Antibodies against Parvovirus B19 (IgG)
Test instructions for the Anti-Parvovirus B19 EUROLINE

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DN 2580-1601 G	Parvovirus B19 antigens	IgG	Ag-coated immunoblot strips	16 x 01 (16)

Indications: Test system for the in vitro determination of antibodies against parvovirus B19 in human serum or plasma for the diagnosis of erythema infectiosum. Synonyms: megaloerythema, Sticker's disease, fifth disease.

Application of the Anti-Parvovirus B19 EUROLINE (IgG): The **Anti-Parvovirus B19 EUROLINE (IgG)** is based on recombinant highly specific antigens purified by affinity chromatography which contain linear and conformational epitopes. By using the complete spectrum of antigens, different manifestations of parvovirus B19 infections can be reliably diagnosed.

Principles of the test: The EUROLINE test kit provides a qualitative in vitro assay for human antibodies of the IgG class against the Parvovirus B19 antigens. The test kit contains test strips coated with highly purified antigens. In the first reaction step, diluted patient samples are incubated with the immunoblot strips. 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	Symbol
1. Test strips coated with antigens: VP1, VLP, VP2 and NS1 from Parvovirus B19	1 x 16 strips	STRIPS
2. Positive control (IgG, human), 50x concentrate	1 x 0.04 ml	POS CONTROL 50x
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human-IgG (goat), 10x concentrate	1 x 3 ml	CONJUGATE 10x
4. Universal buffer 10x concentrate	1 x 50 ml	BUFFER 10x
5. Substrate solution NBT/BCIP, ready for use	1 x 30 ml	SUBSTRATE
6. Incubation tray	2 x 8 channels	TRAY
7. Test instruction	1 booklet	---

LOT Lot — CE — Storage temperature

IVD In vitro determination — Unopened usable until

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers:
ZD 9899-0130 Incubation tray with 30 channels
ZD 9898-0130 Incubation tray with 30 channels (black, for EUROBlotCamera system)
ZD 9898-0144 Incubation tray with 44 channels (black, for EUROBlotCamera system)
ZD 9898-0148 Incubation tray with 48 channels (black, for EUROBlotCamera system)
For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive plastic 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 you wish to perform a **visual evaluation**, you may order the required evaluation protocol under:
ZD 2580-0101 Visual-evaluation protocol Anti-Parvovirus B19 EUROLINE.

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.

- **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 ready for use control the amount required should be removed from the bottle using a clean pipette and diluted 1:51 with ready for use universal buffer. Example: add 30 µl of control to 1.5 ml of ready for use diluted universal buffer and mix thoroughly. The ready for use diluted 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 ready for use enzyme conjugate the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with the ready for use diluted universal buffer. Example: For 1 test strip dilute 0.15 ml anti-human IgG concentrate with 1.35 ml ready for use diluted universal buffer. The ready for use diluted 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 ready for use universal buffer shake the bottle. The amount required should be removed from the bottle using a clean pipette and diluted 1:10 with deionised or distilled water. Example: for 1 test strip add 1.5 ml universal buffer (10x concentrate) to 13.5 ml deionised or distilled water. The ready for use diluted universal buffer should be used at the same working day.

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

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: Undiluted patient samples 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.

Warning: The control serum used has tested negative for HBsAg, and antibodies against HCV, HIV-1 and HIV-2 using enzyme immunoassays or indirect immunofluorescence methods. Nonetheless all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents are toxic (buffer, substrate solution). Avoid contact with skin.

Preparation and stability of the patient samples

Sample material: 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 1 4 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 diluted universal buffer. For example, add 30 µl of serum to 1.5 ml ready for use diluted 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 ready for use 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 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**.

EUROIMMUN Anti-Parvovirus B19 EUROLINE

Incubation protocol

Blocking
Pipette 1.5 ml working strength universal buffer into the incubation channel and insert test strip

15 min — Shake

1. Step: Sample incubation
Aspirate off, pipette 1.5 ml of diluted serum sample (1:51) into the incubation channel

30 min — Shake

Washing
Aspirate off, wash 3 x 5 min with 1.5 ml working strength universal buffer each

2. Step: Conjugate incubation
Aspirate off, pipette 1.5 ml working strength enzyme conjugate into the incubation channel

30 min — Shake

Washing
Aspirate off, wash 3 x 5 min with 1.5 ml working strength universal buffer each

3. Step: Substrate incubation
Aspirate off, pipette 1.5 ml substrate into the incubation channel

10 min — Shake

Stopping
Aspirate off, rinse three times with 1.5 ml distilled water

Evaluation
EUROLineScan (digital)

Evaluation and interpretation of the results obtained by the Anti-Parvovirus B19 EUROLINE (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 (with the membrane chips containing the lipids showing a bright background) are then scanned using a flatbed scanner (EUROIMMUN AG) and evaluated with **EUROLineScan**. 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 **ParvoEL_IgG**.
If a visual evaluation must be performed, place the incubated test strips onto the respective work protocol for visual evaluation. This protocol is available at EUROIMMUN under the order no. ZD 2580-0101.

Note: A correctly performed test for class IgG antibodies against Parvovirus B19 antigens 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:
VP1 (viral protein 1):
Structural protein of human parvovirus B19. Highly specific recombinant antigen with predominantly linear epitopes purified by affinity chromatography.

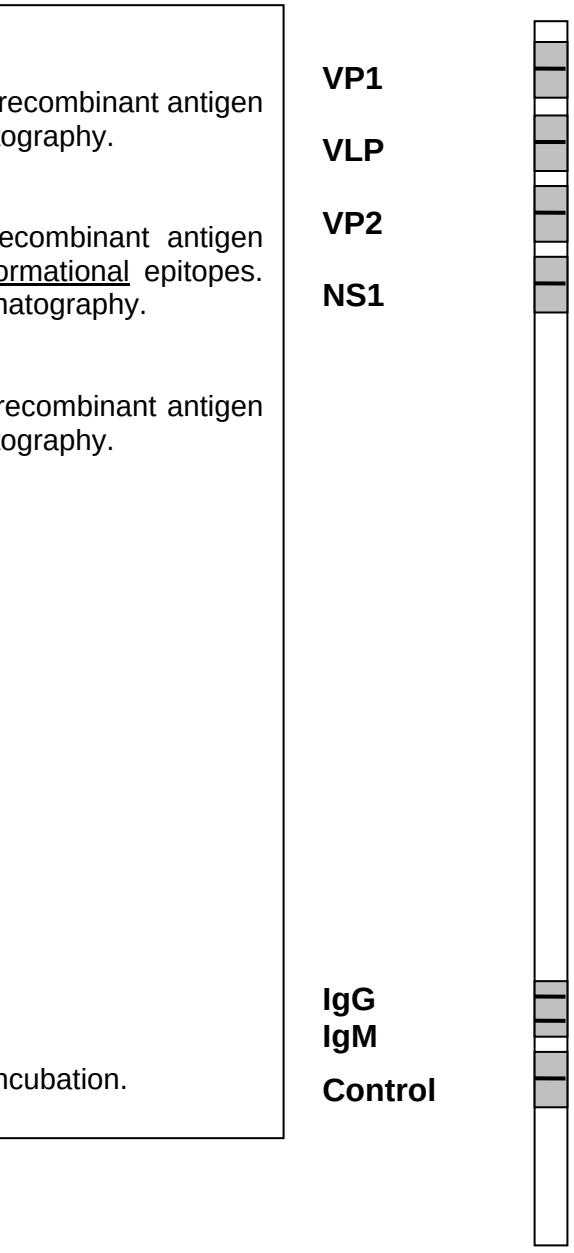
VLP (virus like particle):
Virus particle of human parvovirus B19. Highly specific recombinant antigen purified by affinity chromatography that also contains conformational epitopes. Highly specific recombinant antigens purified by affinity chromatography.

VP2 (viral protein 2):
Structural protein of human parvovirus B19. Highly specific recombinant antigen with predominantly linear epitopes purified by affinity chromatography.

NS1 (non-structural protein 1):
Recombinant antigen purified by affinity chromatography.

Control bands:
IgG or IgM

Control: Incubation control indicating a correctly performed incubation.



Antibodies of class IgG against parvovirus B19

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

Signal	Signal intensity EUROLineScan	Result	
No signal or very weak band	0 - 11	o	Negative
Weak band	12 - 18	(+)	Borderline
Medium to strong band	>18	+	Positive

Evaluation IgG: The results of the Anti-Parvovirus B19 EUROLINE can be divided into negative, borderline and positive results. Antibodies against NS1 have no relevance in the diagnosis but according to literature they can be detected for example in patients with forms of reactive arthritis caused by parvovirus B19.

Result	Characteristics
Negative	No bands or up to two borderline antigen bands at VP1, VLP and/or VP2.
Borderline	Three borderline antigen bands at VP1, VLP and VP2.
Positive	Minimum one positive antigen band at VP1, VLP or VP2.

Test characteristics

Measurement range: The EUROLINE is a qualitative method. No measurement range is provided. The titer limit is given at a dilution of 1:51.

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 repro-ducibility.

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:

Results of interlaboratory tests

n = 65		Labquality/INSTAND/ Reference Institute for Bioanalytics	
		+	-
EUROIMMUN Anti-Parvovirus B19 EUROLINE (IgG)	+	50	0
	(+)	0	0
	-	1	14

65 precharacterised patient samples (Labquality, Finland; INSTAND and Reference Institute for Bioanalytics, Germany) were examined with the EUROIMMUN Anti-Parvovirus B19 EUROLINE (IgG). The test showed a sensitivity of 98% and a specificity of 100%.

Clinical significance

Parvovirus B19 is the smallest (“parvo“) known virus, with a genome length of 5000 to 5500 base pairs. It 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 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 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). 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 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 in development.

Literature reference

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