EUROLINE Anti-TO.R.C.H. 10 Profile (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DN 2410-1601-11 G DN 2410-6401-11 G	separate: Toxoplasma gondii, rubella virus, CMV, HSV-1, HSV-2, Bordetella pertussis, Chlamydia trachomatis, parvovirus B19, Treponema pallidum, VZV	IgG	Ag-coated immunoblot strips	16 x 01 (16) 64 x 01 (64)

Indications: The EUROLINE test kit provides a qualitative in vitro assay for human antibodies of the immunoglobulin class IgG to 10 different TO.R.C.H. antigens, i.e. **Toxoplasma gondii, rubella virus, CMV, HSV-1, HSV-2, Bordetella pertussis, Chlamydia trachomatis, parvovirus B19, Treponema pallidum and VZV** in serum or plasma for the diagnosis of infections.

Application: The EUROLINE Anti-TO.R.C.H. 10 Profile (IgG) allows determination of specific antibodies of class IgG against the pathogens Toxoplasma gondii, rubella virus, CMV, HSV-1, HSV-2, Bordetella pertussis, Chlamydia trachomatis, parvovirus B19, Treponema pallidum and VZV with one test strip. The antibody detection provides useful information for determining the immunity status, assessing the risk in pregnancy and performing continuative monitoring during prenatal care.

Principles of the test: The test kit contains test strips coated with parallel lines of highly purified antigens. In the first reaction step, the immunoblot strips are incubated with diluted patient samples. In the case of positive samples, the specific IgG antibodies (also IgA and IgM) will bind to the corresponding antigenic site. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Format 1601	Format 6401	Symbol
1. Test strips coated with antigens: Toxoplasma gondii, rubella virus, CMV, HSV-1, HSV-2, Bordetella pertussis, Chlamydia trachomatis, parvovirus B19, Treponema pallidum and VZV	16 strips	4 x 16 strips	STRIPS
2. positive control (IgG, human), 50x concentrate	1 x 0.04 ml	4 x 0.04 ml	POS CONTROL 50x
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human-IgG (goat), 10x concentrate	1 x 3 ml	4 x 3 ml	CONJUGATE 10x
4. Universal buffer 10x concentrate	1 x 50 ml	2 x 100 ml	BUFFER 10x
5. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolyl phosphate (NBT/BCIP), ready for use	1 x 30 ml	4 x 30 ml	SUBSTRATE
6. Incubation tray	2 x 8 channels	---	
7. Test instruction	1 booklet	1 booklet	

LOT Lot description — CE 0197 — Storage temperature

IVD In vitro diagnostic medical device — Unopened usable until

Storage and stability: The test kit must be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, controls and incubated test strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers.
Performance of the test requires an **incubation tray**:
ZD 9899-0130 Incubation tray with 30 channels
ZD 9898-0130 Incubation tray with 30 channels (black, for the EUROBlotCamera system)
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and EUROBlotCamera system)
For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
If a **visual evaluation** is to be performed in individual cases, the required evaluation protocol can be ordered under:
ZD 2410-0101-11 G Visual evaluation protocol EUROLINE Anti-TO.R.C.H. 10 Profile (IgG).

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the package with the test strips only when the strips have reached room temperature (+18°C to +25°C) to prevent condensation on the strips. After removal of the strips the package should be sealed tightly and stored at +2°C to +8°C.

- **Positive control:** The control is a 50x concentrate. For the preparation of the working strength control the amount required should be removed from the bottle using a clean pipette and diluted 1:51 with working strength universal buffer. Example: add 30 µl of control to 1.5 ml working strength universal buffer and mix thoroughly. The working strength control should be used at the same working day.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the working strength enzyme conjugate the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with the working strength universal buffer. For one test strip dilute 0.15 ml anti-human IgG concentrate with 1.35 ml working strength universal buffer. The working strength enzyme conjugate should be used on the same working day.

- **Universal buffer:** The universal buffer is supplied as a 10x concentrate. For the preparation of the working strength universal buffer the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with deionised or distilled water. For the incubation of 1 test strip 2.0 ml universal buffer (10x concentrated) should be diluted with 18.0 ml deionised or distilled water. The working strength universal buffer should be used on the same working day.

- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light  .

Warning: The control of human origin has tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: The **patient samples** for analysis are diluted **1:51** with ready for use universal buffer. For example, add 30 µl of sample to 1.5 ml ready for use universal buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Incubation

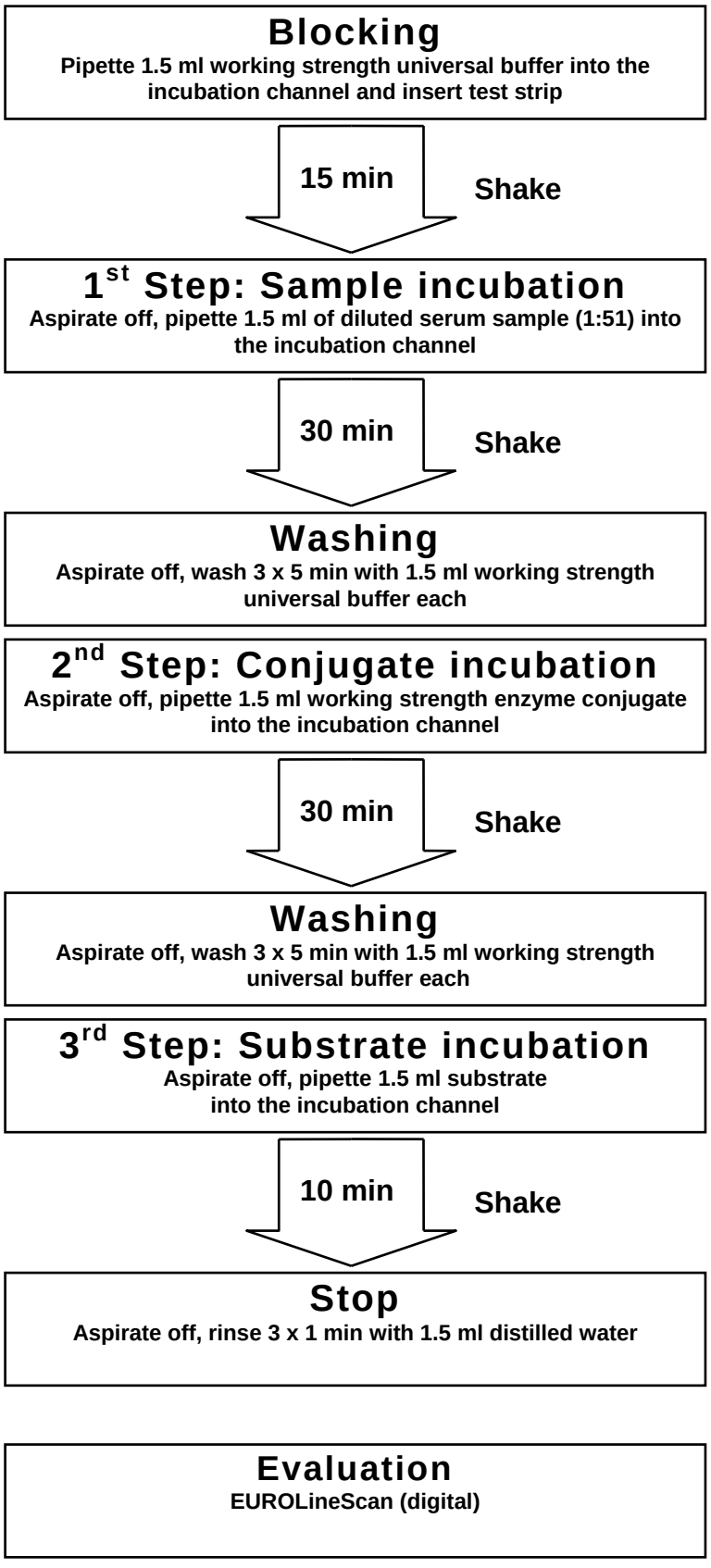
Blocking:	Fill the channels of the incubation tray according to the number of serum samples to be tested with 1.5 ml working strength diluted universal buffer each. Remove the required amount of test strips from the packaging using a pair of tweezers and place them one by one in the channels containing the buffer. The number on the test strip should be visible. Incubate for **15 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.
Sample incubation: (1st step)	Fill each channel with 1.5 ml of the diluted serum samples and incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.
Wash:	Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working strength universal buffer on a rocking shaker.
Conjugate incubation: (2nd step)	Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase conjugated anti-human IgG) into each channel and incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Wash:	Aspirate off the liquid from each channel. Wash as described above.
Substrate incubation: (3rd step)	Pipette 1.5 ml substrate solution into the channels of the incubation tray. Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Stop:	Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with deionised or distilled water.
Evaluate:	Place test strip on the evaluation protocol, air dry and evaluate.

For automated incubation with the **EUROBlotMaster** select the program **Euro02 Inf WB30**.

For automated incubation with the **EUROBlotOne** select the program **Euro01/02**.

EUROLINE Anti-TO.R.C.H. 10 Profile (IgG)

Incubation protocol



Evaluation and interpretation of the results obtained by the EUROLINE Anti-TO.R.C.H. 10 Profile (IgG)

Handling: For the evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned using a flatbed scanner (EUROIMMUN AG) and evaluated with **EUROLineScan**. Alternatively, imaging and evaluation is possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan programme please refer to the EUROLineScan user manual (EUROIMMUN AG). The code for entering the **test** into EUROLineScan is **TORCH 10IgG**.

If a visual evaluation is to be performed in individual cases, place the incubated test strips onto the respective work protocol for visual evaluation. This protocol is available at EUROIMMUN under the order no. ZD 2410-0101-11 G.

Note: A correctly performed test for class IgG antibodies is indicated by a positive reaction of the control band and the IgG band. If one of these bands shows a very weak reaction or none at all, the result must not be used for evaluation.

Antigens and their arrangement on the strips:

Antigens:
Toxoplasma gondii: Lysates of sonicated and gamma irradiated Toxoplasma gondii tachyzoites.

Rubella virus: Purified rubella virus antigens. The antigen source is provided by inactivated cell lysates of Vero cells infected with the “HPV-77“ strain of rubella virus.

CMV: Highly purified recombinant phosphoproteins of cytomegalovirus, expressed in E. coli.

HSV-1: Affinity-purified glycoprotein C1.

HSV-2: Affinity-purified glycoprotein G2.

Bordetella pertussis (PT): Highly purified antigen preparation of pertussis toxin (PT) from Bordetella pertussis.

Chlamydia trachomatis (MOMP): Recombinant major outer membrane protein (MOMP) of Chlamydia trachomatis.

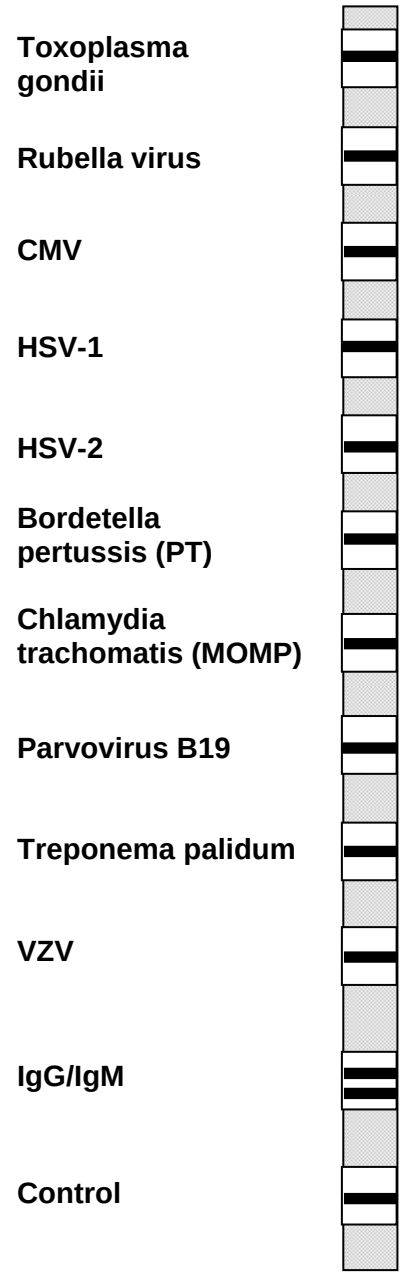
Parvovirus B19: Recombinantly produced, affinity chromatographically purified viral protein 1 (VP1) of human parvovirus B19.

Treponema pallidum: Highly purified recombinant lipoproteins of Treponema pallidum, expressed in E. coli.

VZV: Highly purified glycoproteins of Varicella zoster virus.

Control bands:
IgG or IgM

Control: Incubation control indicating a correctly performed incubation.



Antibodies of class IgG against TO.R.C.H. 10 antigens

It is recommended that the results of the bands be classified according to the signal intensity:

Signal	Result	
No signal or very weak band	o	Negative
Weak band	(+)	Borderline
Medium to strong band	+	Positive

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Measurement range: The EUROLINE is a qualitative method. No measurement range is provided.
The lower detection limit is the mean value of a tenfold determination of an analyte-free sample plus three times the standard deviation and is, on average, EUROLineScan intensity value 2. This intensity value corresponds to a negative result.

Inter- and intra-assay variation: The inter-assay variation and intra-assay variation were determined by multiple analyses of characterised samples over several days. In every case, the intensity of the bands was within the specified range. This EUROLINE displays excellent inter- and intra-assay reproducibility.

Interference: Haemolytic, lipaemic and icteric sera up to a concentration of 5 mg/ml for haemoglobin, of 20 mg/ml for triglycerides and of 0.4 mg/ml for bilirubin showed no effect on the analytical results of the present EUROLINE.

Sensitivity and specificity: Precharacterised sera from interlaboratory tests and 150 sera from healthy blood donors were investigated using the EUROLINE. The following results were achieved:

Antibodies against	n	Sensitivity	Specificity	n	Prevalence
Toxoplasma gondii	19	100%	100%	150	42.7%
Rubella virus	20	100%	100%	150	96.7%
CMV	79	97.9%	100%	150	32.7%
HSV-1	20	100%	100%	150	77.3%
HSV-2	20	100%	100%	150	11.3%
Bordetella pertussis (PT)	10	100%	100%	150	12.7%
Chlamydia trachomatis (MOMP)	13	100%	100%	150	22.0%
Parvovirus B19 (VP1)	20	100%	100%	150	64.0%
Treponema pallidum	20	100%	100%	150	0%
VZV	19	100%	100%	150	96%

Cross reactivity: The cross reactivity was determined using a representative selection of antibodies against infectious agents. Results obtained for parameters with the highest relevance in the assessment of the cross reactivity are given as examples in the following table.

ELISA Antibodies against	pos (n)	EUROLINE Anti-Toxoplasma IgG positive
Rubella IgG	192	0%
CMV IgG	191	0%
HSV-1 IgG	164	0%
HSV-2 IgG	16	0%
Parvovirus B19 IgG	128	0%
VZV IgG	185	0%

ELISA Antibodies against	pos (n)	EUROLINE Anti-Rubella IgG positive
Toxoplasma IgG	5	0%
CMV IgG	12	0%
HSV-1 IgG	10	0%
HSV-2 IgG	1	0%
Parvovirus B19 IgG	7	0%
VZV IgG	12	0%

ELISA Antibodies against	pos (n)	EUROLINE Anti-CMV IgG positive
Toxoplasma IgG	6	0%
Rubella IgG	21	0%
HSV-1 IgG	12	0%
HSV-2 IgG	1	0%
Parvovirus B19 IgG	15	0%
VZV IgG	20	0%

ELISA Antibodies against	pos (n)	EUROLINE Anti-Chlamydia trachomatis IgG positive
Toxoplasma IgG	88	0%
Rubella IgG	261	0%
CMV IgG	268	0%
HSV-1 IgG	236	0%
HSV-2 IgG	21	0%
Parvovirus B19 IgG	181	0%
VZV IgG	257	0%

Samples that were positive for a specific parameter in ELISA showed no reactivity with the respective other parameters in the EUROLINE test. Thus, cross reactions can be excluded.

Correlation analyses: Investigation of 246 sera in relation to the CE-registered reference methods Anti-Toxoplasma gondii ELISA, Anti-Rubella virus ELISA and Anti-CMV ELISA (test systems from EUROIMMUN AG) yielded the following specificities and sensitivities for detection of the corresponding antibodies. Borderline samples were not included in the evaluation.

		Anti-Toxoplasma gondii ELISA (IgG)		
n = 246		positive	borderline	negative
EUROLINE Anti-TO.R.C.H. Profil (IgG)	positive	86	1	2
	borderline	1	0	1
	negative	1	0	154

Specificity: 98.7%
Sensitivity: 98.9%

		Anti-Rubella Virus ELISA (IgG)		
n = 246		positive	borderline	negative
EUROLINE Anti-TO.R.C.H. Profil (IgG)	positive	231	2	2
	borderline	0	0	0
	negative	0	0	11

Specificity: 84.6%
Sensitivity: 100%

		Anti-CMV ELISA (IgG)		
n = 246		positive	borderline	negative
EUROLINE Anti-TO.R.C.H. Profil (IgG)	positive	230	0	0
	borderline	5	0	1
	negative	3	0	7

Specificity: 100%
Sensitivity: 98.7%

Investigation of 92 sera in relation to the CE-registered reference method Anti-Chlamydia trachomatis IgG EUROLINE-WB (test system from EUROIMMUN AG) yielded a specificity of 100% and a sensitivity of 95.5%. Borderline samples were not included in the evaluation.

		Chlamydia trachomatis (IgG) EUROLINE-WB		
n = 92		positive	borderline	negative
EUROLINE Anti-TO.R.C.H. Profil (IgG)	positive	21	2	0
	borderline	1	0	1
	negative	1	1	65

Specificity: 100%
Sensitivity: 95.5%

Clinical significance

Toxoplasma gondii:
The pathogen of toxoplasmosis is the sporozoon Toxoplasma gondii. It was first found in the gundi, an African rodent. However, the main host animal is the cat, in which after peroral infection the parasite lives in intestinal cells and causes oocysts to develop (sexual development cycle). During the asexual development cycle, the Toxoplasma parasites develop in the brain, muscle, liver, spleen and other organs of a warm-blooded animal, where they become encapsulated. Humans are generally infected perorally by ingestion of oocysts (contained in the faeces of infected cats) or from meat products (the raw flesh of infected animals contains cysts with viable trophozoites). Toxoplasma can also be transmitted diaplacentally when a pregnant woman is infected for the first time.

Toxoplasmosis acquired post-natally generally proceeds inapparently. In this case, cysts containing trophozoites, which can persist for years and can sustain immunity, form in the tissue. Depending on the organ manifestation, the symptoms of the disease include fever, lymphadenopathy, encephalitis, chorioretinitis, myositis, myocarditis, pneumonia, hepatosplenomegaly and exanthema. Specific antibodies have even been detected in the brain. In the case of immunologically challenged patients (recipients of trans-plants, tumour patients, HIV-infected patients), a primary infection with Toxoplasma or the reactivation of a toxoplasmosis can lead to life-threatening illness.

After an intrauterine infection in the first trimester, with the placenta and embryo being severely affected, a rejection of the foetus occurs. An infection in the second or third trimester results in foetal symptoms which vary depending on the timepoint of infection, the dose of the infection and the immune status of mother and foetus. In the foreground are: hepatosplenomegaly, pneumonia, myocarditis, purpura, hydrocephalus, chorioretinitis, edema of the optic nerve, intracerebral calcification.

Rubella:
The pathogenic agent of rubella is the rubella virus, which is present worldwide. The disease is transmitted via aerosols and is already contagious during the two to three-week incubation period. The symptoms include headaches, characteristic lymph node swellings, and often a typical blotchy rash. The majority of infections occur between the ages of 5 and 14 years, whereby about 40 to 50% of cases proceed subclinically. A rubella infection leads to life-long immunity. An infection spread of 80 to 90% is assumed for adults in central Europe. This means that 10 to 20% of women of child-bearing age are not immune.

Rubella virus transmitted diaplacentally causes the highest rate of embryonic deformities, through infection in the form of a rubella embryopathy. This leads to serious disorders, such as cataract, inner ear damage, heart defects, and microcephaly, particularly in the first three months of pregnancy. In many countries, an acute rubella infection is considered to be a medical indication for termination of pregnancy.

For this reason, the serological analysis of antibodies against the three most important rubella structure proteins (E1, E2, C), which can be detected two to three days after the onset of the exanthema, is of particular importance in pregnant women. In case of a negative result, an unknown immunisation status or the presence of specific IgM, passive immunisation can be given within 7 days after exposition in the early stage of pregnancy.

Various inoculation strategies have been employed worldwide to prevent rubella infections. Since active immunisation is well tolerated, vaccination programs aim to protect all young persons before puberty using a two-stage rubella vaccination.

CMV:
The majority of cytomegalovirus infections proceed inapparently. The disease can manifest itself in almost all organs, in the foreground are, however, hepatitis and pneumonia, which are accompanied by a prolonged fever. Of importance is connatal CMV infection, which causes damage in particular to the liver, the spleen and the central nervous system. Approximately 1% of all foetal cases are infected in utero; in 20% of these cases IgM class antibodies against CMV can be detected in the foetal blood, correlating with severe damage in the foetus or newborn.

Antibodies against CMV can be detected in the serum of nearly all patients after the disease has taken its course. Life-long immunity normally develops. The prevalence of infection in adults amounts to about 80%. Past infections can be reactivated if the immunological defence mechanisms are weakened. The diagnosis of CMV infections in pregnant women is based on the determination of IgG and IgM antibodies. In order to determine the time of the onset of the disease, the IgG avidity must be determined. False-positive IgM antibody results have been found in Epstein-Barr virus infections.

In the case of immunologically challenged seronegative patients, such as tumour patients and recipients of transplants, passive immunisation with specific immunoglobulin concentrates is frequently indicated. Such patients and infants (particularly premature children), should not be given blood products from CMV-infected (CMV-antibody positive) blood donors.

HSV-1/HSV-2:
Herpes simplex is a disease which causes the formation of blisters on the skin and mucous membranes. It is prevalent worldwide and normally proceeds blandly. Complications result when internal organs are also affected and start to necrotise. Herpes simplex virus 1 (HSV-1) prefer the area of the mouth and nose, herpes simplex virus 2 (HSV-2) the genitals. Provocable relapses are typical.

Antibodies against HSV-2 can be detected in the serum of nearly all patients after the disease has taken its course. More than 90% of the population are infected with HSV-1, whereas antibodies against HSV-2 can only be found in 7 to 20%. However, the antibodies are not able to prevent a relapse or re-infection. In primary HSV-2 infections, cephalalgia and neck stiffness are frequent symptoms, whereas meningitis is rare. If newborns are infected with HSV-2 passing the birth canal, blisters of the skin but also on the mouth and eyes are frequently observed, often accompanied by hepatomegaly, splenomegaly, loss of kidney function, icterus and encephalitis. Without treatment the disease is fatal in most cases.

Bordetella pertussis:
The genus Bordetella (B.) encompasses four known species: B. pertussis, B. parapertussis, B. bronchiseptica and B. avium. The first three pathogens are genetically so closely related that they are also referred to as subspecies of the same type. The highly contagious B. pertussis (which causes whooping cough) is distributed worldwide and transmitted among humans by droplet infection. The disease is not seasonal. It occurs sporadically or epidemically. An infection confers specific immunity, which reduces after decades. The disease is known in adults, but is rarely diagnosed, even though coughing adults can infect their surroundings. In 2003 around 17 million people worldwide were infected with whooping cough, 90% of these in developing countries. In the same year, approximately 280,000 fatal cases of whooping cough were recorded.

After an incubation time of around 7 to 14 days, Bordetella pertussis infections begin with an uncharacteristic catarrhal stage, which lasts for about 1 to 2 weeks. Then the convulsive stage develops, lasting for 2 to 3 weeks with typical paroxysmal, staccato coughing attacks, frequently followed by stridor with possible vomiting. Nocturnal attacks are frequent. During both of these stages the pathogen is coughed out. Transmission via contaminated objects cannot be excluded. Following this is the decrimenti stage, which lasts for several weeks, with continual diminishment of coughing attacks.

Complications such as secondary pneumonia or otitis media are possible, especially in children under the age of 2 years. There is no difference in morbidity between boys and girls. Season and climate have no influence on the frequency of the disease. Re-infection in persons over 60 years of age can be life-threatening.

In Germany, the Standing Commission for Vaccination (STIKO) recommends vaccination at the ages of 2, 3 and 4 months, further vaccination at the age of 11 to 14 months, booster vaccinations in pre-school and adolescent ages and, moreover, the vaccination of adults, particularly elderly persons. The vaccination of pregnant women without specific antibodies in the first trimester is indicated due to the high mortality rate of infected newborns and infants under 3 months old. This improves the immunisation status of the child from birth to first vaccination. Specific antibodies can be detected in serum by IFT, blot methods or ELISA.

Chlamydia trachomatis:
The infectious agent Chlamydia trachomatis belongs to the human pathogenic Chlamydia genus, together with Chlamydia pneumoniae and Chlamydia psittaci. It is one of the smallest intracellular, gram-negative bacteria. It subsists as an energy parasite on the ATP of infected cells. Around 700 million people are infected worldwide, with approximately 50 million new infections taking place each year. In the USA the prevalence of mainly asymptomatic C. trachomatis infections in 16- to 25-year-old women is 22%, and in Western Europe 2.7% (in Italy) to 8% (in Iceland) according to the WHO. The disease is transmitted by contact with infected humans.

Chlamydia trachomatis is the pathogenic agent of non-gonorrheal urethritis, lymphogranuloma venereum, trachoma, inclusion conjunctivitis, Reiter's syndrome and neonatal pneumonia.

Sexually transmitted non-gonorrheal urethritis is the most frequent venereal disease. In Germany, these infections are caused by the C. trachomatis serotypes D to K. The bacteria live mostly in the cells of the urethra, in men also in the prostate and the seminal vesicles and in women in the cervix or oviducts (salpinx). In men they cause urethritis, epididymitis and prostatitis, in women urethritis, cervicitis and salpingitis/adnexitis. In male patients, C. trachomatis infections often proceed asymptomatically; in female patients, however, they cause itching, pains and abnormal vaginal discharge and, if the inner genital organs are affected, lead to sterility in many cases. In Germany, more than 100,000 women suffer from Chlamydia-caused infertility. Secondary infertility in men has also been shown. There is an evident connection between acute C. trachomatis infections during the first three months of pregnancy and early abortions, premature deliveries or stillbirths (32nd to 34th week of pregnancy).

In tropical regions, C. trachomatis leads to trachoma (serotypes A, B, Ba and C), an eye infection of varying severity which is also known as trachomatous conjunctivitis, granular conjunctivitis or Egyptian ophthalmia. It is caused by direct contact between the mucous membranes of the eye, nose and mouth or may be transmitted by the mutual use of towels or washcloths. The first symptoms of severe conjunctivitis occur after an incubation period of 5 to 12 days. Around 400 million people suffer from trachoma, which is the most frequent cause of blindness worldwide (trachoma blindness).

In 1 to 3% of cases, an urogenital infection with C. trachomatis is followed by reactive arthritis (Reiter's disease with the triad urethritis, conjunctivitis and arthritis). This is an oligoarthritis which predominantly affects the lower extremities, particularly the knee and ankle joints, causing local swelling. The distal interphalangeal joints and the spine (inflammatory back pain) are also frequently involved.

In newborns, particularly premature infants, prenatally or perinatally transmitted C. trachomatis causes conjunctivitis (ophthalmia neonatorum) and pneumonia (serotypes D to K). The latter is noticeably often accompanied by pneumothorax and lifelong health problems.

Studies throughout Europe show that immunological detection methods are suited to confirming C. trachomatis-induced infertility in women and men by the determination of C. trachomatis specific serum IgA and IgG antibodies. C. trachomatis specific IgA and IgG antibodies are frequently found in women who have had a premature delivery or a silent birth, mostly in connection with high IgM titers. Recognised medical centres therefore recommend that a C. trachomatis screening be carried out for both parents before pregnancy, or for pregnant women in the first trimester at the latest, e.g. during extended TORCH analysis.

Serologically detected Chlamydia infections can generally be cured with various antibiotics within 7 days, even during pregnancy. In reactive arthritis, a long-term, differentiated treatment is required, which acts locally and systemically.

Parvovirus B19:
Parvovirus B19, the smallest (“parvo“) known virus, is a single-stranded DNA virus from the family of Parvoviridae. The virus consists of two viral structural protein types (major and minor structural protein species), which form an icosaedric capsid. Its replication takes place predominantly in haematopoietic cells. Up until now three different genotypes (genotypes 1 to 3) have been identified. Parvovirus B19 is characterised by a very high stability with regards to environmental factors and detergents. The virus attacks a receptor on erythrocytes, the globoside blood group P antigen.

B19 infections (fifth disease, erythema infectiosum, megaloerythema, Sticker’s disease) occur worldwide, mainly in spring. They occur in local epidemics, especially in child day care centres, schools, families and hospitals. In central Europe they can be described as endemic.

Parvovirus B19 is transmitted by droplets, skin contact, via blood or blood products or diaplacentally. The incubation time is 4 to 14 days, occasionally 3 to 17 days. The virus can be detected in the serum of the infected person between the 3rd and 16th day after infection. When the exanthema appears the patient is no longer infectious.

Typically headaches, itching, myalgia and fever occur in the prodrome phase. Fresh B19 infections (anti-B19 IgM) can occur in all age groups. Acute infections are found most frequently in 6 to 15 year olds. The prevalence of antibodies against parvovirus B19 (anti-B19 IgG) increases with age. In Germany this amounts to around 35% for 4 to 6 year olds, 58% for 10 to 15 year olds, 70% for 25 to 29 year olds and 79% for 65 to 69 year olds.

In children parvovirus B19 causes fifth disease. The exanthema generally begins with an intense redness and swelling on the cheeks (butterfly form; "slapped cheek"). Individual large areas of bright red colour are found on the forehead and around the ears. The exanthema extends to the extensor side of the arms, as well as the buttocks and legs. The extremities are most severely affected; surfaces of the hands and feet can also be afflicted. The trunk is the least affected. Mucous membranes remain free from exanthema. The exanthema is characteristically garland-shaped or net-like. It lasts for 6 to 21 days and subsides with an undulating form. As well as exanthema, lymph node swelling and flu-like symptoms are frequently observed. Accompanying symptoms are occasionally pruritus, subfebrile temperature and arthralgia. Symmetrical arthritis of the small joints can occur as a complication in children. An acute B10 infection can also proceed with purpura Schoenlein-Henoch or trigger various diseases, such as pseudoappendicitis, coxitis, enteritis, myocarditis, neuropathy of the brachial plexus, and erythema nodosum.

In adults the infection can trigger acral erythema and arthritis (acute symmetrical polyarthropathy), which is difficult to differentiate clinically from chronic polyarthritis. 17 to 33% of all heart muscle inflammation cases can be attributed to parvovirus B19.

Parvovirus B19 multiplies in erythroblastocytes, causing temporary anaemia. The infection can lead to complications and even death in immunocompromised patients. The condition "pure red cell aplasia" described in AIDS patients is caused by chronic B19 infection.

Diaplacental B19 infections during pregnancy can lead, via inhibition of foetal erythropoiesis, to anaemia, hypoxia and in extreme cases to hydrops fetalis (in around 12% of cases) and foetal death. Further symptoms are caused by hypoproteinaemia: oedema, percardial and pleural effusion, ascites.

Since fifth disease is difficult to distinguish clinically from rubella, serology is often used for clarification. In adults, in particular, fifth disease often proceeds with atypical exanthema.

Banked blood is not currently tested for B19 virus. Since B19 infected persons are mostly still asymptomatic in the viraemic stage, B19 virus infections via transfusion can occur. Tests in Germany and France showed a prevalence of B19 virus in banked blood of approximately 0.01 to 0.03%. Since the detection of B19 antigen is time-consuming, high-risk patients should only be given blood that has tested positive for anti-B19 IgG. Anti-B19 IgG positive blood generally no longer contains B19 virus.

Due to the differing manifestations of a B19 infection it is necessary to confirm or exclude an acute B19 infection. The detection of B19 antigen or DNA (PCR) play a secondary role in diagnosis, since patients in the viraemic stage of a B19 infection are mostly asymptomatic. Thus, the detection of B1-specific antibodies (Anti-B19 IgG and IgM) is of particular significance. Diagnostics for a B19 infection is performed using ELISA or immunoblot, which selectively detect anti-B19 IgG or anti-B19 IgM using a viral structural protein as antigen.

The detection of anti-B19 IgM indicates a fresh B19 infection. Anti-B19 IgM can be detected from around 10 days up to 3 to 5 months after infection. Anti-B19 IgG appears at the end of the 3rd week after infection at the earliest and is assumed to persist lifelong. Therapeutic measures are limited to treating the symptoms. With hydrops fetalis an intrauterine exchange transfusion can substantially improve prognosis.

Treponema pallidum:
Treponema pallidum pallidum is a helically wound bacteria of the Spirochaeta family. This family includes five genera: Borrelia, Spirochaeta, Cristispira, Treponema and Leptospira. Treponema pallidum is the causative agent of syphilis or lues, a chronic infectious disease. The subspecies T. pallidum endemicum causes veneric syphilis; T. pallidum pertenue leads to a non-veneric infection occuring in tropical regions called framboesia; T. pallidum carateum is the causative agent of Pinta.

Syphilis is transmitted from human to human during sexual acts via the mucosa. Indirect transmission by blood transfusions and wounds is also possible. During pregnancy and at birth the baby can become infected by the mother (syphilis connata). Syphilis is a known risk factor for abortions and stillbirths as they occur 21% more often in pregnant women with syphilis. 15% of newborns show signs of congenital syphilis.

The disease has three stages. The primary lesion of syphilis (stage I), the so-called ulcus durum (or hard-edged ulcer) normally develops three weeks after the infections at the place of entry of the pathogen (e.g. penis). It is a painless ulcer, which contains large quantities of the pathogen and is therefore highly contagious. Typically, the clearly defined fibrous or crusted erosion has a raised hard edge. The possible swelling of the regional lymph nodes is painless and the lymph nodes remain displaceable. After 2 to 6 weeks the ulcer heals leaving a scar. The infection generally persists and develops into stage II.

Approximately 8 weeks after the infection, the secondary stage manifests with flu-like symptoms such as fever, fatigue or head and joint pains. In addition to a generalised swelling of the lymph nodes, 90% of patients show local or generalised skin disorders, which are accompanied by weak or no itching. At first, light pink patches form, which further evolve into hard, coppery nodules (papules). In the foreground are condylomata lata, broad papules, which mainly affect skin folds. The liquid excreted by open and weeping papules is highly contagious. Additionally, various organ disorders may develop, for example, ketaritis, iritis, hepatitis, vasculitis, and myocardial disorders.

All skin disorders (syphilids) heal after approximately 4 months. Secondary syphilis is followed by a clinically silent, but seroactive stage (syphilis latens), which can last for years. Patients are infectious for around one year after infection (early latent phase), but not in the late latent phase.

Typical manifestations of a Treponema pallidum infection in stage III are large papules and ulcers on the skin and mucous membranes, as well as organ or visceral syphilis, including gummatous and interstitial inflammation, perivasculitis, cardiovascular syphilis, neurosyphilis (asymtomatic and symptomatic form), osteitis, and periostitis. Ten to thirty years after an untreated infection, 8% to 10% of patients experience severe neurological disorders such as neurosyphilis with progressive paralysis and Tabes dorsalis with severe mental and vegetative disorders.

The diagnosis of syphilis is based on clinical findings according to the disease stage, microscopic detection of the infectious agent (dark field), and serological detection of antibodies against Treponema pallidum.

Antibodies against Treponema pallidum can be detected in serum and in CSF. This is diagnostically relevant, for example, in children with congenital syphilis. The intrathecal agent-specific antibody production is defined by the relative CSF/serum quotient CSQrel. (synonym: antibody specificity index).

VZV:
Varicella zoster virus (VZV), synonym: human-pathogenic herpes virus 3 (HHV3), is the causative agent of chicken pox (varicella). After first manifestation, the virus establishes latency in sensory nerve cells where it may be reactivated at a later stage causing herpes zoster (shingles) as second manifestation. Humans are the only host for the virus. Chicken pox a very contagious disease, has traditionally been regarded as a benign, inevitable disease among children (25% in 1 to 4 year olds, 43% in 5 to 8 year olds, 27% in 9 to 18 year olds), with typical blister-like rash of the entire skin. Now we know that varicella is a serious infection in childhood, but especially in young and older adults and during pregnancy.

Zoster is the endogenous recurrence of an earlier varicella infection or the result of a reinfection in persons with existing residual immunity. The incidence of herpes zoster in Europe per year is 3 cases per 1000 persons in general and 10 cases per 1000 persons over 80 years old. The entire viral genome is present in the latently infected ganglia. VZV is latent in multiple ganglia along the entire human neuraxis. In zoster, the rash affects the distribution area of one or several sensitive nerve roots, especially T3-L3 and N. trigeminus. Both primary infection and reactivation of VZV may provoke complications of the central nervous system (CNS). More serious manifestations arise when reactivated VZV infects the spinal cord or the cerebral arteries, causing diseases such as myelitis, focal vasculopathy and encephalitis.

Varicella causes serious infections during pregnancy with severe outcome for the foetus. Varicella zoster infection in the mother is life-threatening for the newborn at birth. If infection occurs between the fourth day ante partum and the second day post partum the neonatal mortality rate is 20 to 30%. Patients with congenital varicella syndrome generally show the following clinical symptoms: skin lesions, neurological defects, eye complaints and/or hypoplasia of the extremities. Manifestations of the brain or eye have also been observed in individual cases.

VZV myelitis or VZV encephalitis are diagnosed by the determination of antibodies against VZV in CSF and serum.

CNS involvement results in the intrathecal synthesis of antibodies against VZV in cerebro-spinal fluid (CSF). Due to the fact that specific antibodies can pass from the serum through the blood-brain barrier into the CSF by diffusion, a relative CSF/serum quotient (CSQrel., synonym: antibody specificity index) is determined.

Infection or successful vaccination generally confer life-long immunity. A passive immunisation with specific immunoglobulin concentrates is usually given to immunocompromised seronegative individuals, such as tumour patients and recipients of transplants, as well as to seronegative pregnant women after exposure to the virus.

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