legionella culture. Despite a lack of sensitivity, it should at least be used for at-risk patients. The PCR and culture form an ideal basis for strain typing and epidemiological studies.
The standard test for the detection of antibodies is the IIFT. Titer > 1:100 are considered diagnostically relevant. A rise in the concentration of specific antibodies by a factor of 10 after 2-3 weeks is serologically indicative of a legionella infection. As the antibody formation partially occurs with a delay, a further sample should be analysed after 6-8 weeks where necessary. A single high titer is not evidence of a legionella infection, this is occasionally also found in the healthy population. Cross-reactivities between the individual species can occur. A negative antibody detection does not exclude a legionella infection. Serology is also of epidemiological importance. In Germany, a reporting obligation exists in accordance

with Section 7 (1) no. 26 of the German Infection Protection Act.

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Robert-Koch-Institut Berlin (2012) Epidemiologisches Bulletin 50:500-510

Leishmania ssp.

W. STÖCKER

Classification. Order: Kinetoplastida; Family: Trypanosomatidae; Genus: *Leishmania* (*L.*); Species: *L. donovani*, *L. infantum*, *L. tropica*, *L. major*, *L. aethiopica*, *L. chagasi*, *L. peruviana*, *L. braziliensis*, *L. panamensis*, *L. guyanensis*, *L. mexicana*, *L. amazonensis* and others.

Description of the pathogen. Leishmania belong to the Trypanosomatidae family, which is divided into nine genera, of which *Trypanosoma* and *Leishmania* are human pathogens. Besides *L. infantum* and *L. chagasi*, *L. donovani* also belongs to the pathogens of visceral leishmaniasis.
Leishmania are parasitic protozoa. Their cell morphology changes drastically during their two-stage life cycle: In the intestine of insect vectors (drain flies) they are present as promastigotes. After they are transmitted to the mammal host, they transform to amastigotes in the macrophages. Promastigotes have a spindle-shaped cell body 10-20 µm long, in which a 20 µm-long flagellum is anchored at the front end. By contrast, amastigotes are spherical cells with a diameter of just 2-4 µm and a much shorter flagellum, which remains concealed in the flagellar pocket.

Diseases. Depending on the type of leishmania, leishmaniasis manifests in three different main forms. Cutaneous leishmaniasis (CL) occurs in both the “old” (Africa, Asia and Europe) as well as the “new” world (America); it is the most common form of the disease, accounting for 75% of cases. The lesions remain localised at the point of entry and often heal spontaneously. Mucocutaneous leishmaniasis (MCL) is only found in the “new” world. The parasite enters the mouth, nose and throat via the lymph and blood vessels. The destruction of the mucous membrane and surrounding tissue leads to the severe disfigurement of the person suffering from the disease. If left untreated, MCL, generally triggered by super infections, can lead to death. The most serious manifestation is visceral leishmaniasis (VL), which is also referred to as kala azar or Dumdum fever. It is caused by *L. donovani*, *L. infantum* and *L. chagasi*. The parasite attacks the entire reticuloendothelial system, including the spleen, liver and bone marrow, and leads to spleno- and hepatomegaly, anaemia and weight loss - left untreated, patients die within 2 years. 90% of all VL cases registered worldwide occur in India, Nepal, Bangladesh, Sudan and Brazil.
No vaccination and no chemoprophylaxis against leishmaniasis currently exist. Prevention measures include wearing long clothing as well as the use of repellents and impregnated anti-insect nets. Pentavalent antimony compounds, amphotericin B, miltefosine and paromomycin are used as drugs. The treatment options are often limited due to severe side effects and the development of resistances.
Leishmaniasis is an anthropozoonosis, for which dogs and small rodents are often the reservoir hosts. Leishmania primarily occur in agricultural regions of the tropical and subtropical parts of the world. The distribution extends to all continents except for Australia. Of the 88 affected countries, 72 are developing countries. However, the parasites (*L. infantum* and *L. tropica*) are also found in 16 European countries, including France, Spain, Italy and Greece. Two million new leishmania infections are recorded every year, 12 million people currently suffer from the disease and 350 million people are at constant risk of infection. In Central Europe, the risk of infection is low, as drain flies of the *Phlebotomus* genus, which are used as vectors by leishmania in the “old” world, are scarce here. However, at-risk groups include travellers, migrants or refugees from endemic areas.

Analytics. Direct pathogen detection: Culture, microscopy and molecular biological methods (polymerase chain reaction).
Serology: Antibody detection by indirect immunofluorescence (immunofluorescence, indirect), enzyme-linked immunosorbent assay (ELISA) and immunoblot (Western blot). Rapid tests, amongst others, those based on particle agglutination, are available for field testing.

Sample material and sample stability. Direct detection and culture: Bone marrow puncture fluid, biopsy material (lymph nodes, liver, spleen) and citrate or EDTA blood (buffy coat) are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C (cerebrospinal fluid, one week), or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Visceral leishmaniasis is primarily diagnosed by direct pathogen or antigen detection. The microscopic direct detection of the generally amastigotic leishmania forms in Giemsa-stained smear or swab preparations is difficult. The morphological differentiation of the leishmania species is generally not possible. Culturing of leishmania on special cultivation media (e.g. NNN medium) is generally very successful, but can take up to 3 weeks depending on the type, starting content and division rate.
These days, molecular biological detection methods play a key role in leishmania diagnosis: The PCR is sensitive, specific and quick and enables the leishmania to be distinguished.
The detection of specific antibodies in serum is an established detection method and is used as a screening test where leishmaniasis is suspected. High antibody concentrations, whose diagnostic predictive value exceeds 90%, can particularly be found in immunocompetent patients after a visceral leishmania infection. However, a negative finding does not exclude an infection.
The indirect immunofluorescence assay is particularly sensitive and specific for the leishmaniasis serology, but does not permit a serological differentiation of the leishmania species. Cross-reactions have been described with Chagas disease, narcolepsy, malaria, leprosy and tuberculosis.
Differential diagnosis: Diseases associated with fever and splenomegaly, tuberculosis, malaria, brucellosis, typhus, bilharzia, miliary tuberculosis, mononucleosis, histoplasmosis and haemoblastosis.

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Listeria monocytogenes

W. Stöcker

Classification. Family: Listeriaceae
Genus: *Listeria* (*L.*)
Species: *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri, L. innocua*, *L.grayi*, *L. rocourtiae*

Description of the pathogen. The genus *Listeria* includes various species of which only *Listeria monocytogenes* is relevant as a human pathogen and, in very rare cases, *Listeria ivanovii* or *seeligeri*. *Listeria monocytogenes* can be divided into 13 serovars. Serovars 1/2a, 1/2b and 4b are primarily associated with human diseases.
Listeria are short, mobile (up to 28 °C), aerobic or facultative anaerobic, gram-positive rod bacteria. They are non-encapsulated, do not form spores and their reproduction is facultative intracellular. Their ability to grow, even at 4 °C, is used for selective enrichment.

Diseases. Listeria are unassuming bacteria that occur around the world. The host spectrum is diverse. *L. monocytogenes* is a facultative-pathogenic bacterium for animals (domestic and wild animals, rodents, birds, reptiles, fish, crustaceans, arthropods, etc.) as well as for humans. Transmission primarily occurs via contaminated food, such as cabbage, lettuce, raw milk products, meat and fish. Although listeria they widespread, they rarely lead to manifest listeriosis, which can have dramatic consequences, especially for pregnant women and their unborn children (connatal listeriosis), newborns, as well as immunosuppressed and old people. Professionally exposed persons, such as butchers or veterinarians also have a higher risk of infection. Besides uncharacteristic general infections, enteritis and local wound infection, serious courses of the disease with meningitis, encephalitis and septicaemia are possible. Infections during pregnancy can lead to miscarriages, premature births, deficiencies and stillbirths. In 2015, the Robert Koch Institute registered 662 cases of listeriosis. This included 22 cases of listeriosis during pregnancy, which involved the death of 5 children. The overall mortality amounted to 7%.
Contact with listeria is generally impossible to avoid, they reproduce in biofilms on the surface of various items of food, even at refrigerator temperature. However, the risk of an infection can be minimised by complying with hygiene standards during food production. At-risk patients should wash raw vegetables particularly thoroughly before eating and avoid consuming raw products, such as raw milk, unpasteurised cheese, salami, ground pork and seafood.
Listeria are sensitive to various antibiotics, such as amoxicillin, aminoglycosides, erythromycin, co-trimoxazole; however, cephalosporins and fluoroquinolones are ineffective. Due to the unclear symptoms, treatment is often started too late. In addition, the bacteria can partially evade the attack of antibiotics through intracellular reproduction in the host. Defence against the infection is primarily T cell-mediated and via macrophages.

Analytics. The direct detection of listeria in patient samples using molecular biology techniques (such as polymerase chain reaction) is possible, but cultural detection systems are mostly used. In a phase contrast microscope, listeria are identified by their staggering movement. Smears can be taken and prepared for gram-staining. Cultivation of the unassuming bacteria on blood and selective culture media is uncomplicated. Cold enrichment is helpful in samples with accompanying bacterial flora. The isolated strains are biochemically characterised and typed. *L. monocytogenes* can be distinguished from other listeria species by the sugar metabolism pattern (rhamnose +, xylose -) and the positive CAMP test.
Only *L. monocytogenes* displays a β haemolysis on blood agar and is positive in the CAMP test against β-haemolysin-producing *Staphylococcus aureus* (strong) and against *Rhodococcus equi* (weak). β haemolysis is an important pathogenicity characteristic with listeriolysin as the virulence factor. A classification into serovars is performed with oligoclonal specific antibodies against O and H antigens. The fast and reliable fine typing of isolates is possible using molecular biological methods.
The serum antibodies can be detected using the Widal test, the complement fixation test, indirect immunofluorescence (immunofluorescence, indirect) and other methods, but these are not helpful from a diagnostic perspective.

Sample material and sample stability. Smear and culture: Blood, cerebrospinal fluid, amniotic fluid, meconium, biopsy material and pus, vaginal secretion. Stool samples are less suitable, as listeria can also occur in healthy persons (~10 %). Due to their environmental stability, listeria do not have any specific conditions for sampling, transport and storage. Sample material can be transported at 4 °C and stored for up to 24 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C (cerebrospinal fluid, one week), or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. PCR direct detection is primarily used for identifying listeria in food and environmental samples. As a possible pathogen of meningitis, encephalitis, septicaemia or intrauterine infections in pregnancy, *Listeria monocytogenes* should be culturally identified and an antibiogram prepared for antibiotic treatment. Serology plays a secondary role for diagnosis. It is reportable in accordance with Section 7 of the German Infection Protection Act (FSG).

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Measles viruses

W. Stöcker

Description of the pathogen. Morbilli virus from the family Paramyxoviridae. The virus particles have a pleomorphic structure and are 110–250 nm in size. They contain an unsegmented, single-stranded RNA genome with a negative polarity. Together with nucleocapsid protein, phosphoprotein and polymerase, the RNA forms a helical ribonucleoprotein complex, which is enclosed in a lipid envelope. The inside is lined with a matrix protein, while the outer spikes consist of haemagglutinin and fusion protein. In contrast to other paramyxoviruses, the measles virus does not contain any neuraminidase. It only exists in a single serotype, with a high antigen stability. The virus is extremely sensitive to heat, light, UV radiation, detergents and disinfectants.

Diseases. Measles are a global, high-fever and serious infectious disease, which primarily occurs during childhood. Morbidity and mortality are particularly high in developing countries. According to the WHO, 14,400 people died from measles in 2015. In Europe, the number of measles cases has fallen sharply since the introduction of the vaccination, from 850,000 (1980) to just around 3,000 (2015). 6% of the reported cases were in Germany. However, local epidemics continue to occur. The only natural reservoirs of measles virus are humans. The transmission of the highly contagious pathogen occurs via droplet infection as well as via contact with nasopharyngeal secretion. Acute measles start after an incubation time of approx. 10 days with a catarrhal prodromal stage (fever, rhinitis, pharyngitis, coughing, conjunctivitis). The Koplik spots on the buccal mucosa are pathognomonic. The characteristic maculopapular measles exanthema occurs on the 14th-15th incubation day together with an increased rise in fever. It initially occurs behind the ears and on the face, quickly spreads to the entire body and subsides after 5-7 days. Generalised lymphadenopathy is generally present. In addition, measles lead to a transient weakening of the immune system and therefore to a predisposition for bacterial secondary infections with otitis media, bronchitis, pneumonia, myocarditis and diarrhoea. Meningoencephalitis only occurs in about 0.1% of patients with the disease, but this is fatal in one in five cases, while permanent brain damage is caused in a further 30%.
The most serious complications include acute postinfectious encephalitis (incidence: 1:1,000 measles cases) and the always fatal subacute sclerosing panencephalitis (SSPE; latency: 7-10 years; incidence: 7-11 per 100,000 measles cases). A measles infection during pregnancy can lead to abortion, premature birth or stillbirth.
Treatment is symptomatic. Active immunisation with attenuated vaccine is recommended, generally as a combination vaccine: measles, mumps, rubella and varicella (MMRV vaccine). Suspicion of the disease, cases of measles and death as a result of measles as well as the direct or indirect detection of the pathogen are reportable.

Analytics. Direct detection of the measles virus with RT-PCR (polymerase chain reaction), direct immunofluorescence or antigen ELISA. Cultures of monkey kidney cells are used for virus cultivation. A cytopathic effect (with syncytium formation) is observed in the positive case.
Serology: Antibody detection with enzyme immunoassays (enzyme-linked immunosorbent assay, chemiluminescence immunoassays), indirect immunofluorescence (immunofluorescence, indirect) using measles virus-infected culture cells, haemagglutination inhibition test, complement fixation test or neutralisation assay.

Sample material and sample stability. Direct detection and culture: The virus can generally not be directly detected. Nasal, pharyngeal and bronchial secretion as well as conjunctival fluid are analysed for differential diagnosis. The material must be stored at +4 °C to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Measles can often be diagnosed due to the typical clinical symptoms, sometimes in connection with the current epidemic situation. However, laboratory diagnosis is gaining in importance due to the increasing scarcity of the clinical picture. Rapid virus detection is possible in the early stage of the disease (start of exanthema) using RT-PCR. In the case of a positive RNA detection, the pathogen's genotype can also be identified, which allows the sources of infection and transmission paths to be determined and a distinction to be made between wild and vaccine strains. By contrast, virus cultivation is time-consuming and not very reliable. A negative result does not exclude measles for either RNA detection nor for virus isolation.
The most reliable marker of acute measles are virus-specific IgM antibodies. They can be detected in 50% of patients just 3 days after the start of exanthema and within 10 days in over 90% of patients. The positive detection of IgM should be confirmed with a virus-specific IgG test. A seroconversion or a significant titer rise in the specific IgG are considered evidence of a fresh infection. In case of doubt, the avidity of the specific IgG is analysed; if it is high, an acute measles infection is unlikely. If measles encephalitis is suspected, specific, intrathecally synthesised IgG antibodies are detected in the cerebrospinal fluid: SSPE patients display an extremely high IgG titer in serum and cerebrospinal fluid. The possibility of cross-reactions with other paramyxoviruses must be taken into account. Scarlet fever, rubella, Kawasaki disease and a drug eruption must be considered in differential diagnosis.

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Description of the pathogen. Family: Coronaviridae; Genus: Betacoronavirus; Species: Middle East Respiratory Syndrome Coronavirus; positive-strand RNA genome, enveloped.

Diseases. Distribution: The virus was first identified in Saudi Arabia in 2012 and has spread primarily on the Arabian Peninsula.
Transmission: The precise transmission paths are currently unknown; dromedaries as the intermediate host are the most likely source of the pathogen for humans, while the human-to-human transmission of the virus seems to be difficult.
Symptoms: The incubation time amounts to 1-2 weeks; the disease starts with acute, flu-like symptoms, similar to those of “severe acute respiratory syndrome” (SARS), while gastrointestinal complaints can also arise. Pneumonia can develop in severe forms of the disease. Possible complications are acute respiratory distress syndrome or kidney failure. About 36% of reported cases are fatal; this primarily relates to people with a weakened immune systems and chronic pre-existing conditions.

Analytics. Direct detection: Detection of viral RNA by RT-PCR (polymerase chain reaction)
Serology: Detection of specific antibodies (IgG, IgM) in serum through indirect immunofluorescence (IIFT; immunofluorescence, indirect), enzyme-linked immunosorbent assay (ELISA), or neutralisation assay.

Sample material. Direct detection: Smear from the airways, serum, stool, urine; the material must be stored at +4 and analysed within 72 hours.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The direct detection of MERS-CoV (PCR within 1-2 weeks of the start of symptoms) and the detection of antibodies against MERS-CoV (IIFT, ELISA, neutralisation assay) are the most important laboratory diagnostic methods for confirming suspected cases of a MERS-CoV infection. A 2-stage strategy (1st - screening using an ELISA or IIFT; 2nd - confirmation using a neutralisation assay) as well as an analysis of a serum pair is recommended for the serological detection. The acute serum must be taken about a week after the start of symptoms and the subsequent sample is taken at least 28 days after the start of symptoms. A seroconversion or an at least four-fold titer increase are adequate to confirm a MERS-CoV infection. If only a single sample can be analysed, the sample must be taken no earlier than day 14 after the onset of symptoms.

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MRSA

W. Stöcker

Synonym(s). Methicillin-resistant *Staphylococcus aureus*; ORSA; Oxacillin-resistant *Staphylococcus aureus*

Definition. *S. aureus* strains with acquired methicillin resistance.

Description of the pathogens. In general, *S. aureus*, within the staphylococci genus (gram-positive, facultative anaerobic coccus), are expressly pathogenic, but the pathogen also colonises the skin, especially in the area of the nasal atrium and the perineum, in about 20-50% of the healthy population. It causes three quarters of all wound infections, 50% of all osteomyelitis, 30% of all cases of septicaemia and endocarditis and 10% of all pneumonia.
The first MRSA strains occurred in 1961, shortly after the introduction of β-lactamase-resistant penicillins (methicillin, oxacillin). The methicillin resistance is based on the *mec* determinants, consisting of the mecA gene and regulatory sections, which are localised on a mobile genetic element, the "Staphylococcus cassette chromosome mec" (SCCmec). MecA codes for a modified penicillin binding protein PBP2A, which has a very low affinity for β-lactam antibiotics – methicillin-resistant staphylococci are therefore resistant to all β-lactam antibiotics (penicillins, cephalosporins and carbapenems).
MRSA often display multiple resistances against a range of different other groups of antibiotics, such as aminoglycosides, fluoroquinolones, macrolides and lincosamides, which is the reason why treatment options may be limited to a few reserve antibiotics (e.g. glycopeptides or newer substances, such as linezolid, daptomycin or tigecycline).
MRSA occur worldwide and play a key role as the pathogens of nosocomial infections. A distinction is made between *S. aureus* strains, which can be acquired during stays in hospitals or care facilities (healthcare-associated MRSA, haMRSA) and the strains acquired outside care facilities (community-acquired MRSA, caMRSA). The haMRSA prevalences vary significantly from country to country. While the percentage of MRSA of all analysed *S. aureus* isolates is below 1% in the Netherlands and in Scandinavia, due to a good hospital hygiene standard, south and Western Europe have high prevalence rates of over 40%. In Germany, the prevalence rose from about 8% to 20% from 1995 to 2001.
The community-acquired caMRSA strains are being increasingly reported in the past few years, they often have the virulence factor PVL (Panton-Valentine leucocidin), due to which they have an increased pathogenicity. MRSA infections are associated with a high morbidity and mortality as well as significant costs for care and treatment. Prevention is therefore considered extremely important.

Laboratory diagnosis. The diagnosis MRSA requires the relevant isolate as well as the *S. aureus* species diagnosis to be ensured and its oxacillin- or cefoxitin-resistance to be clearly verified. The analysis of methicillin resistance is made more difficult by the fact that its phenotypical expression in-vitro can only occur in a part of the bacteria population. This is not reliably shown by the standard antibiogram with oxacillin; cefoxitin test plates are better suited in this case. The reference method is the determination of the minimum inhibitory concentration (MIC) in accordance with DIN (58940) or CLSI (M100-S15, MIC testing). Alternative methods are screening tests using Müller-Hinton agar with 4% NaCl and 6 mg/L oxacillin or nutrient agar with the addition of cefoxitin and chromogenic substrate for alkaline phosphatase (DIN 58940-31).
A commercially available agglutination test enables the confirmation of the methicillin resistance by detection of PBP 2a. The gold standard is the molecular detection of the mecA gene using the polymerase chain reaction. Molecular test kits are now also available, which detect the mecA gene and also include the *S. aureus* species differentiation.

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Mumps viruses

W. Stöcker

Description of the pathogen. The mumps virus belongs to the Paramyxoviridae family. The virus particles are 150–200 nm in size and contain a single-stranded RNA genome with a negative polarity, which is enclosed by a helical capsid. The inside of the viral envelope is lined with a matrix protein, while the outer spikes consist of haemagglutinin-neuraminidase protein and fusion protein. At the genome level, a distinction can be made between several mumps strains, which differ in their biological characteristics, such as with regard to their neurovirulence.

Diseases. Mumps (parotitis epidemica) is a highly contagious disease that is endemic worldwide. Its prevalence has fallen sharply in Germany since the introduction of vaccination and waves of infections are now rare. It predominantly affects children and adolescents. The mumps virus is exclusively transmitted from person to person via droplet infection or direct contact and is primarily introduced via the mucosa in the oral cavity and the nasopharynx. More than a third of all mumps infections are asymptomatic. The disease starts after an incubation time of 16-18 days with non-specific prodromes (fever, headache, nausea, muscle pains, respiratory symptoms). The main symptom of the disease is a painful, single- or double-sided inflammatory swelling of the parotid gland, which lasts for 3-7 days. The involvement of the submandibular and sublingual salivary glands is possible. Complications may arise independent of the occurrence of manifest parotitis, especially in adults:

- serous meningitis,
- pancreatitis,
- orchitis,
- oophoritis,
- mastitis,
- occasionally, meningoencephalitis,
- inner ear hearing loss
- and deafness.

Diseases during pregnancy (especially in the 1st trimester) are a possible cause of spontaneous abortions, but not of foetal malformations. Mumps infections are exclusively treated symptomatically (analgesics, antipyretics, bed rest). Active vaccination with attenuated vaccine is recommended for prevention. A combined immunisation against measles, mumps, rubella and varicella (MMRV vaccine) is generally administered.

Analytics. Direct detection of the mumps virus is possible by RT-PCR (polymerase chain reaction) and direct immunofluorescence test. Virus cultivation takes place in embryonated chicken eggs or in cultures of monkey kidney cells (cytopathic effect with syncytium formation).
Serology: Antibody detection with enzyme immunoassays (enzyme-linked immunosorbent assay, chemiluminescence immunoassays), indirect immunofluorescence (immunofluorescence, indirect) using mumps virus-infected culture cells, haemagglutination inhibition test, complement fixation test or neutralisation assay.

Sample material and sample stability. Direct detection and culture: Throat swabs, saliva, blood, cerebrospinal fluid, urine and biopsies are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Diagnosis occurs in the event of typical symptoms based on the clinical picture (parotitis) and is only confirmed by laboratory analysis in the event of the atypical course of the disease. Direct pathogen detection and virus isolation are possible in the acute infection phase, but are only required in special cases (e.g. CNS manifestation).
Virus-specific IgM antibodies can generally be detected shortly after the start of the disease. A seroconversion or a significant IgG titer rise confirms a fresh infection. If the involvement of the central nervous system is suspected, the antibodies are simultaneously detected in cerebrospinal fluid and serum and the specific cerebrospinal fluid-serum ratio is calculated.
The control of the vaccination titer only makes sense 4 months after a mumps vaccination at the earliest, as this is when the vaccine-induced humoral immunity is fully established. This requires the use of reagents that contain antigens of the wild type as well as of the vaccine virus. The possibility of cross-reactivities with other paramyxoviruses must be taken into account. Parotitis swelling can also occur with other viral infections (e.g. influenza A, parainfluenza, Coxsackie, HIV, EBV), the build-up of secretion with salivary stones or tumours in the parotid gland. Therefore, these conditions must be considered in differential diagnosis.
A general reporting obligation exists for mumps diseases under the German Infection Protection Act.

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Description of the pathogen. Family: *Flaviviridae;* Genus: *Flavivirus;* Species: Murray Valley encephalitis virus, positive-strand RNA genome, enveloped; MVEV belongs to the Japanese encephalitis virus serocomplex

Diseases. Distribution: Australia, Papua New Guinea and Western New Guinea
Vectors: *Culex annulirostris*
Hosts: Water birds (virus reservoir), humans
Symptoms: A distinction is made between 2 courses of the disease. Mild course: Headache, fever, often also including neck stiffness, nausea, vomiting, diarrhoea. The symptoms stabilise within about a week. Serious course: Encephalitis with neck stiffness, sensitivity to light, cramps, seizures, respiratory insufficiency, paralyses, disturbances of consciousness, long-term neurological effects (behavioural disorders, personality changes, ataxias, signs of paralysis) in up to 50% of affected patients; encephalitis is fatal in 15-30%.
About 1 in 1,000 infected persons is seriously affected, especially infants.

Analytics. Direct detection: Virus isolation or detection of viral RNA by RT-PCR (polymerase chain reaction)
Serology: Detection of specific antibodies (IgM, IgG) in serum through indirect immunofluorescence (immunofluorescence, indirect) or enzyme-linked immunosorbent assay.

Sample material. Direct detection: Blood or cerebrospinal fluid. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The anamnesis, especially information on extended stays in endemic areas, is important. The detection of specific antibodies (IgM, IgG) is considered the standard method for diagnosing MVEV. A significant rise in the IgG titer is a reliable confirmation of an infection. Possible cross-reactivities with antibodies against other flaviviruses must be taken into account.

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Mycoplasma hominis

W. STÖCKER

Description of the pathogen. Mycoplasma are amongst the smallest self-reproducing bacteria. They do not have a rigid cell wall (murein deficit) and are therefore resistant against cell wall-active antibiotics. More than 12 species of the *Mycoplasma* genus have been found in humans, including *M. hominis*.

Diseases. *M. hominis* is predominantly found with urethritis, cervicitis and vaginitis. It occasionally causes mild bacteraemia (e.g. after births, gynaecological operations and abortions), wound infections, salpingitis, amnionitis and infections in newborns. Transmission primarily occurs via the genitals.

Analytics. Direct detection by nucleic acid amplification tests (e.g. polymerase chain reaction (PCR)). The pathogen can be cultivated on horse serum-containing special cultures within 4 days under anaerobic conditions, under CO_2- and N_2-containing gas mixtures.
Serology: Detection of antibodies against *M. hominis* by indirect immunofluorescence (substrate: *mycoplasma*-infected culture cells) or enzyme immunoassay.

Sample material and sample stability. Direct detection and culture: Smears or secretions of the urogenital tract are used as sample material. A sucrose phosphate buffer transport medium (SP2 medium) is used. It must be refrigerated for transport and analysed within 4 h. Rapid transport is required, as a reduction in the bacterial count by a factor of 10 must be assumed after just 24 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Diagnosis is based on the detection of a high bacterial count of the pathogen in the urogenital tract. Antibody tests for infections with *M. hominis* only play a limited diagnostic role due to the wide distribution of the pathogen as part of the commensal flora.

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Mycoplasma pneumoniae

W. STÖCKER

Description of the pathogen. Mycoplasma are amongst the smallest self-reproducing bacteria. They do not have a rigid cell wall (murein deficit) and are therefore resistant against cell wall-active antibiotics. More than 12 species of the *Mycoplasma* genus have been found in humans, including *M. pneumoniae*.

Diseases. *M. pneumoniae* is a species that is present worldwide and the pathogen of 15% of all community-acquired acute respiratory infections (tracheitis, bronchitis, primary-atypical pneumonia). Humans are the only reservoir; the pathogen is transmitted aerogenically via droplets. Epidemic spreads are possible. It particularly affects children and young people (prevalence of 40% in children below 5 years of age). In schools and military facilities, the prevalence can increase to up to 70%. One part of the infection occurs without symptoms and heals spontaneously without antibiotics.
M. pneumoniae can cause community-acquired pneumonia (CAP) and acute respiratory distress syndrome (ARDS). The pathogen is sensitive to macrolides and tetracyclines.

Analytics. Direct detection by nucleic acid amplification tests (e.g. polymerase chain reaction (PCR)). The culture requires a great deal of technical expertise and is only successful, if at all, on special media, whose main component is horse serum as the source of cholesterol. A negative culture result therefore has no influence on the therapy decision.
Serology: Detection of antibodies against *M. pneumoniae* by indirect immunofluorescence (infected culture cells as substrate) or enzyme immunoassay (incl. enzyme-linked immunosorbent assays and chemiluminescence assays).

Sample material and sample stability. Direct detection and culture: Nasopharyngeal secretion, sputum or bronchial lavage fluid. The material must be refrigerated for transport and analysed within 4 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Laboratory diagnosis plays an important role, as an infection with *M. pneumoniae* does not result in typical symptoms. The cultivation of the pathogen is difficult, time-consuming (6-15 days) and prone to errors. Pathogen detection by PCR is quick and reliable. The serology is most reliable if a serum sample from the acute stage of the disease and a sample, taken 2-3 weeks later, are analysed for specific IgM and IgG. The prevalence of the antibodies does not necessarily correlate to the detection of the pathogen, but the serology remains important for diagnosis and the therapy decision.

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Parainfluenza viruses

W. Stöcker, C. Krüger

Description of the pathogen. The parainfluenza viruses belong to the family *Paramyxoviridae* and the subfamily *Paramyxovirinae*. A distinction is made between 4 serotypes, which fall in two different genera. The human parainfluenza viruses 1 and 3 belong to the genus *Paramyxovirus*, while serotypes 2, 4a and 4b belong to the genus *Rubulavirus*.

Diseases. Infections with parainfluenza viruses primarily occur in infants. The infection rate in children up to 10 years of age is 90%. The viruses are present worldwide and all serotypes, except for serotype 4, occur frequently. Endemic as well as epidemic infections are possible.
They only known natural host are humans. Transmission occurs as a result of direct personal contact or via droplet infection. The incubation time amounts to 2-6 days. The viruses cause flu-like symptoms (parainfluenza). The lower respiratory tract is often affected, which results in feverish laryngotracheobronchitis, bronchitis, bronchiolitis or bronchopneumonia. In serious forms of the disease, pseudocroup can occur in infants, possibly with an allergic component. Further complications include otitis media and bacterial superinfections with pneumococcus, staphylococcus or *Haemophilus influenzae*. Parainfluenza infections can be fatal for immunocompromised patients with systemic diseases. Normal adults only develop a mild catarrh in the upper respiratory tract after infection. Symptomatic therapy to support the lung and cardiovascular function is indicated for serious forms of the disease.

Analytics. **Direct detection**: Antigen detection in infected cells of the respiratory tract is possible by direct immunofluorescence or enzyme-linked immunosorbent assay (ELISA). Parainfluenza viruses can also be detected by polymerase chain reaction (PCR) (reverse transcriptase PCR, RT-PCR).
Culture: Virus cultivation takes place on suitable cell cultures (monkey kidney and Vero cells) and identification occurs by testing various properties, such as haemadsorption, haemagglutination, haemagglutination inhibition, haemolysis, direct immunofluorescence or ELISA.
Serology: Serum antibodies against parainfluenza viruses are detected with ELISA, indirect immunofluorescence (immunofluorescence, indirect), complement fixation tests, haemagglutination inhibition assays, neutralisation assays or complement fixation.

Sample material and sample stability. **Direct detection and culture**: Nasopharynx secretion, throat washings, throat smears and other human samples (PCR). The samples must be refrigerated for transport and analysed within 6 h (PCR) and 24 h (culture, direct immunofluorescence).
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The virus culture and antigen detection are essential, as the serological diagnosis is impaired due to the wide distribution of parainfluenza viruses and due to cross-reactions between different paramyxoviruses. The specific IgM detection permits an early diagnosis, while a significant rise in the specific IgG within 1-3 weeks enables a retrospective serological diagnosis.

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Parvovirus B19

W. STÖCKER

Synonym(s). Fifth disease; erythema infectiosum

Description of the pathogen. Family: *Parvoviridae*; Genus: *Erythrovirus*

Disease. Parvovirus B19 infections cause local epidemics, primarily in the spring, and tend to occur in kindergartens and schools. The viruses are primarily transmitted via the respiratory tract, but can also be transmitted by blood or blood products as well as diaplacentally. About 30% of infections in children occur without symptoms, otherwise a non-specific prodromal stage with fever, headaches, nausea and diarrhoea is followed by characteristic exanthema ("fifth disease"). In all parvovirus B19 infections, the reticulocyte and haemoglobin values fall due to the destruction of erythrocyte precursor cells. Complications such as arthritis, persisting thrombo- and neutropenia, encephalitis, vasculitis and myocarditis occasionally occur. In general, the course of the disease is much more serious in adults than in children.
The seroprevalence in childbearing age amounts to 60-70%. Infections during pregnancy can be transmitted to the foetus, which has serious consequences, especially in the first 20 weeks of pregnancy: from the 10th week, the virus can reproduce in the pronormoblasts of the foetal liver and destroy it, which leads to anaemia and hydrops fetalis. The symptoms arise with a delay of about 2-6 weeks in the foetus, sometimes up to 18 weeks after the mother is infected. Severe anaemia in the foetus (Hb < 8g/dL) can be treated by blood transfusions.

Analytics. **Direct detection**: Direct virus detection by polymerase chain reaction (PCR) is possible about 2-3 days after contact with the virus. Neutralising antibodies eliminate the pathogen so that the PCR turns negative generally 3-4 weeks after infection in infected children. By contrast, in adults, the viraemia can persist for weeks and months. Viral DNA can occasionally be detected after eliminating the pathogen in various tissues, which makes it more difficult to clarify ambiguous clinical pictures.
Serology: Specific class IgM antibodies can be detected in the serum from about the 10th day after infection, generally associated with exanthema. A few days later, the class IgG titers against the viral proteins VP1 and VP2 also rise, which persist for the person's entire life. Almost all of the target antigens used for serological methods are based on recombinant viral structural and non-structural proteins, as it is difficult to efficiently cultivate parvovirus B19 in vitro. Besides 7enzyme immunoassays (enzyme-linked immunosorbent assays, chemiluminescence immunoassays), various blot systems (immunoblot) are used, which enable the parallel detection of antibodies against various virus proteins.

Sample material and sample stability. **Direct detection and culture**: Blood, saliva, amniotic fluid and chorionic villi biopsies are analysed. The material must be stored at +4 °C to +8 °C within 24 h until further processing. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The classic childhood parvovirus infection generally does not require diagnosis, as the course of the disease is asymptomatic or, at worst, bland. Exanthema suggests a clinical diagnosis, but it can easily be confused with rubella. If complications occur, diagnostic confirmation is obtained by serology and PCR. The analysis of blood reserves is useful in order to prevent infections caused by transfusions.
The ascertainment of immunity at the start of pregnancy is advisable as part of the pregnancy diagnostics. Seronegative patients are a risk group. After contact with persons suffering from the disease or if there are clinical indications of an acute infection, the diagnosis should always consist of a combination of serology (IgG, low-avidity IgG and IgM) and PCR, as the IgM titer can sometimes decrease rapidly. If an acute infection is diagnosed during pregnancy, the continuous monitoring of the foetus by Doppler sonography is indicated to promptly identify hydrops fetalis and, where applicable, treat this with intrauterine transfusions.

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Description of the pathogen. Family: *Togaviridae;* Genus: *Alphavirus*; Species: Eastern equine encephalitis virus. Positive-strand RNA genome, enveloped, 60-70 nm diameter.

Diseases: Distribution: From the east coast of the USA and Canada to the northern part of South America
Vectors: Mosquitoes (*Aedes* ssp., *Coquilletidia* ssp., *Culex* ssp., in birds *Culiseta melanura*)
Hosts: Birds (virus reservoir), horses, humans
Symptoms: High fever, nausea and vomiting; in about 6% of infected children and 2.5% of infected adults it leads to the development of encephalitis with muscle weakness and stiffness, reduced reflexes, neck stiffness, spasms, sensitivity disturbances, flaccid or spastic pareses, while personality disorders are also possible; convalescence can take years, long-term neurological damage is possible; the mortality rate in the event of encephalitis amounts to 30-75%.

Analytics. Culture: Virus cultivation
Direct detection: Detection of viral RNA by RT-PCR (polymerase chain reaction)
Serology: Detection of specific antibodies (IgM, IgG) through indirect immunofluorescence (immunofluorescence, indirect), enzyme-linked immunosorbent assay, or neutralisation assay.

Sample material. Direct detection: Blood and blood components, tissue or cerebrospinal fluid. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum, plasma or cerebrospinal fluid. Serum samples for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The anamnesis is important. Direct detection is only possible during the first acute days of the disease. Specific antibodies (IgG, IgM) are found in the serum or cerebrospinal fluid after a few days. A four-fold rise in the specific antibody titer is a clear indication of an acute infection.
Differential diagnosis: Infections with herpes viruses, Coxsackie viruses or other arboviruses that attack the CNS.

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Description of the pathogen. Family: *Togaviridae;* Genus: *Alphavirus*, Species: Venezuelan equine encephalitis virus. Positive-strand RNA genome.

Diseases. Distribution: Northern South America, Central America
Vectors: Mosquitoes (*Culex* ssp., *Aedes* ssp., *Haemagogus* ssp.)
Hosts: Various warm-blooded animals act as the virus reservoir (rodents: enzootic infection cycle, horses: epizootic infection cycle). Epidemics in horses are significant, as they may be caused by epidemics in human populations.
Symptoms: In most cases, infections are asymptomatic or with mild symptoms with slight headaches; serious courses of the disease with high fever, meningitis and encephalitis occur in about 1:100 cases; the mortality rate for these patients lies at 10%; long-term neurological damage is possible in survivors.

Analytics. Direct detection: Detection of viral RNA by RT-PCR (polymerase chain reaction), virus cultivation
Serology: Detection of specific antibodies (IgM, IgG) in serum or cerebrospinal fluid by enzyme-linked immunosorbent assays, radioimmunoassays or neutralisation assays, among others.

Sample material. Direct detection: Blood and blood components, tissue or cerebrospinal fluid. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The anamnesis and detection of specific antibodies (IgG, IgM) are of primary importance for a diagnosis. The use of special laboratories is recommended for the serological analysis.
Differential diagnosis: Infections with herpes viruses, Coxsackie viruses, dengue viruses or other arboviruses that attack the CNS.

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Robert-Koch-Institut, Berlin (2011) Steckbriefe seltener und importierter Infektionskrankheiten

Description of the pathogen. Family: *Togaviridiae*, Genus: *Alphavirus*, Species: Western equine encephalitis virus. Positive-strand RNA genome, enveloped, 60-70 nm diameter.

Diseases. Distribution: West coast of the USA and Canada as well as Mexico, Central and South America; rural areas
Vectors: Mosquitoes (*Culex* ssp., e.g. *Culex tarsalis*, in some cases *Aedes* ssp.)
Hosts: Birds (virus reservoir), horses, humans (secondary hosts); epidemics in horses are significant, as they may be caused by epidemics in human populations.
Symptoms: Western equine encephalitis is similar to Eastern equine encephalitis, but the course of the infection is generally milder. Severe courses are characterised by fever, nausea, vomiting, etc.; in about 2% of infected children and 0.1% of infected adults it leads to the development of encephalitis (muscle weakness/stiffness, reduced reflexes, neck stiffness, spasms, sensitivity disturbances, pareses); 3-7% mortality with encephalitis; convalescence can take years, long-term neurological damage is possible.

Analytics. Direct detection: Detection of viral RNA by RT-PCR (polymerase chain reaction), virus cultivation.
Serology: Detection of specific antibodies (IgM, IgG) in serum or cerebrospinal fluid through indirect immunofluorescence, enzyme-linked immunosorbent assay, or neutralisation assay.

Sample material. Direct detection: Blood and blood components, tissue or cerebrospinal fluid. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Direct detection is only possible during the first acute days of the disease. Specific antibodies (IgG, IgM) are found in the serum after a few days. Only a four-fold rise in the specific antibody titer is a clear indication of an acute infection.
Differential diagnosis: Infections with herpes viruses, Coxsackie virus or other arboviruses that attack the CNS.

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Robert-Koch-Institut, Berlin (2011) Steckbriefe seltener und importierter Infektionskrankheiten

Plasmodia

W. Stöcker

Description of the pathogen. Single-cell blood parasites.
Domain: Eukaryota; Phylum: Alveolata, Strain: Apicomplexa, Class: Haematozoea, Order: Haemosporidia; Family: Plasmodiidae; Genus: *Plasmodium*, Species: *Plasmodium falciparum*, *vivax*, *ovale*, *malariae*, *knowlesi* and others.

Diseases. Malaria tropica (*Plasmodium falciparum*), tertiana (*Plasmodium ovale* and *vivax*), quartana (*Plasmodium malariae*), *Plasmodium knowlesi* malaria.

Distribution:
P. falciparum: Worldwide in tropical and subtropical regions
P. vivax: Asia, Latin America and some regions of Africa
P. ovale: Africa, islands in the Western Pacific
P. malariae: Worldwide
P. knowlesi: South-East Asia
Vectors: Mosquitoes (*Anopheles* genus). Transmission, including transplacental, and via blood transfusion or organ transplantation is also possible.
Host: Humans
Symptoms: Attacks of fever with chills and outbreaks of sweating – with malaria tropica and *P. knowlesi* malaria, in short and irregular intervals; with malaria tertiana in a 48 h cycle; with malaria quartana in a 72 h cycle. In addition, clouding of consciousness up to coma, anaemia, splenomegaly, diarrhoea, pulmonary oedema, kidney failure. Due to the persistence of the pathogen in the blood, relapses are possible many years after a remission. A malaria infection during pregnancy leads to anaemia, premature birth or impaired maturity of the foetus. In some cases, the woman may not feel any symptoms during pregnancy.
Treatment and prophylaxis: These days, numerous drugs are available to treat malaria. Due to the constantly changing resistance situation, (in Germany) the current recommendations of the German Society for Tropical Medicine and International Health (www.dtg.org) with regard to prophylaxis and treatment should be followed.
No vaccination has proven to be effective to date. Prevention consists of avoiding mosquito bites, combatting the vectors and chemoprophylaxis. It is recommended that people obtain expert advice before travelling to an endemic region. The use of malaria prophylaxis does not exclude malaria.

Analytics. **Direct detection**: The parasites can be microscopically detected directly in the blood – in Giemsa-stained “thick film” (obsolete) or, much more sensitively and reliably, in a haematocrit tube, which is filled with whole blood and centrifuged directly after acridine fluorescence staining. The fluorescing parasites collect at the boundary layer between erythrocytes and plasma (buffy coat), where they are easy to identify. In addition, detection using PCR methods (polymerase chain reaction) is also possible and rapid malaria tests for the immunochromatographic detection of plasmodia-specific antigens (“histidine-rich protein 2”, lactate dehydrogenase, aldolase) are available.
Serology: Detection of specific antibodies in serum to identify dormant, asymptomatic and chronic infections as well as for screening blood reserves by indirect immunofluorescence and enzyme immunoassays.

Sample material/sampling conditions. **Direct detection**: Whole blood. The patient samples must be stored at +4 °C to +8 °C after they are taken and transported to the laboratory within six hours where possible.
Serology: Serum. Patient samples for antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. For the direct detection of the pathogen, the blood must be taken during a parasitaemic (fever) phase. PCR detection is useful for specific diagnostic questions (forensic analyses, epidemiological studies, genetic basis of resistances) and in case of infections with low parasitaemia. Rapid antigen tests are generally less sensitive and they also do not detect all plasmodium species.
The detection of antibodies against plasmodia is part of the serological differential diagnosis of tropical fevers. Many European blood donation organisations regularly test their reserves for anti-plasmodium antibodies. The latency between the time of infection and the reactivity in the antibody test must be taken into account.
Differential diagnoses: bacterial, viral and other parasitic fevers.

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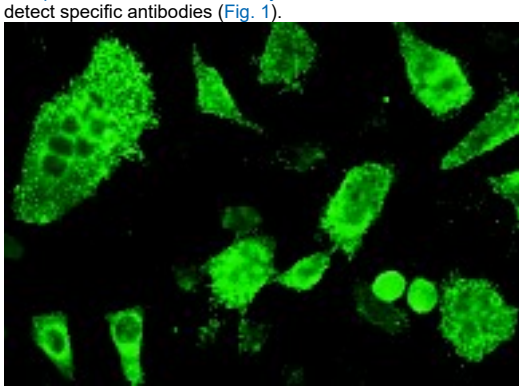
Respiratory syncytial virus

W. Stöcker

Description of the pathogen. The respiratory syncytial virus (RSV) is a polymorphic RNA virus that belongs to the *Paramyxoviridae* family. It was identified and characterised in 1956 by Robert M. Chanock. In the cell culture, RSV causes characteristic syncytia formation with eosinophilic cytoplasmic inclusions.

Diseases. RSV is the most prominent pathogen of recurring airway infections in babies and infants, such as croup, bronchitis, bronchiolitis or interstitial pneumonia. Half of the population has already survived an initial RSV infection in the first year of their life; everyone has been affected by the time they turn three. An outbreak of the infections with the highly contagious pathogen occurs every winter and is transmitted by droplets on the mucosae. Humans are the only RSV reservoir. Immunity only lasts for a short period, so reinfections are common. RSV is the most relevant cause of nosocomial infections in in-patient paediatrics.
Treatment is symptomatic; ribavirin as an aerosol is used in severe cases. Children with weakened immunity can be passively immunised with hyperimmune sera or monoclonal antibodies as prophylaxis.

Analytics. Virus detection is possible by polymerase chain reaction or enzyme and fluorescence staining, alternatively or additionally with a virus culture.
Complement fixation tests, enzyme-linked immunosorbent assays or indirect immunofluorescence (immunofluorescence, indirect) are used to detect specific antibodies (Fig. 1).



Respiratory syncytial viruses. Fig. 1. Indirect immunofluorescence: Antibodies against RSV

Sample material and sample stability. **Direct detection and culture**: Nasopharyngeal secretion is used as the sample material for virus detection. Fresh material, which has not been contaminated with other pathogens (e.g. fungi), is suitable for virus culture. It must be refrigerated for transport and analysed within 4 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Laboratory diagnosis plays an important role, as an infection with RSV does not result in typical symptoms. The RT-PCR as well as other direct detection methods are considered quick and reliable methods for the clinical practice. Virus culture requires specialists and is time-consuming, as the cytopathic effects can only be detected after a minimum of 4 days.
Antibody detection is more appropriate for epidemiological evaluations than for acute diagnostics. The complement fixation test is criticised due to its inability to distinguish between antibody classes. A suspected diagnosis can be confirmed by an increase in the concentration of the specific IgG within 2-3 weeks.

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Rift Valley fever virus

W. STÖCKER

Description of the pathogen. Family: *Bunyaviridae*; Genus: *Phlebovirus*; Species: *Rift Valley fever virus*
Negative-strand RNA genome, enveloped, 80-120 nm diameter

Diseases. Rift Valley fever
Distribution: Africa, Arabian Peninsula
Vectors: Mosquitoes (*Aedes* and *Culex* genera, sandflies).
Hosts: Ruminants (cattle, sheep), rodents, humans (risk groups: People working in agriculture or in slaughterhouses – risk caused by aerosols).
Transmission is also possible as a result of direct contact with bodily fluids or tissue of infected animals.
Symptoms: Sudden high fever and flu-like symptoms. In 1% of cases: Haemorrhaging and hepatitis, in rare cases meningoencephalitis, with a high mortality; as a late complication, uveo/retinopathy.
Treatment and prophylaxis: Only symptomatic treatment is possible to date; no vaccination is (yet) available in human medicine. Prevention of mosquito bites, combatting the vectors, avoidance of contact with infected animals. Vaccination of livestock.

Analytics. Working with the pathogen requires the use of a laboratory of safety class 3.
Direct detection of the virus in the blood or tissue with RT-PCR (polymerase chain reaction) or virus cultivation in cell culture. Serology: Detection of specific antibodies (IgG, IgM) through indirect immunofluorescence (immunofluorescence, indirect), enzyme-linked immunosorbent assay (ELISA), haemagglutination inhibition assay, complement fixation test (obsolete) and Western blot (immunoblot).

Sample material and sample stability. Direct detection: Blood and tissue samples are analysed. They must be stored at +4 °C to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The detection of virus components as well as specific antibodies enables complete diagnosis – in certain phases of the disease, the presence of a specific infection can only be confirmed based on one of the two diagnostic principles. The reproduction in cell culture and the subsequent positive specific immune response as well as a positive PCR confirm the presence of the virus. However, an infection can also not be ruled out in the negative case, especially as the human body forms specific antibodies, which neutralise the virus, within just a few days.
Direct detection is possible during the first 2-7 days of the disease. Specific IgM, which is formed from the 2nd-4th day can be reliably detected by indirect immunofluorescence. The IgM peaks about 2 weeks after the onset of the symptoms of the disease and remains present for 2-6 months. In the event of an infection, specific IgG does not appear before the 9th day of the disease. In cases of doubt, a significant rise in the specific IgG titer within 2 weeks is a clear sign of a fresh infection.
Differential diagnoses: Other viral haemorrhagic fevers (Crimean-Congo, dengue, Ebola, Marburg, Lassa haemorrhagic fever) and other infections that can be associated with haemorrhagic manifestations (rickettsiosis, leptospirosis, louse-borne relapsing fever, malaria, meningococcal infections).
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya virus, dengue viruses, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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Ross River viruses (RRV)

Description of the pathogen. Family: *Togaviridiae*, Genus: *Alphavirus*, Species: Ross River virus. Positive-strand RNA genome, enveloped, 60-70 nm diameter; closely related to the Barmah Forest virus

Diseases. Distribution: Australia
Vectors: Mosquitoes (*Aedes* ssp., especially *Aedes vigilax*, *Culex* spp., e.g. *Culex annulirostris*)
Hosts: Humans; wallabies and the Australian dusky rat (*Rattus colletti*) presumably act as virus reservoirs
Symptoms: Endemic polyarthritis; with about 5,000 registered cases every year, RRV infection is the most prominent arboviral infectious disease in Australia. An incubation time of 7-9 days may be followed by fever, arthralgia, often with arthritis, myalgia, skin rash and lethargy; more often than with BFV (Barmah Forest virus) infections, arthralgia and myalgia persist for longer than 6 months.

Analytics. Direct detection: Virus isolation or detection of viral RNA by RT-PCR (polymerase chain reaction)
Serology: Detection of specific antibodies (IgM, IgG) in serum through indirect immunofluorescence, enzyme-linked immunosorbent assay, or neutralisation assay.

Sample material. Direct detection: Blood and blood components, tissue. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The anamnesis, especially information on extended stays in endemic areas, is important. Direct detection of the virus is essentially possible, but serological tests for the detection of antibodies are preferred. Specific IgM antibodies are present in the majority of patients when symptoms start. A significant rise in the anti-RRV IgG titer, compared to the first sample, measures in a second serum sample (interval of approx. 2 weeks between the blood collections) is a reliable verification of an infection. Cross-reactivities with antibodies against co-endemic alphaviruses are possible.

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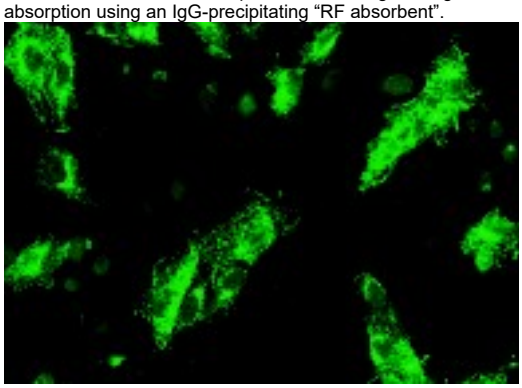
Rubella virus

W. Stöcker

Description of the pathogen. Family: *Togaviridae*; Genus: *Rubivirus*

Diseases. Rubella is transmitted via droplets. About 50% of infections in childhood are asymptomatic. The clinical image is associated with swelling of the nuchal and retroauricular lymph nodes as well as the typical spots up to maculopapular exanthema, which starts in the face and spreads to the body and extremities before disappearing after 1-3 days. Complications, such as encephalitis and arthritis, are reported with a prevalence of 1:6,000. Rubella during pregnancy leads to severe embryopathy, depending on the time of infection. Heart abnormalities, cataracts, inner ear hearing loss and spontaneous abortions occur in 90% of infections in the first 12 weeks of pregnancy (WOP). Individual manifestations are found in about 20% of cases in the event of infections in WOP 11-17. The risk of embryopathy is low after the 20th WOP. The seroprevalence in women of childbearing age amounts to 97%, while the incidence of rubella embryopathy is just 0.1/100,000.
Active immunisation with attenuated vaccines is possible as a preventive measure; these days it is combined in a simultaneous vaccination against measles, mumps and varicella. Initial immunisation takes place at 12-14 months of age and is repeated after a year. Boys should also be immunised against rubella in order to contain its prevalence.
In seronegative pregnant women, passive immunisation by administering a specific hyperimmunoglobulin is recommended as a post-exposure prophylaxis up to 48 h after contact with a rubella patient.

Analytics. **Direct detection**: Cultivation of the cell culture, after which the rubella antigens are immunocytologically displayed. The results of the real-time PCR (polymerase chain reaction) are available much more quickly and it has become the generally established method, especially for invasive prenatal diagnostics.
Serology: Detection of postnatal infections and determination of the immunity situation by haemagglutination inhibition assay (HIA), haemolysis in gel assay (HiG) or more modern test systems, which can be used to identify the immunoglobulin classes of the antibodies: indirect immunofluorescence (immunofluorescence, indirect, Fig. 1) or enzyme immunoassays (incl. enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassays). Principle of the HIA: Antibodies against rubella haemagglutinin contained in the serum prevent the agglutination of detection (chicken) erythrocytes when the antigen is added. Immunity can be assumed from a HIA titer of 1:32. The HiG is rarely used these days. In this case, the antibodies of a reactive patient serum induce the complement-dependent lysis of antigen-coated erythrocytes. The results of the ELISA are indicated in international units, immunity can be assumed from 15 IU/ml in the IgG. Blot techniques (immunoblot; immunodot) enable a separate detection of antibodies against rubella glycoproteins E1 and E2: Antibodies against the E2 protein are only formed late in the course of a disease; their presence rules out an acute disease.
Specific class IgM antibodies or low-avidity class IgG antibodies indicate an acute infection, while the sameholds true for an IgG titer rise within two weeks.
Before detecting the specific IgM antibodies, any existing specific IgG must be eliminated in order to counter false-positive reactions caused by rheumatoid factors and to prevent false-negative IgM reactions due to competing IgG. Separation is achieved by ultracentrifugation or by absorption using an IgG-precipitating “RF absorbent”.



Rubella virus. Fig. 1. Indirect immunofluorescence: Antibodies against Rubella virus

Sample material and sample stability. **Direct detection and culture**: Amniotic fluid, chorionic villi biopsies, abortion material, foetal EDTA blood, cerebrospinal fluid, pharyngeal smears and urine are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The diagnosis of an acute rubella infection is confirmed by detection of specific class IgM rubella antibodies and a significant rise of the IgG within two weeks. The detection of the avidity of the specific IgG is also significant. If the involvement of the central nervous system is suspected, the specific antibodies and the whole antibody are simultaneously detected in cerebrospinal fluid and serum and the specific cerebrospinal fluid-serum ratio is calculated. A value significantly > 1 indicates an intrathecal antibody synthesis.
Indirect immunofluorescence and enzyme immunoassays enable the immunoglobulin classes to be distinguished. They must therefore be preferred to the HIA and HiG assays.
EBV and parvovirus infections can have symptoms similar to rubella. A serological distinction sometimes also has to be made from other viral exanthema (HHV 6, measles, parvovirus B19) as well as drug eruptions and other allergic skin reactions.
Analytics during pregnancy help to prevent rubella embryopathies. The combination of PCR, detection of the specific IgM, and avidity determination of the specific IgG enable a congenital infection to be confirmed or ruled out at the prenatal or postnatal stage.
Acute rubella diseases are reportable in accordance with the German Infection Protection Act.

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Sandfly fever viruses

W. Stöcker

Description of the pathogen. Family: *Bunyaviridae*; Genus: Phlebovirus; Species: Sandfly fever virus, important serotypes: Sicilian (SFSV),

Naples (SFNV), Toscana (TOSV), and Cyprus (CYPV). Double and triple infections are common. Negative-strand RNA virus, enveloped, 80-120 nm diameter.

Diseases. Sandfly fever (pappataci fever).
Distribution: Mediterranean region to Southern China.
Vector: Mosquitoes (sandflies, especially *Phlebotomus papatasi*).
Hosts: Livestock (especially ruminants), rodents, bats, humans.
Symptoms: Most infections are subclinical. The disease starts with sudden high fever and flu-like symptoms, with TOSV and SFSV, it is often also associated with aseptic meningitis or meningoencephalitis with lymphocytic pleocytosis and specific intrathecal antibody production as well as neurological disorders and cephalgia, which persists for weeks or months. In rare cases also haemorrhaging.
Treatment and prophylaxis: Treatment is currently only symptomatic. No vaccine is available. Prevention: protection against mosquito bites, combatting the vectors.

Analytics. Working with the pathogen requires the use of a laboratory of safety class 3.
Direct detection: RT-PCR (polymerase chain reaction) or virus cultivation in cell culture.
Serology: Detection of specific antibodies (IgM, IgG) through indirect immunofluorescence, ELISA (enzyme-linked immunosorbent assay) or neutralisation assay.

Sample material and stability. **Direct detection and culture**: Blood components, cerebrospinal fluid or biopsy material are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C (cerebrospinal fluid only one week), or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Direct detection of the pathogen is only performed in exceptional cases. The reproduction in cell culture and the subsequent positive specific immune response as well as a positive PCR confirm the presence of the virus. However, an infection can also not be ruled out in the negative case, especially as the human body forms specific antibodies, which neutralise the virus, within just a few days.
Serology: Specific antibodies (IgG, IgM) appear in the serum from the 5th day of the disease. The IgG concentration peaks in the convalescence phase and persists for several years. A four-fold rise in the specific IgG titer within 2-3 weeks can be considered a clear indication of a fresh infection. Due to the significant genetic differences, immunity against a certain serotype does not protect against an infection by another. The use of BIOCHIP Mosaics with different serotypes is useful in indirect immunofluorescence and enables identification of the current serotype by comparing the reaction strengths.
Differential diagnoses: West Nile fever viruses, Rift Valley fever viruses, dengue fever viruses, influenza viruses
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya virus, dengue viruses, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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SARS coronavirus

W. Stöcker, C. Krüger

Synonym(s). Severe acute respiratory syndrome-associated coronavirus

Description of the pathogen. Family: *Coronaviridae*; Subfamily: *Coronavirinae*; Genus: *Betacoronavirus*; Positive-strand RNA genome, enveloped, 80-90 nm diameter.
The pathogen was newly identified in 2003. Due to the gene sequences, it is assumed that a known coronavirus either mutated or that a type of virus that previously only attacked animals “jumped” across to humans.

Diseases. SARS is an infectious disease caused by the SARS coronavirus (SARS CoV). The main symptoms of the disease are fever, pharyngitis, bronchitis, dyspnoea and pneumonia. The mortality rate amounted to 10%, or up to 50% for people over 65 years of age. The pathogen was primarily transmitted directly via droplet infection from a short distance or via contact infection. Infected animals, e.g. cockroaches, also transmitted the disease. The incubation time amounted to 2-7 days.
The single major outbreak of the disease was the SARS pandemic of 2002/2003 with 8,098 people falling ill and 744 deaths. The largest number of SARS cases was reported in Asia, where the virus is endemic. However, cases were also reported in North America and Europe. At the time, physicians initially administered the nucleoside analogue ribavirin as well as cortisone as virostatic agent. The patients then generally received a cocktail of different antibiotics in order to combat the accompanying bacterial infections of the respiratory tract.
As a preventive measure, those with the disease and contact persons were isolated, mass gatherings were cancelled for the public, cinemas and theatres were closed, hospital visits prohibited and everything was consistently disinfected.

Analytics. **Direct detection**: Detection of SARS coronaviruses by RT-PCR (reverse transcriptase PCR; polymerase chain reaction).
Culture : Virus isolation and reproduction in cell culture (e.g. vero cell culture).
Serology: Illustration of a seroconversion by indirect immunofluorescence (IIFT, Fig. 1) or enzyme-linked immunosorbent assay (ELISA).

SARS coronavirus. Fig. 1. Indirect immunofluorescence: Antibodies against SARS coronavirus

Sample material and sample stability. **Direct detection and culture**: Nasopharynx secretion, throat washings, throat smears and other human samples (PCR). The samples must be refrigerated for transport and analysed within 6 h (PCR) and 24 h (culture, IFT).
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The complete laboratory diagnosis of a virus infection includes the detection of virus components (direct detection) as well as specific antibodies in the serum. In a large part of SARS cases 2002/2003, the presence of an infection could only be confirmed with one of the two diagnostic principles in certain stages of the disease. At least two samples (different sample material or the same material taken on two different days) must test positive for viral RNA by RT-PCR for a positive SARS finding. Alternatively, a verified seroconversion using ELISA or IIFT, or a four-fold titer rise between 2 samples from the acute and convalescence phase, indicates an infection.

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Schistosoma spp.

Description of the pathogen. Class: *Trematoda* (flukes); Family: *Schistosomatidae*; Genus: Schistosoma (blood flukes). Species*: S. haematobium, S. mansoni, S. intercalatum, S. japonicum, S. mekongi*

Diseases. Schistosomiasis (bilharzia)

Distribution: Tropical and subtropical regions in Africa, Latin America as well as South West and South East Asia

S. haematobium: Africa, Near and Middle East

S. mansoni: Africa, Arabian Peninsula, South America, occasionally in the Caribbean

S. intercalatum: West Africa, regional in Cameroon, Gabon and in the Congo

S. japonicum: China, Philippines, Indonesia, occasionally in Japan

S. mekongi: Laos, Cambodia, Thailand

Host: Fresh water snails (intermediate host), the final host/natural reservoir are mammals and birds, among others
Transmission/development: Fertilised eggs enter the water with the stool or urine of the final host. The ciliated larvae (miracidium) hatch, actively enter the intermediate host and develop into sporocysts. The sporocysts give rise to hundreds of infectious cercaria that enter the water. They penetrate into the skin of the final host (penetration phase) and migrate into their bloodstream. Development to the sexually mature parasites and mating takes place in the final host.
Disease: The incubation time until cercarial dermatitis takes 6-48 hours. In particular, infections with *S. japonicum* and *S. mekongi* can lead to an acute feverish clinical picture after 2-8 weeks (Katayama fever with chills, coughing, and headaches). Symptoms of chronic schistosomiasis (incl. blood in the urine/stool, abdominal pains) can arise after years. The paired parasites settle in the veins, primarily in the bladder (*S. haematobium*), the mesentery (*S. mansoni, S. japonicum, S. mekongi*) or the rectum (*S. intercalatum*), but, in principle, all organs in the body can be attacked. With an increasing number of deposited eggs, the capillaries become occluded, which leads to chronic, inflammatory immune reactions against the eggs in the affected organs (bladder bilharzia, hepatolienal bilharzia, bowel bilharzia). As a consequence, granulomas up to fibroses can form.

Analytics. Direct detection: Detection of the eggs in the urine, stool or mucosa biopsies from the bladder or intestine; detection of the DNA by polymerase chain reaction in special laboratories
Serology: Detection of specific antibodies (IgM, IgG) in serum through indirect immunofluorescence (immunofluorescence, indirect) or enzyme-linked immunosorbent assay.

Sample material. Direct detection: Urine, stool, mucosa biopsy
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The patient's anamnesis can provide important information (trips to at-risk regions, contact with inland waters, occurrence of skin reactions). The direct detection of parasite eggs is possible after 4-10 weeks at the earliest. Eosinophilia can be detected in the haemogram at an earlier stage. The serology particularly plays an important role for travellers returning from endemic regions, as, in the case of an initial infection with Schistosoma ssp., the egg production is often intermittent and an infection can only be detected by antibody detection, including during the prepatency period (approx. 3 monthsWith successful treatment with praziquantel, a decrease in the antibody titer can be measured after about 6-12 weeks. Cross-reactions with antibodies against other parasites are possible.
Differential diagnoses:
Cercaria dermatitis: Allergies.
Acute schistosomiasis: Typhus abdominalis, malaria, brucellosis, amoebiasis, ancylostomiasis, lymphatic filariasis, trichomonads, visceral leishmania.
Chronic schistosomiasis: Different types of bladder, intestinal and liver diseases depending on the localisation.

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Description of the pathogen. Family: *Togaviridae;* Genus: *Alphavirus*, Species: Sindbis virus; Subtypes: Ockelbo virus, Babanki virus; positive-strand RNA genome, enveloped

Diseases. Distribution: South Africa, Egypt, India, Philippines, South East Asia, Central Asia, Russia, Australia, northern Europe.
Transmission: Mosquitoes (ornithophile *Culex* ssp., especially *C. pipiens* and *C. torrentium*, *Aedes* ssp., etc.); various species of birds, including migrating birds, serve as the reservoir.
Symptoms: Sindbis fever, febrile illness with headaches, arthritis/arthralgia, exanthema, which lasts for about a week. Joint complaints only persist in a small number of patients.

Analytics. Culture: Virus cultivation
Serology: Detection of specific antibodies (IgA, IgG, IgM) in serum through indirect immunofluorescence (immunofluorescence, indirect), enzyme-linked immunosorbent assay (ELISA), radioimmunoassays, neutralisation assay or haemagglutination inhibition assay

Sample material. Direct detection: Blood or blood components. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Also in Germany may the Sindbis virus be the cause of fever accompanied by skin rash and joint pains. Direct detection of the virus from the blood is possible during the first few days of the illness. Specific antibodies (IgM, IgG) can be detected from the 8-10th day after the symptoms arise. Cross-reactions with antibodies against related viruses are possible.
Differential diagnosis: Viral infections with joint involvement (e.g. dengue and chikungunya fever), which also occur in the infection areas, potentially rheumatoid arthritis and reactive arthritis.
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya virus, dengue viruses, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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Description of the pathogen. Family: *Flaviviridae;* Genus: *Flavivirus;* Species: St. Louis encephalitis virus. Positive-strand RNA genome, approx. 40 nm diameter

Diseases. Distribution: North America, Caribbean, Central and South America.
Vectors: Mosquitoes (various *Culex* species)
Hosts: Birds (virus reservoir), humans (final host)
Symptoms: Over 99% of patients show no or only mild symptoms. If it leads to meningoencephalitis (often in older patients) or encephalitis, this is initially associated with fever, headaches, neck stiffness, confusion and drowsiness. The subsequent course of the disease may lead to tumours, spontaneous nystagmus, myoclonia, rigor, paralyses and ataxia up to a coma. Further complications: Bronchopneumonia, gastrointestinal bleeding. The mortality rate in young patients is below 5%, but increases to 15% depending on age. Long-term neuropsychiatric consequences, such as fatigue, forgetfulness and concentration disorders are possible.

Analytics. Direct detection: Detection of viral RNA by RT-PCR (polymerase chain reaction), virus cultivation
Serology: Detection of specific antibodies (IgM, IgG) in serum through, e.g., indirect immunofluorescence or enzyme-linked immunosorbent assay.

Sample material. Direct detection: Blood plasma, cerebrospinal fluid or tissue. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. In the USA, SLEV are one of the most common causes of viral encephalitis epidemics. Direct detection of the virus shortly after infection is possible, but difficult. Detection of specific IgM antibodies is possible from the 3rd-5th day of the disease; attention must be paid to cross reactions with antibodies against other flaviviruses (TBE, yellow fever, dengue, West Nile, Zika, Powassan viruses, etc.). A four-fold rise in the specific antibody titer in a second serum sample is a clear indication of an infection.
Differential diagnosis: West Nile fever, other bacterial or viral CNS infections with meningitis or encephalitis.

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Tetanus

W. Stöcker

Description of the pathogen. *Clostridium tetani* is present worldwide as a gram-positive, obligate anaerobic, mobile rod bacterium from the Bacillaceae family. It forms spores, which are extremely resistant to heat, drying and disinfectants. After infection, *Clostridium tetani* reproduces very quickly in the low-oxygen wound environment and produces two exotoxins, haemolytic tetanolysin and the highly potent neurotoxic tetanospasmin (LD_{50} approx. 1-2 ng/kg). The latter prevents the release of inhibiting neurotransmitters and therefore blocks the inhibition of spinal motor neurons. This results in an increase in the tonicity, hyperexcitability of the musculature and cramps.

Diseases. Tetanus (lockjaw) arises from wound infections. Morbidity and mortality are particularly high in warm and humid countries with low vaccination rates as well as poor medical care. Globally, the disease is responsible for over a million deaths every year. In Germany, 10-15 cases occur every year, primarily in adults. The pathogen (*Clostridium tetani*) is ubiquitous in the intestines of many animals, as well as in the soil and dust, and penetrates into the skin through wounds of any kind, e.g. wood splinters, nails, thorns or bite injuries, secondary infection after burns and via the navel. The incubation time takes between 3 days and 3 weeks, in some cases up to several months. Short incubation times (high toxin level) are typical of serious courses and a high mortality.
Generalised tetanus is associated with spasms in the mimic facial musculature (risus sardonicus), the jaw musculature (trismus) as well as the neck and back musculature (opisthotonus). Painful tonic-clonic cramp attacks occur, which are caused by minor stimuli and are experienced in full consciousness. The paralysis of the diaphragm and the intercostal musculature can lead to suffocation. Left untreated, the mortality rate is extremely high, but can be reduced to 10-20% with adequate treatment. The less common local form of tetanus only leads to muscular rigidity in the wound area, but not to general cramps. In newborns of inadequately immunised mothers, navel infections lead to tetanus neonatorum.
Following the outbreak of tetanus, a high dose of tetanus immunoglobulin is administered as soon as possible in order to neutralise the circulating toxin. This is joined by wound excision, antibiotic treatment and active immunisation. Intensive therapy (sedation, muscle relaxation and artificial respiration) is often required. The most important prevention measure is active immunisation with tetanus toxoid. In the event of a negative or unclear vaccination status, simultaneous active and passive immunisation is recommended after injuries. No reporting obligation exists for tetanus.

Analytics. Microscopically, *Clostridium tetani* is displayed as gram-positive, mobile rods with terminal endospores (drumsticks). However, the analysis of microscopic direct preparations in tetanus diagnostics is not sensible. The cultural detection of the pathogen is rarely successful and is practically meaningless for a diagnosis. Cultivation takes place on supplemented cultivation media (liver/thioglycolate broth, blood agar) under anaerobic conditions at 37 °C.
The diagnosis is based on the detection of the toxin using an in-vivo neutralisation test in animal testing (mouse). To do so, mice are injected with wound material, patient serum or culture filtrate. The toxin leads to typical symptoms (tetanus of the back legs) and to the death of the untreated mouse within a few days, while immunised comparison animals do not display any symptoms under otherwise identical treatment.
In serology, antibodies against the tetanus toxin are detected using enzyme-linked immunosorbent assays (ELISA), for example to check the vaccination status.

Sample material and sample stability. Direct detection, culture and toxin detection: Wound material and serum are analysed. The material must be stored at +4 °C to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C (cerebrospinal fluid only one week), or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Tetanus is primarily diagnosed by the clinical picture as well as the injury and vaccination history. Toxin detection in animal testing (currently) remains the method of choice to confirm a diagnosis. The quantitative determination of the IgG antibodies against the tetanus toxin using ELISA is considered a standardised test method and is primarily used to check the immune status. Hypercalcaemic tetany, rabies, meningitis, brain tumours and strychnine poisoning must be considered in terms of differential diagnosis.

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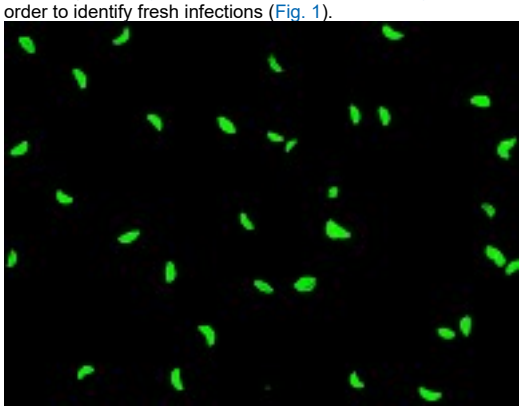
Toxoplasma gondii

W. Stöcker

Description of the pathogen. Protozoan; Class: Sporozoa, Strain: Apicomplexa.
Toxoplasma gondii was first discovered as a parasite in the gundi in Tunisia in 1908. Due to its crescent shape, it was named *Toxoplasma* (Greek, toxon: bow) by its discoverers Nicolle and Manceaux.

Diseases. *T. gondii* is present worldwide and infects numerous farm and wild animals. The parasite reproduces vegetatively and forms cysts. A generative cycle in the intestine only occurs in felines, e.g. house cats, which can lead to the excretion of oocysts in the faeces.
Humans can be infected by the oral ingestion of oocysts or by consuming cyst-containing, inadequately heated meat. Laboratory infections when handling infectious material are possible; this is theoretically also true for a transmission via blood transfusion at the time of parasitaemia.
Postnatal infections of immunologically healthy persons are only symptomatic in 10% of cases. Local lymphadenopathies are the most common symptom. In immunosuppressed patients, the dissemination of the pathogen can severely damage individual or multiple organs, in the event of a latent toxoplasma infection, antibiotics may need to be administered throughout the person's life to prevent a reactivation.
In the event of an initial infection in pregnancy, a diaplacental transmission to the foetus may occur, but only in rare cases in the event of a reactivation of a latent infection.
The probability of transmission during pregnancy depends on the time of the initial infection: In the first trimester, this amounts to 15%, in the second 30% and in the third 60%. Infections in the first trimester cause more serious damage to the child than infections at a later time during pregnancy. Manifest connatal toxoplasmosis is reflected in a low birth weight, hepatomegaly, feeding problems, cerebral seizures, development retardation or squinting. The classic triad of hydrocephalus, cerebral calcification and retinochoroiditis is found in about 5% of cases. Without antibiotic treatment, retinochoroiditis can develop over time with deterioration in the visual acuity up to blindness. In more favourable cases, infected newborns initially seem unaffected, but symptoms may still occur after an interval of several years.

Analytics. **Direct detection**: Attacked lymph nodes are histologically analysed. These days, the pathogens can be cultivated in culture, but the PCR method (polymerase chain reaction) is commonly used, especially if an infection is suspected during pregnancy. The sensitivity amounts to up to 65%.
Serology: The Sabin-Feldmann test analyses the neutralising property of the serum compared to the cultivated toxoplasma. The immunosorbent agglutination assay (ISAGA) detects class IgA and IgM antibodies with a high sensitivity and specificity: This method uses the µ-capture technique to isolate the antibodies, which then agglutinate the intact toxoplasma in the positive case. Both methods involve the handling of *toxoplasma* cultures. Various enzyme immunoassays, which can separately analyse class IgA, IgG or IgM antibodies against toxoplasma, have become very popular (incl. enzyme-linked immunosorbent assay, chemiluminescence immunoassays). In addition, low-avidity specific IgG can be detected in order to identify fresh infections (Fig. 1).



Toxoplasma gondii. Fig. 1. Indirect immunofluorescence: Antibodies against Toxoplasma gondii

Sample material and sample stability. **Direct detection and culture**: Amniotic fluid, biopsies of chorionic villi, brain tissue or heart muscle, EDTA blood, bronchoalveolar lavage fluid, aqueous humour and cerebrospinal fluid are analysed. The material must be stored at +4 °C to +8 °C until further processing. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. In the few symptomatic *toxoplasma* infections in adults with full immunocompetence, diagnosis primarily serves to distinguish it from other infections. In case of reactivations, as can occur in immunosuppressed patients, drugs (incl. spiramycin, pyrimethamine, sulfadiazine) are available, which can be administered after a corresponding diagnosis. However, they are only effective for the vegetative form, not for cysts.
Antibody detection in early pregnancy or even beforehand enables seronegative women to be identified and a seroconversion can be immediately identified, where applicable. The prompt diagnosis of a new infection during pregnancy can have a considerable effect on the fate of the child, as it is possible to administer effective antibiotics during pregnancy. Seronegative pregnant women are encouraged to take measures to prevent exposure.

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Treponema pallidum

W. STÖCKER

Definition. The name Treponema (Greek: turned thread) describes the characteristic movement of the thin screw bacterium, which was given the attribute “pallidum” (Latin: pale) by its discoverers Fritz Schaudinn and Erich Hoffmann (1905) due to its low colourability.

Description of the pathogen. The *Treponema* genus contains gram-negative, helical wound bacteria (6-14 windings) from the *Spirochaetaceae* family. Endoflagella enable the specific motility (rotation around the longitudinal axis) of the spirochaetes.

Diseases. Confirmed human pathogens are: *T. pallidum subspec. pallidum* (syphilis) as well as the pathogens of non-venereal treponematoses: *T. pallidum subspec. endemicum* (endemic syphilis, bejel), *T. pallidum subspec. pertenue* (yaws) and *T. carateum* (pinta).
Syphilis or lues (Latin for plague) is a general infection that occurs worldwide and is predominantly transmitted by sexual contact. This is followed by incubation, primary affection, generalisation and organ manifestation. Clinicians divide the disease into different stages. The early stages of primary syphilis (lues I) and secondary syphilis (lues II) can be followed by latent syphilis (lues latens), which may be dormant for many years. An untreated infection transfers to the late stages of tertiary syphilis (lues III) and neurosyphilis (quaternary stage, lues IV). The diaplacental transmission of the pathogen in pregnancy leads to lues connata, which is subdivided into the *praecox* (newborns and infants) and *tarda* stages(children > 3 years).
The therapeutic agent of choice for all stages of the disease is penicillin. A pathogen resistance has not yet been identified, but treatment failures are sometimes observed.

Analytics. **Direct detection and culture**: The direct detection of *Treponema pallidum* can be attempted from the primary affection (syphilis: coarse, indolent, attack of regional lymph nodes) with the insensitive, but specific dark-field microscopy. An alternative, more sensitive pathogen detection is direct immunofluorescence using fluorescence-labelled, monoclonal antibodies. A diagnosis using polymerase chain reaction is still faced with certain issues. The cultural detection of *Treponema pallidum* on artificial culture media has not been successful to date.
Serology: Detection of pathogen-specific antibodies in serum or cerebrospinal fluid by indirect immunofluorescence (immunofluorescence, indirect), enzyme immunoassays, immunoblot assays and the haemagglutination inhibition assay.

Sample material and sample stability. **Direct detection**: Stimulus secretion from lesions (primary affection). The material must be analysed as soon as possible (dark-field microscopy) or stored at +4 °C to +8 °C until further processing. It must be analysed within 4 h.
Serology: Serum, plasma or cerebrospinal fluid for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Laboratory diagnosis is focussed on antibody detection. The step-by-step diagnosis involves: Screening, confirmation and, in the event of a positive result, assessment of the activity. Screening tests are the *Treponema pallidum*-specific particle immunoassay (TPPA) or the haemagglutination assay (TPHA). Principle: Latex particles or erythrocytes charged with treponema antigens agglutinate when positive patient sera are added. However, they are increasingly being replaced by enzyme immunoassays (incl. enzyme-linked immunosorbent assay, chemiluminescence immunoassays), which also provide information on the immunoglobulin classes of the specific antibodies.
Reactive results in the screening test can be confirmed by immunoblots on which the specific antigens of *Treponema pallidum* are displayed separately. Indirect immunofluorescence (immunofluorescence, indirect) is also considered a confirmatory test, in which the patient serum is displaced with lysed *Treponema phagedenis extract*, which neutralises non-specific reactants (“FTA-Abs”). Before detecting the specific IgM antibodies, any existing specific IgG must be eliminated in order to counter false-positive reactions caused by rheumatoid factors and to prevent false-negative IgM reactions due to competing IgG. Separation is achieved by ultracentrifugation (19S IgM FTA-Abs test) or by absorption using an IgG-precipitating “RF absorbent”. Alternatively, the μ-capture method can be used, in which the sample’s IgM is selectively bound to a surface with the specific test antigen prior to the reaction.
In the case of a positive confirmatory test, the disease activity must be assessed. In laboratory diagnosis, this can take place via a quantitative lipoid (cardiolipin) antibody flocculation reaction [formerly “Wassermann reaction”, now VDRL (“venereal disease research laboratory”) test, among others]. Lipoid antibodies are not specific for a *Treponema pallidum* infection, but are used as an activity marker and are helpful for assessing the course of the disease. A reduction by a factor of 10 or more under treatment indicates the curing of the infection.
If the involvement of the central nervous system is suspected, the specific and whole antibodies are simultaneously detected in cerebrospinal fluid and serum and the specific cerebrospinal fluid-serum ratio (cerebrospinal fluid/serum IgA ratio; cerebrospinal fluid/serum IgG ratio; cerebrospinal fluid/serum IgM ratio) is calculated. A value significantly > 1 indicates an intrathecal antibody synthesis and therefore neurosyphilis.

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Description of the pathogen. Genus: *Trypanosoma;* Species: *Trypanosoma cruzi*, single-cell flagellate

The single-cell parasite *Trypanosoma cruzi* (*T. cruzi*) is the pathogen of Chagas disease (American/South American trypanosomiasis, Chagas disease). The pathogen is transmitted to mammals and humans via a host change from 3-4 cm assassin bugs (Reduviidae).

Diseases. Chagas disease
Distribution: Particularly in Central and South America, especially rural areas
Transmission: Contamination of small skin lesions or mucosae (especially in the eye) with pathogen-containing faeces of assassin bugs; wild and domestic animals as the pathogen reservoir; human-to-human transmission via blood transfusions and organ transplants as well as transplacentally is possible.
Symptoms: Acute symptoms in 30-40% of infected persons, often in children; Chagas disease passes through several stages:
1. Local swelling after a bug bite at the pathogen's entry site, often near the eye (Romana's sign).
2. Acute phase after a few days, with fever, breathlessness, oedema, diarrhoea, abdominal pains, swelling of the lymph nodes, seizures (brain participation) and cardiac enlargement. The mortality rate with cardiac or cerebral complications in this phase amounts to 5-10%.
3. Largely asymptomatic latency phase which can last for several years.
4. With chronic disease (10-30% of those infected), this leads to manifestations, especially on the heart (cardiac enlargement, heart failure, conduction system disorders) and on the digestive tract (destruction of nerve cells, megaoesophagus, megacolon). Central nervous symptoms occur in rare cases.
It is estimated that 7 million people are infected with *T. cruzi* worldwide, while over 10,000 people die from Chagas disease every year.

Analytics. Direct detection: Microscopic detection of the pathogen in the blood (stained blood smear); detection of the pathogen DNA by polymerase chain reaction (PCR); xenodiagnosis (laboratory-cultivated pathogen-free assassin bugs receive a blood meal on the patient's skin; after 2-4 weeks, the faeces of the assassin bug is analysed for the pathogen)
Serology: Detection of specific antibodies (IgG) in serum through enzyme immunoassays (enzyme-linked immunosorbent assay, chemiluminescence immunoassay), indirect immunofluorescence (IIFT; immunofluorescence, indirect)

Sample material. Direct detection: Blood or blood components. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. In the acute phase, the parasite can be detected microscopically or by PCR. In the latent and, in particular, in the chronic phase of Chagas disease, the detection of the IgG against *T. cruzi* in serum is significant for diagnosis; enzyme immunoassays or IIFT are primarily used for this purpose. Chagas disease is curable if it is promptly diagnosed and treated.
Differential diagnosis: Typhoid fever, influenza, visceral leishmaniasis, malaria, brucellosis.

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Ureaplasma urealyticum

W. STÖCKER

Description of the pathogen. The *Ureaplasma* genus of the Mycoplasmataceae is amongst the smallest self-reproducing bacteria. They do not have a rigid cell wall (murein deficit) and are therefore resistant against cell wall-active antibiotics. A human pathogenic species is *Ureaplasma urealyticum*.

Diseases. *Ureaplasma urealyticum* settles in the urinary and genital tract, e.g. urethra, vagina, cervix, prostate or epididymis. Infections often remain asymptomatic. The involvement of the pathogen in ascending chorioamnionitis, which can sometimes lead to abortions or premature births, has been observed. In particular, respiratory infections and meningitis caused by *Ureaplasma urealyticum* are occasionally observed in underweight newborns.
The lower genital tract is infected with *Ureaplasma urealyticum* in 40-80% of women and 5-20% of men. Transmission occurs via sexual contact and during birth.
The antibiotics of choice are tetracyclines and macrolides.

Analytics. The pathogens can be cultivated anaerobically or under CO_2 on horse serum-containing special cultures within 1-5 days. Identification takes place based on the morphology of the microcolonies and urease detection.

Sample material and sample stability. Direct detection and culture: Smears or secretions from the urogenital tract are used as sample materials. A sucrose phosphate buffer transport medium (SP2 medium) or commercially available, pre-filled indicator transport media are used. Rapid transport is required, as a reduction in the bacterial count by a factor of 10 must be assumed after just 24 h.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The diagnosis of *U. urealyticum* infections is based on the detection of a high bacterial count of the pathogen in the urogenital tract. Antibody tests only have a low diagnostic value due to the widespread distribution of the pathogen as part of the commensal flora.

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Description of the pathogen. Family: *Flaviviridae;* Genus: *Flavivirus;* Species: Usutu virus, positive-strand RNA genome, enveloped; USUV belongs to the Japanese encephalitis virus serocomplex.

Diseases. Distribution: The USUV originates from Africa; it presumably first occurred in Europe in 1996; in 2001 it was connected to the sudden death of a large number of birds in Austria and has since spread on the continent.
Vector: Mosquitoes (primarily *Culex pipiens*, also *Aedes albopictus*)
Hosts: The main hosts are birds, but also mammals, especially humans, horses and rodents; blackbirds display the highest mortality from USUV infections amongst birds.
Symptoms: Patients infected with USUV often suffer from fever and skin rashes. Neurological manifestations are also possible in immunosuppressed persons. The world's first acute neuroinvasive USUV infection (USUV meningoencephalitis) was diagnosed in Italy in 2009 by detecting viral RNA in a cerebrospinal fluid sample.

Analytics. Direct detection: Detection of viral RNA by RT-PCR (polymerase chain reaction)
Serology: Detection of specific antibodies (IgG, IgM) in serum through enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IIFT; immunofluorescence, indirect). Cross-reactions with antibodies against other flaviviruses are described.

Sample material. Direct detection: Blood or cerebrospinal fluid. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. Direct virus detection is only possible a few days after the onset of the disease, during the short viraemic phase. USUV-specific antibodies can subsequently be detected by IIFT and/or ELISA. Cross-reactions with antibodies against other flaviviruses must be taken into account, so a parallel screening for antibodies against various flaviviruses of significance for differentiation purposes (such as per IIFT BIOCHIP Mosaic) can make an important contribution to providing a diagnosis. The patient's anamnesis (vaccinations, trips to endemic areas) can provide important additional information.
Differential diagnosis: Tropical febrile illnesses, such as West Nile fever, dengue fever, yellow fever, chikungunya fever, Japanese encephalitis, sandfly fever or malaria.
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya virus, dengue viruses, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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Varicella zoster viruses

W. STÖCKER

Synonym(s). VZV

Description of the pathogen. Family: *Herpesviridae*; Subfamily: *Alphaherpesviridae*; Genus *Varicella zoster virus*.
VZV is also referred to as human herpes virus 3 (HHV-3).

Diseases. Varicella (chickenpox) and herpes zoster (shingles). The viruses are transmitted from human-to-human via droplet or smear infection. VZV is considered particularly contagious ("chickenpox"), so the natural seroprevalence is very high (80-90% in 20-year-olds). In Germany, 700,000 people become infected every year.
In the case of an initial infection with VZV, the virus reproduces in the regional lymph nodes, followed by an initial viraemia after 4-6 days. A second viraemic phase introduces the prodromal stage with non-specific symptoms, such as headaches, fever and fatigue. By attacking the skin and mucosae, this subsequently causes intermittent exanthema (macular, papular, vesicular, encrusted; the presence of all stages at the same time is typical, in contrast to pox with similar efflorescences). An infection can lead to numerous complications:

- Cerebellitis (1:4,000)
- Encephalitis (1:25,000)
- Meningitis
- Thrombocytopenia
- Pneumonia
- Secondary bacterial infections

The complication rate is particularly high in children ≤ 1 year; it decreases in toddlers and then increases once again from the age of four. Varicella can be life-threatening for immunosuppressed patients. Treatment is symptomatic for mild forms, potentially also with virostatic agents if necessary.
If seronegative women (prevalence of around 5% in Europe) are infected in the first 20 weeks of pregnancy (WOP), this leads to foetal (congenital) varicella syndrome, with microcephaly, cataracts, microphthalmia, chorioretinitis, hypoplasia of the extremities, skin defects, malformations of the intestinal and urogenital tract as well as skeletal and muscular hypoplasia, in about 2% of the children of these women (600 cases per year in Germany!).
The newborn develops connatal (neonatal) varicella in the first 2 weeks after birth, if the mother catches varicella within the last 3 WOP. The disease benefits from a lack of maternal antibodies as well as an immature immune system in the newborn. Left untreated, the mortality rate is up to 30%. In Germany, around 40-90 cases of varicella can be assumed every year.
Postnatal varicella occurs after the 12th day of life. While no complications are expected with fully-developed children, they can take a serious course in premature babies in the first weeks of life.
Herpes zoster (shingles) occurs by reactivating persisting viruses in the nerve ganglia, resulting in localised neuritis in connection with typical efflorescences and pains in the corresponding dermatome (75% thorax region). Complications are post-zoster neuralgias, zoster meningoencephalitis and bacterial superinfections. In immunosuppressed patients, zoster can lead to a protracted course and relapse.
Since 2004, active immunisation against VZV has been recommended in Germany by the Standing Vaccination Committee. The attenuated vaccine is administered separately or as part of the measles, mumps, rubella and varicella vaccination. Adults with a negative serostatus can also elect to be immunised, especially women intending to have children. Attenuated wild strains are used for the vaccination and occasional breakthrough infections, with atypical symptoms and a mild course, but which can still be contagious, must be expected. If seronegative pregnant women come into contact with a source of infection, passive immunisation should be performed within 96 hours (preferably 48 hours). Women of child-bearing age without VZV immunity should not work in kindergartens (remedy: active vaccination before the start of pregnancy).

Analytics. Direct detection: The electron microscopic detection of the virus is possible. Virus cultivation is time-consuming (1-4 weeks) and difficult due to the lability of the VZV. The polymerase chain reaction has a better sensitivity (90%; sensitivity, diagnostic) and specificity (99%; specificity, diagnostic).
Serology: Indirect immunofluorescence (immunofluorescence, indirect) and enzyme immunoassay (incl. enzyme-linked immunosorbent assay, chemiluminescence immunoassays) are established methods. Quantification takes place in international units of a WHO standard serum.

Sample material and sample stability. Direct detection and culture: Blister content, tissue, amniotic fluid, bronchoalveolar lavage fluid (with pneumonia) and cerebrospinal fluid (if varicella encephalitis is suspected) are analysed; for the PCR, also smears from atypical lesions, especially for immunosuppressed patients. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. In the case of varicella, specific antibodies can be detected about 3-4 days after the onset of exanthema. In the case of a primary infection, specific class IgM and IgA antibodies are generally detected first. A seroconversion or a significant titer rise in the specific IgG within 7-10 days confirms the diagnosis. A reactivation is generally associated with a sharp rise in the specific class IgA antibodies. The determination of the avidity of the specific IgG is also an established method and helps to serologically distinguish primary from secondary infections.
Infections with other neurotropic viruses, herpes simplex viruses, pox and blister-forming autoimmune dermatoses must be included in the differential diagnosis. The serology plays an important role in determining the immunity before and during pregnancy.
Acute varicella diseases are reportable in accordance with the German Infection Protection Act.

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West Nile fever virus

W. STÖCKER

Description of the pathogen. Family: *Flaviviridae*, Genus: *Flavivirus*, Species: West Nile virus (WNV).
Positive-strand RNA genome, enveloped, 50 nm diameter; considered an “emerging virus”.

Disease. West Nile fever
Distribution: Africa, Near and Middle East, Central Asia, Europe, North and South America; Subtype: Kunjin virus in Australia and Oceania.
Vectors: Mosquitoes (*Culex*, *Aedes* and *Mansonia* species).
Hosts: Birds, mammals (especially horses), humans (risk groups: children, the elderly and immunosuppressed persons).
Transmission is also possible via blood transfusion or organ transplants as well as transplacentally or via breast milk.
Symptoms: The infection may occur asymptomatically (70-80% of cases). In the case of symptomatic infections, sudden high fever and flu-like symptoms, exanthema, in about 1% of cases, especially in older people, encephalitis or meningoencephalitis with neurological symptoms, such as generalised pareses, often with long-term effects, mortality with encephalitis of about 10%; occasionally: Myocarditis, hepatitis, pancreatitis, haemorrhaging;
Treatment and prophylaxis: No specific therapeutic agents currently exist, only symptomatic treatment is possible. A vaccine for humans is still in development (horses can already be vaccinated against West Nile viruses). Prevention: protection against mosquito bites, combatting the vectors.

Analytics. Working with the pathogen requires the use of a laboratory of safety class 3. **Direct detection**: RT-PCR (polymerase chain reaction) or immunochromatographic rapid antigen test, virus cultivation in cell culture.
Serology: Detection of specific antibodies (IgG, IgM) in serum or cerebrospinal fluid through indirect immunofluorescence (IIFT, immunofluorescence, indirect), enzyme-linked immunosorbent assay (ELISA), neutralisation assay or haemagglutination inhibition assay.

Sample material and sample stability. **Direct detection and culture**: Blood components, cerebrospinal fluid or biopsies are analysed. The material must be stored at +4 to +8 °C until further processing. Direct detections must take place within 24 h and cultures must be prepared within 6 h. The material must be frozen for longer transport times.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C (cerebrospinal fluid, one week), or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The detection of both virus components as well as specific antibodies is part of a complete diagnosis - in certain phases of the disease, the presence of a specific infection can only be confirmed based on one of the two diagnostic principles. The reproduction in cell culture and the subsequent positive specific immune response as well as a positive PCR confirm the presence of the virus. However, an infection can also not be ruled out in the negative case, especially as the human body forms specific antibodies, which neutralise the virus, within just a few days of infection.
Direct detection is only possible during the acute phase of the disease and is often negative due to the short viraemia and low virus titer.
IgM class antibodies can be detected in serum from the 2nd day after the onset of the disease. If the IgM test is negative, although the symptoms indicate West Nile fever, a second serum sample should be re-tested for specific IgG antibodies after 2 weeks, in which case a combination of ELISA and IIFT provides almost 100% certainty. Anti-WNV IgM antibodies persist for 2-3 months, often for more than a year.
Class IgG antibodies follow the IgM with an interval of about 2 days. A rise in the respective antibody class by a factor of 10 is considered to be indicative of an infection. An additional distinction between fresh infections and those that have existed for some time is ensured by detection of low-avidity class IgG antibodies: If high-avidity antibodies are found in the sample, this indicates a past or reactivated infection. The IgG avidity can be detected by an ELISA as well as by indirect immunofluorescence.
Cross-reactions with other flaviviruses (TBE, dengue, Zika, Japanese encephalitis, St. Louis encephalitis, yellow fever viruses, etc.) must be observed. In the case of a positive finding, the recommendation is to titrate the sample and analyse this at the same time as other relevant flavivirus substrates for cross-reactions. Comparing the titer amounts allows the initial finding to be confirmed or rejected by a second detection and enables another flavivirus infection to be identified as the cause of the disease.
PCR tests or serological methods are used for screening blood transfusions for viral RNA or for specific antibodies.
Differential diagnoses: Dengue fever, Zika infection and other arbovirus diseases, malaria, with encephalitis, other viral and bacterial meningoencephalitis pathogens.
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya virus, dengue viruses, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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Yersinia enterocolitica

W. STÖCKER

Description of the pathogen. Classification: Family: Enterobacteriaceae; Genus: *Yersinia* (*Y.*)
The genus *Yersinia*, which belongs to the family of Enterobacteriaceae, currently includes 14 species. Obligate human pathogens are *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*. The species *Yersinia enterocolitica* forms a heterogeneous group of pathogenic and apathogenic strains, which can be subdifferentiated into over 60 different serovars.
Microscopically, the bacteria are gram-negative, coccoid to pleomorphic, generally alkali-stable, psychrophilic (cold-loving) short rods with monotrichous to peritrichous flagellation.

Diseases. *Yersinia enterocolitica* is distributed around the world in temperate and subtropical climate zones. The bacterium occurs in the intestine (occasionally in the throat) of many warm-blooded wild, domestic and farm animals, in their excretions as well as in the environment. Pigs are the most important reservoir for human infection. Infection occurs via inadequately heated animal products, primarily raw pork and milk. Contaminated blood reserves as well as the direct handling of pigs or domestic animals also present an infection risk.
Enteral yersiniosis in humans caused by pathogenic *Y. enterocolitica* strains is one of the most common bacteria-related gastrointestinal infections in Germany (3364 cases in 2010). Secondary extraintestinal, immunologically-induced reactions, such as erythema nodosum, uveitis, reactive arthritis (Reiter's arthritis), glomerulonephritis, thyroiditis or myocarditis may occur.
Prophylaxis against infection primarily involves complying with hygiene standards during food production and preparation. Treatment of an acute infection is generally limited to symptomatic measures, such as the replacement of fluid and salt depletion caused by vomiting and diarrhoea. Drugs, which prevent vomiting or influence the intestinal activity, may be used as supporting measures. In severe cases with positive pathogen detection, treatment with antibiotics may be administered (broad-spectrum cephalosporin plus aminoglycoside).

Analytics. Pathogen detection takes place by cultivation from stool and non-faecal samples, such as blood or biopsies, on selective cultivation media. Cold enrichment (4 °C, 1–3 weeks) is performed first if the number of bacteria in the sample is too low. Due to the heterogeneity within the species, the biochemical confirmation and biotyping as well as the serological pathogenicity determination of the isolates are important to identify virulent strains. Direct pathogen detection using molecular biological methods (polymerase chain reaction) is becoming increasingly important, especially because this allows the pathogenicity genes to be identified. However, initial selective cultivation is also beneficial in this case due to the accompanying bacterial flora.
A yersinia infection induces the formation of specific serum antibodies in immunoglobulin classes IgA, IgG and IgM, which are detected by immunoblots, immunofluorescence assays (immunofluorescence, indirect) and enzyme immunoassays based on virulence factors (Yop D, E, H, M). Other detection methods are the complement fixation test and Widal reaction. Yersinioses are reportable in accordance with Section 7 (1) of the German Infection Protection Act.

Sample material and sample stability. **Culture and PCR**: Stool, blood, biopsy, lymph node smear.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The direct detection of the pathogen in the stool is the method of choice for a diagnosis with an acute infection with *Yersinia enterocolitica*. Antibody detection is primarily used to clarify yersinia-associated long-term diseases, especially reactive arthritis.

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Zika virus

Description of the pathogen. Family: *Flaviviridae;* Genus: *Flavivirus;* Species: Zika virus, positive-strand RNA genome, enveloped.
The virus was first isolated from a rhesus monkey in Uganda in 1947. The virus has only become a focus of research since 2007, after a series of major epidemics outside Africa and especially since a serious outbreak in Brazil in 2015.

Diseases. Distribution: South and Central America, South-East Asia
Transmission: The virus is primarily transmitted to humans by the sting of infected mosquitoes of the genus *Aedes*. Perinatal transmission, i.e. the transmission of the virus from an infected mother to the foetus, is possible. Transmissions have also been described as a result of sexual intercourse.
Symptoms: A Zika virus infection is asymptomatic in about 80% of cases, while, in about 20% of cases, a skin rash, fever, headache, joint pains and conjunctivitis occur 3-12 days after an infection. The symptoms persist for 2-7 days and the disease is generally self-limiting. In Brazil and a number of other countries, a significant rise in neurological diseases, especially Guillain-Barré syndrome, was recorded during the Zika epidemic of 2015/2016. In addition, an unusually high number of babies with microcephaly were born. The connection between a Zika virus infection and the occurrence of neurological diseases and foetal malformations (congenital Zika syndrome) has now been confirmed.

Analytics. Direct detection: Detection of viral RNA by RT-PCR (polymerase chain reaction) from serum, urine and sperm.
Serology: Detection of specific antibodies (IgA, IgG, IgM) in serum through indirect immunofluorescence (substrate: ZIKV-infected cells, Fig. 1) and enzyme-linked immunosorbent assay (ELISA). In the ELISA, the use of non-structural protein 1 (NS1) has proven to be a highly specific and sensitive target antigen.

Sample material. Direct detection: Blood, urine, sperm. The material must be stored at +4 to +8 °C until further processing.
Serology: Serum or plasma for detection of the antibodies are stable for up to 2 weeks at +4 °C, or for months and years at –20 °C. 80% buffered glycerin can be added to the samples for frozen preservation of the IgM.

Diagnostic value. The most appropriate method for detecting a Zika virus infection depends on the stage of the disease. Detection of viral RNA is possible during the early stage of the infection: Zika virus can be detected by RT-PCR in the blood until about a week after symptoms start. In infected pregnant women, the virus can occasionally also be detected several weeks later. Virus detection by PCR in the urine can be possible for up to 4 weeks. However, if an infection occurred more than 7 days ago, serological tests, such as ELISA or indirect immunofluorescence assays are recommended. Antibodies can be detected in the patient's blood from the 5th day. The close relationship of the flaviviruses must be taken into account when interpreting the results. Cross-reactions between the specific antibodies may occur, if previous infections or vaccinations with another flavivirus exist.
The regulation to align the reporting obligations to the epidemic situation in accordance with the German Infection Protection Act (IfSG Reporting Obligation Alignment Ordinance), which entered into force on 01/05/2016, extended the reporting obligations for laboratories, in accordance with Section 7 (1) sentence 1 IfSG, to the direct or indirect detection of chikungunya virus, dengue viruses, West Nile fever virus, Zika virus and other arboviruses, if the detection indicates an acute infection. Additional general non-pathogen or -disease-specific reporting obligations may also exist.

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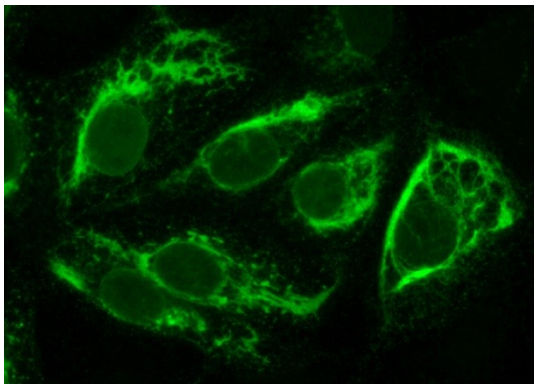

Zika virus. Fig. 1. Indirect immunofluorescence: Antibodies against Zika virus.