# Anti-Parvovirus B19 ELISA (IgG)
# Test instruction

| ORDER NO. | ANTIBODIES AGAINST | IG CLASS | SUBSTRATE | FORMAT |
|---|---|---|---|---|
| EI 2580-9601 G | Parvovirus B19 | IgG | Ag-coated microplate wells | 96 x 01 (96) |

**Indication:** The ELISA test kit provides semiquantitative or quantitative in vitro determination of human antibodies of the immunoglobulin class IgG against parvoviruses B19 in serum or plasma to support the diagnosis of erythema infectiosum. Synonyms: megaloerythema, Sticker's disease, fifth disease.

**Application:** The determination of anti-parvovirus B19 antibodies of classes IgG and IgM, e.g. using ELISA, is after direct virus detection the most important method for diagnosis of a parvovirus B19 infection. Assessment of immunity against parvovirus B19 infection is of particular significance in pregnant women. Detection of virus-specific IgG antibodies together with negative IgM and negative direct virus detection indicate a past parvovirus B19 infection and existing immunity.

**Principles of the test:** The test kit contains microtiter strips each with 8 break-off reagent wells coated with parvovirus antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. 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 | Colour | Format | Symbol |
|---|---|---|---|
| **1. Microplate wells coated with antigens**<br>12 microplate strips each containing 8 individual break-off wells in a frame, ready for use | --- | 12 x 8 | STRIPS |
| **2. Calibrator 1**<br>100 IU/ml (IgG, human), ready for use | red coloured in decreasing intensity | 1 x 2.0 ml | CAL 1 |
| **3. Calibrator 2**<br>25 IU/ml (IgG, human), ready for use | | 1 x 2.0 ml | CAL 2 |
| **4. Calibrator 3**<br>5 IU/ml (IgG, human), ready for use | | 1 x 2.0 ml | CAL 3 |
| **5. Calibrator 4**<br>1 IU/ml (IgG, human), ready for use | | 1 x 2.0 ml | CAL 4 |
| **6. Positive control**<br>(IgG, human), ready for use | blue | 1 x 2.0 ml | POS CONTROL |
| **7. Negative control**<br>(IgG, human), ready for use | green | 1 x 2.0 ml | NEG CONTROL |
| **8. Enzyme conjugate**<br>peroxidase-labelled anti-human IgG (rabbit), ready for use | green | 1 x 12 ml | CONJUGATE |
| **9. Sample buffer**, ready for use | light blue | 1 x 100 ml | SAMPLE BUFFER |
| **10. Wash buffer**, 10x concentrate | colourless | 1 x 100 ml | WASH BUFFER 10x |
| **11. Chromogen/substrate solution**<br>TMB/$H_2O_2$, ready for use | colourless | 1 x 12 ml | SUBSTRATE |
| **12. Stop solution**<br>0.5 M sulphuric acid, ready for use | colourless | 1 x 12 ml | STOP SOLUTION |
| **13. Test instruction** | --- | 1 booklet | |
| **14. Quality control certificate** | --- | 1 protocol | |
| **15. Protective foil** | --- | 2 pieces | FOIL |

LOT Lot description — CE — Storage temperature

IVD In vitro diagnostic medical device — Unopened usable until

**Modifications to the former version are marked in grey.**

## 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. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.
The thermostat-adjusted ELISA incubator must be set at +37°C ± 1°C.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working-strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light ✳. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue-coloured.

- **Stop solution:** Ready for use.

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

**Waste disposal:** Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

**Warning:** The calibrators and controls of human origin have 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 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: Patient samples** are diluted **1:101** in sample buffer. For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by votexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.

## Incubation

For **semiquantative analysis** incubate **calibrator 3** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1 to 4** along with the positive and negative controls and patient samples.

**(Partly) manual test performance**

**Sample incubation:** (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
For manual test performance cover the reagent wells with the protective foil. For automated test performance follow the recommendations of the instrument manufacturer.
Incubate for **60 minutes at +37°C ± 1°C**.

**Washing:**
Manual: Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µl of working-strength wash buffer for each wash.
Automatic: Remove the protective foil and wash the reagent wells 3 times with 450 µl of working-strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Mode”).

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings. Insufficient washing (e.g. less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction readings.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

**Conjugate incubation:** (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

**Washing:**
Empty the wells. Wash as described above.

**Substrate incubation:** (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C, protect from direct sunlight).

**Stopping:**
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

**Measurement:**
**Photometric measurement** of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

**Test performance using fully automated analysis devices**

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.

## Pipetting protocol

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | C 3 | P 6 | P 14 | P 22 | | | C 1 | P 3 | P 11 | P 19 | | |
| B | pos. | P 7 | P 15 | P 23 | | | C 2 | P 4 | P 12 | P 20 | | |
| C | neg. | P 8 | P 16 | P 24 | | | C 3 | P 5 | P 13 | P 21 | | |
| D | P 1 | P 9 | P 17 | | | | C 4 | P 6 | P 14 | P 22 | | |
| E | P 2 | P 10 | P 18 | | | | pos. | P 7 | P 15 | P 23 | | |
| F | P 3 | P 11 | P 19 | | | | neg. | P 8 | P 16 | P 24 | | |
| G | P 4 | P 12 | P 20 | | | | P 1 | P 9 | P 17 | | | |
| H | P 5 | P 13 | P 21 | | | | P 2 | P 10 | P 18 | | | |

The pipetting protocol for microtiter strips 1 to 4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7 to 10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 4), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. Therefore, the number of tests performed can be matched to the number of samples, minimising reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

## Calculation of results

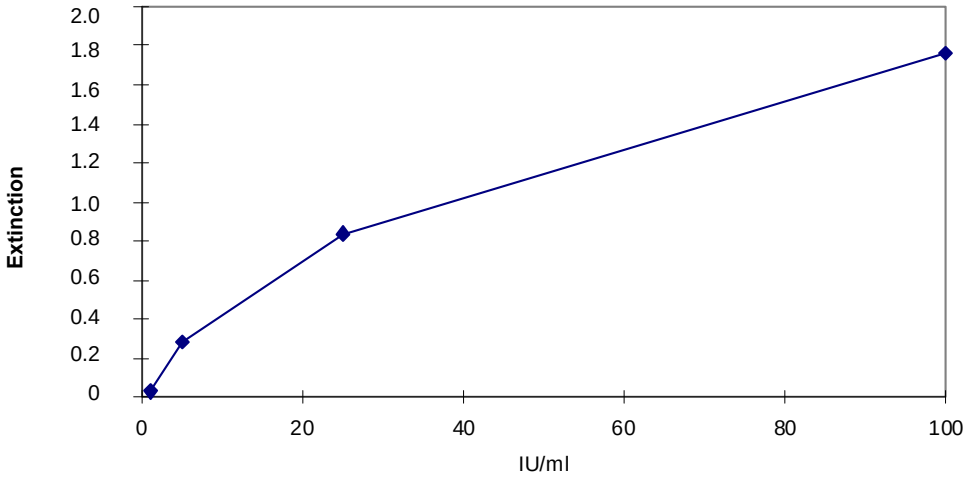
**Semiquantitative:** Results can be evaluated semiquantitatively by calculating a ratio of the extinction of the control or patient sample over the extinction of calibrator 3. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 3}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

| | |
|---|---|
| **Ratio <0.8:** | **negative** |
| **Ratio ≥0.8 to <1.1:** | **borderline** |
| **Ratio ≥1.1:** | **positive** |

**Quantitative:** The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction readings measured for the 4 calibrators against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.




If the extinction for a patient sample lies above the extinction of calibrator 1 (corresponding to 100 IU/ml), the result should be reported as “>100 IU/ml”. It is recommended that the sample be retested at a dilution of e.g. 1:404. The result in IU/ml read from the calibration curve for this sample must then be multiplied by factor 4.

The upper limit of the reference range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **5 international units (IU)/ml**. EUROIMMUN recommends interpreting results as follows:

| | |
|---|---|
| **<4 IU/ml:** | **negative** |
| **≥4 to <5.5 IU/ml:** | **borderline** |
| **≥5.5 IU/ml:** | **positive** |

For duplicate determinations, the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant IgG titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after at least 7 to 10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

## Test characteristics

**Calibration:** The controls of the Anti-Parvovirus B19 ELISA (IgG) were calibrated using the WHO Interantional Standard, International Standard for anti-parvovirus B19 plasma, human calibrated (NIBSC code 01/602). The material contains 77 international units (IU) per ampoule by definition and was resuspended in a concentration of 77 IU/ml.

For every group of tests performed, the extinction readings of the calibrators and the international units determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

**Antigen:** The antigen source is a recombinant viral structural protein expressed in eukaryotic cells.

**Linearity:** The linearity of the Anti-Parvovirus B19 ELISA (IgG) was determined by assaying at least 4 serial dilutions of different patient samples. The Anti-Parvovirus B19 ELISA (IgG) is linear at least in the tested concentration range (1 IU/ml to 100 IU/ml).

**Detection limit:** The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-Parvovirus B19 ELISA (IgG) is 0.1 IU/ml.

**Cross-reactivity:** The quality of the antigen used ensures a high specificity of the ELISA. Sera from patients with infections caused by various agents were investigated with the EUROIMMUN Anti-Parvovirus B19 ELISA (IgG).

| Antibodies against | n | Anti-Parvovirus B19 ELISA (IgG) positive |
|---|---|---|
| **Adenovirus** | 12 | 0% |
| **Bordetella pertussis** | 12 | 0% |
| **Chlamydia pneumoniae** | 12 | 0% |
| **CMV** | 12 | 0% |
| **EBV-CA** | 12 | 0% |
| **Helicobacter pylori** | 12 | 0% |
| **HSV-1** | 12 | 0% |
| **Influenza virus A** | 12 | 0% |
| **Influenza virus B** | 12 | 0% |
| **Measles virus** | 12 | 0% |
| **Mumps virus** | 12 | 0% |
| **Mycoplasma pneumoniae** | 12 | 0% |
| **Parainfluenza virus Pool** | 12 | 0% |
| **RSV** | 12 | 0% |
| **Rubella virus** | 12 | 0% |
| **Toxoplasma gondii** | 12 | 0% |
| **VZV** | 12 | 0% |
| **Yersinia enterocolitica** | 12 | 0% |

**Interference:** Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

**Reproducibility:** The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

| ***Intra-assay variation, n = 20*** | | |
|---|---|---|
| **Sample** | **Mean value (IU/ml)** | **CV (%)** |
| 1 | 21 | 7.7 |
| 2 | 18 | 2.5 |
| 3 | 10 | 2.7 |

| ***Inter-assay variation, n = 4 x 6*** | | |
|---|---|---|
| **Sample** | **Mean value (IU/ml)** | **CV (%)** |
| 1 | 23 | 6.0 |
| 2 | 18 | 1.6 |
| 3 | 10 | 2.1 |

**Sensitivity and specificity:**
Study I: 68 pre-characterised patient samples (origin: Europe; reference method: commercially available ELISA of another manufacturer) were examined with the EUROIMMUN Anti-Parvovirus B19 ELISA (IgG). The sensitivity amounted to 100%, with a specificity of 100%. Borderline results were not included in the calculation.

| n = 68 | | **ELISA of another manufacturer** | | |
|---|---|---|---|---|
| | | positive | borderline | negative |
| **EUROIMMUN Anti-Parvovirus B19 ELISA (IgG)** | positive | 48 | 0 | 0 |
| | negative | 0 | 1 | 19 |

Study II: 273 clinically pre-characterised patient samples (INSTAND, Labquality, NEQAS, RfB and IfQ) were investigated with the EUROIMMUN Anti-Parvovirus B19 ELISA (IgG). The sensitivity amounted to 100%, with a specificity of 98.8%. Borderline results were not included in the calculation.

| n = 273 | | **INSTAND/Labquality/NEQAS/RfB/IfQ** | | |
|---|---|---|---|---|
| | | positive | borderline | negative |
| **EUROIMMUN Anti-Parvovirus B19 ELISA (IgG)** | positive | 185 | 0 | 1 |
| | borderline | 0 | 2 | 0 |
| | negative | 0 | 0 | 85 |

**Reference range:** The levels of anti-parvovirus B19 antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 5 IU/ml, 67.8% of the blood donors were anti-parvovirus B19 positive (IgG).

## Clinical significance

Parvovirus B19 is the smallest ("parvo") known virus, with a genome length of 5000 to 5500 base pairs. It is a single-stranded DNA virus from the family of Parvoviridae and has a diameter of 21 to 23 nm. The virus consists of two viral structural protein types (major and minor structural protein species), which form an icosaedric capsid. Until the discovery of human bocavirus in 2005, parvovirus B19 was the only known strictly human pathogenic virus from the genus Erythroviruses. Its replication takes place predominantly in haematopoietic cells. Parvovirus was discovered in blood donors in 1974 by the Australian virologist Yvonne Cossart. It obtained its name from sample B19 in which it was found by coincidence. The virus has a low sequence variability. Up until now three different genotypes (genotypes 1-3) 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 not greatly affected, and 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 B19 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 hypoproteinemia: oedema, pericardial and pleural effusion, ascites.

Clinically, fifth disease is often difficult to distinguish from rubella. Therefore, clinicians often rely on serology (anti-B19 IgM/IgG). Particularly in adults, fifth disease often proceeds with atypical exanthema.

Banked blood is currently not 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 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 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) plays a secondary role in diagnosis, since patients in the viraemic stage of a B19 infection are mostly asymptomatic. Thus, the detection of B19-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. To narrow down the time of infection, the avidity of specific IgG antibodies (anti-B19 IgG avidity) is determined using microtiter ELISA. This method provides reliable results, in particular when anti-B19 IgM is absent. High avidity excludes infections within the last 4 to 6 weeks.

Therapeutic measures are limited to treating the symptoms. With hydrops fetalis an intrauterine exchange transfusion can substantially improve prognosis. A vaccine is being developed.

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