RIDA® LINE Parvovirus B19 IgG, IgM

Art. No.: LB6023 (IgG)
LB6033 (IgM)
1. Intended use

For *in vitro* diagnostic use. The RIDA® LINE Parvovirus B19 is a qualitative in-vitro test for the detection and identification of antibodies (IgM and IgG) against the parvovirus B19 in human serum or plasma.

Due to the separately coated recombinant components of the virus protein, this test procedure, in contrast to others, allows a statement about the presence of antibodies with different specificity. This allows a better interpretation of the different stages of the illness.

2. General

The human parvovirus B19 was discovered by Cossart and co-workers in England in 1975. They discovered the virus while screening the sera of blood donors for hepatitis B. They named this accidentally found isolate B19.

The parvovirus B19 is a small, non-enveloped DNA virus with a diameter of 18 - 26 nm. The genome consists of single-stranded DNA with a length of 5.4 kb. The capsid consists of two polypeptide proteins of about 84 and 58 kD (VP1 and VP2). Both structural proteins are encoded in a common reading frame on the viral genome, whereby the shorter VP2 has a later transcription start in the reading frame of the VP1 (see pt. 12). This means that both proteins are identical, except for one additional N-terminal part of VP1 which has a size of approximately 26 kD. Furthermore, a „main-non-structure protein“ (NS-1) is encoded in the genome; it is necessary for the virus replication and multiplication. The B19 virus has an icosahedral structure and is very heat-stable. It can sustain a temperature of 60 °C for up to 12 h.

The genus of the parvoviruses comprises - beside the only known human parvovirus B19 - animal pathogens like the canine parvovirus and the feline panleukopenia virus. Together with the denso viruses and the dependo viruses, that need helper viruses (adeno or herpes viruses) for replication, the parvoviruses form the family of parvoviridae.

One significant characteristic of the parvoviruses is their ability to selectively replicate in self-dividing cells such as bone marrow, intestinal and embryonic cells.

Infections with the worldwide distributed human parvovirus B19 occur more frequently during the winter and early summer seasons. It may arise to epidemic outbreaks especially in day care and schools. The transmission is mainly caused via the respiratory system (droplet infection), but it can also be transmitted by blood and blood products. Parvovirus infections occur in all age groups, but are most common between the ages of 6 and 15 years. The prevalence increases with age and reaches a value of 40 - 70% for adults. Women show a higher prevalence than men.

The incubation time lasts 1 - 3 weeks. The viremic phase in which the patient is considered infectious, is limited to 3 - 7 days. During this time, the patient can be asymptomatic or he can complain about some prodromal illnesses such as headache, itchiness, myalgia and fever. Approximately two weeks after infection, an erythema infectiosum (fifth disease) can occur. Children usually show typical exanthemas (redness of cheeks, lattice-like febrile purpura
especially along the extensor surfaces of the extremities), but these are rather untypical for adults. Further symptoms of a B19 infection are arthralgias and arthritis (60%), respiratory symptoms (22%) as well as diarrhea (15 - 35%). Only approximately 20% of all parvovirus B19 infections are symptomless. Women are affected by joint pain twice as much as men. In most cases, the arthropathies disappear within two to four weeks. In a few cases, the symptoms can persist intermittently over years. So that if chronic arthropathies should be verified, a persistent parvovirus B19 infection must also be considered.

The inhibition of erythropoiesis (duration 1 - 4 weeks), which is caused by parvovirus B19, usually remains subclinical in healthy persons, but it can cause an aplastic crisis in patients with chronic hemolytic anaemia (sickle cell anaemia, thalassaemia, pyruvate kinase deficiency, spherocytosis, autoimmune hemolytic anaemia). Such patients require transfusions and should be submitted to a hospital. Additional organ manifestations such as hepatic dysfunctions or a B19 associated myocarditis have also been reported recently.

A further complication following a B19 infection can occur in sero-negative pregnant women. The parvovirus B19 can be transmitted via the placenta to the fetus. Due to the short viability of the erythrocytes up to the 20th week of pregnancy and a missing efficient immune defense, an infection can lead to a severe acute or chronic anaemia and subsequent spontaneous abortion. A further complication after infection during the second trimester, is the development of a hydrops fetalis. However, more than 80% of the pregnancies course without complications. Embryopathies have not been observed so far.

Antibodies of the IgM group can be detected approximately 10 days after the infection and usually occur with the beginning of the symptoms. IgG antibodies can already be found some days later and usually remain for life. However, IgM antibodies cannot be detected after 30 - 60 days. Elisa systems, on the basis of recombinant VP1/VP2 peptides, are suitable for the detection of such antibodies. Because of a more specific statement regarding the possible stages of the illness, due to the detection of different antibodies, a blot such as the RIDA® LINE Parvovirus B19, which uses different recombinantly produced proteins of the virus antigen, should always be used additionally.

3. Test principle

The RIDA® LINE Parvovirus uses six recombinant parvovirus B19 antigens, which are produced in E. coli. This involves parts of the two structural proteins, VP1 and VP2, and the „main-non-structure protein“ NS-1 (see Ch. 12.).

The cleaned, recombinant antigens are applied to a nitro-cellulose membrane. Following that, the membrane is cut into individual blot strips. These strips are incubated with the sample (serum or plasma) that is to be examined. During incubation, the specific antibodies present in the sample will bind to the corresponding protein bands. After removal of unbound antibodies by a washing step, a second incubation with peroxidase-conjugate (anti-human IgM or IgG) and subsequent color reaction, specifically bound antibodies against the Parvovirus B19 antigens become visible as blue bands on the strips.
4. Reagents provided

The reagents of one pack are sufficient for 20 determinations.

Table 1: Pack contents

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Description</th>
<th>LB6023 IgG</th>
<th>LB6033 IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strip</td>
<td>20</td>
<td>Numbered test strips (1 - 20); coated with recombinant Parvovirus B19 antigens</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Wash/Diluent</td>
<td>100 ml</td>
<td>Wash- and sample dilution buffer; 10 fold concentrated phosphate buffer; contains NaCl, KCl, detergent, Methylisothiazolone and Oxypyrione</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>MP</td>
<td>5 g</td>
<td>Skim milk powder</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Conjugate IgG</td>
<td>100 μl</td>
<td>Anti-human IgG conjugate; 100 fold concentrate; POD-conjugated antibody (rabbit); contains NaN₃</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Conjugate IgM</td>
<td>100 μl</td>
<td>Anti-human IgM conjugate; 100 fold concentrate; POD-conjugated antibody (rabbit); contains NaN₃</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Substrate</td>
<td>40 ml</td>
<td>Substrate; ready for use; contains Tetramethylbenzidin</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Instructions</td>
<td>1</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Evaluation sheet</td>
<td>1</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

5. Storage instructions

All reagents have to be stored at 2 – 8 °C and can be used up to the expiry date printed on the labels. The concentrated conjugates have to be diluted freshly in required amounts. The diluted buffer can be used up to for weeks if it is stored at 2 – 8 °C. Microbial contamination has to be avoided.

Reagents from kits with different lot numbers should not be interchanged.

6. Materials required but not provided

6.1. Reagents

– Deionized water
6.2. Accessories

- Incubation trays (please order with Art. No. LB0003 if required)
- Vacuum pump to remove liquid out of the incubation wells
- Pipets for 20 μl, 100 - 1000 μl and 1 - 5 ml
- Measuring cylinder for 100 ml and 500 ml
- Shaker
- Laboratory balance
- Plastic tweezers to handle the strips
- Perhaps: RIDA® Aviditätsreagenz, Art. No. LB0023 (see 12.5.)

7. Warnings and precautions for the users

For in vitro diagnostic use only.

The test has to be used only by experienced laboratory staff. Please refer to guidelines for safety regulations in medical laboratories. The test protocol must be followed strictly.

Do not mouth pipette samples or reagents. Avoid contact with injured skin or mucous membranes. Wear disposable gloves while handling samples and wash hands after assay is complete. Do not smoke, eat or drink in areas where specimen or kit reagents are handled.

The conjugates contain NaN₃. Contact with skin or mucous membranes should be avoided. Contact with lead or copper pipes can form explosive metal azides.

Patient samples should be considered potentially infectious and be treated with respect to the given national safety precautions.

All reagents and materials coming in contact with potential infectious specimens must be treated with disinfectants or autoclaved at 121 °C for at least one hour.
8. Specimen collection and storage

The RIDA® LINE Parvovirus B19 can be carried out with serum or plasma. Heat inactivated samples can lead to higher background reactions. Repeated freezing and thawing of the samples as well as microbial contamination must be avoided. The application of lipemic, hemolytic or turbid samples can cause a dark background coloration of the strips. Furthermore, these samples can lead to false results and should not be used.
The samples can be stored for up to 2 weeks at 2–8 °C if the test cannot be carried out immediately. A prolonged storage of samples is possible at -20 °C.

9. Test procedure

9.1. Preliminary comments

Bring all kit components to room temperature (18 – 25 °C) prior to use for at least 30 minutes. The vial containing the strips [Strip] should be opened thereafter to avoid formation of condensed water. The strips that are not required remain in the vial and continue to be stored at 2-8°C (vial should be closed tightly, strips have to remain dry prior to use!).
Remove only the volume of reagents that is needed for test procedure. Do not pour reagents back into vials as reagent contamination may occur.
Strips and reagents must not be used if pouch is damaged or vials are leaking.
The strips do not have to be used in their order of numeration. But it is important to keep a record about which strip is incubated with the corresponding sample.
The reproducibility of the blot results depend very strongly on the consistency of washing the strips. The washing steps described below should always be adhered to.
Control bands are applied to each strip: reaction control, immunoglobulin control (IgG, IgM), cut off control. Therefore, an incubation of additional test strips with separate control sera is not necessary.

9.2. Preparation of the ready to use wash/dilution buffer

This buffer [Wash/Diluent] 10x is used for both serum and conjugate dilution as well as for the washing steps.
Prior to dilution, the volume of wash buffer required for the corresponding number of tests must be determined. See table 2 for the amounts required. Volumes for numbers of test strips not listed in the table have to be determined by calculation.
The skim milk powder [MP] is first dissolved in wash buffer concentrate. This mixture is then filled up with deionised water to the final volume (dilution: 1:10). The ready to use buffer can be stored at 2–8 °C for four weeks.
Table 2: Wash/dilution buffer per applied strip

<table>
<thead>
<tr>
<th>applied strips</th>
<th>milk powder</th>
<th>concentrated buffer</th>
<th>deionized water</th>
<th>ready to use buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1 g</td>
<td>2 ml</td>
<td>18 ml</td>
<td>20 ml</td>
</tr>
<tr>
<td>2</td>
<td>0.2 g</td>
<td>4 ml</td>
<td>36 ml</td>
<td>40 ml</td>
</tr>
<tr>
<td>3</td>
<td>0.3 g</td>
<td>6 ml</td>
<td>54 ml</td>
<td>60 ml</td>
</tr>
<tr>
<td>5</td>
<td>0.5 g</td>
<td>10 ml</td>
<td>90 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td>10</td>
<td>1.0 g</td>
<td>20 ml</td>
<td>180 ml</td>
<td>200 ml</td>
</tr>
<tr>
<td>15</td>
<td>1.5 g</td>
<td>30 ml</td>
<td>270 ml</td>
<td>300 ml</td>
</tr>
<tr>
<td>20</td>
<td>2.0 g</td>
<td>40 ml</td>
<td>360 ml</td>
<td>400 ml</td>
</tr>
</tbody>
</table>

9.3. Preparation of the conjugate solution

The conjugate enclosed in the kits [Conjugate IgG | 100x] or [Conjugate IgM | 100x] is provided as a concentrate. For its application to the test, it must be diluted with the ready to use buffer. Since the diluted conjugate is stable only for a limited period of time, it must be prepared freshly before starting a test.

One part of conjugate (IgG resp. IgM) concentrate is diluted with 99 parts of ready to use buffer (1:100). See table 3 for the amounts required. Volumes for numbers of test strips not listed in the table have to be determined by calculation.

Table 3: Preparation of the ready to use conjugate solutions

<table>
<thead>
<tr>
<th>applied strips*</th>
<th>conjugate concentrate IgG resp. IgM</th>
<th>ready to use wash/dilution buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 µl</td>
<td>2 ml</td>
</tr>
<tr>
<td>2</td>
<td>40 µl</td>
<td>4 ml</td>
</tr>
<tr>
<td>3</td>
<td>60 µl</td>
<td>6 ml</td>
</tr>
<tr>
<td>5</td>
<td>100 µl</td>
<td>10 ml</td>
</tr>
<tr>
<td>10</td>
<td>200 µl</td>
<td>20 ml</td>
</tr>
<tr>
<td>15</td>
<td>300 µl</td>
<td>30 ml</td>
</tr>
<tr>
<td>20</td>
<td>400 µl</td>
<td>40 ml</td>
</tr>
</tbody>
</table>

* The volumes have been calculated without dead volume. Depending on handling (manually or by machine processing) please prepare conjugate solution for additional 1 to 3 strips.
9.4. First Incubation

For each determination, use one well of the incubation tray (see 6.2.) and fill it with 2 ml diluted wash/dilution buffer. For each determination, one blot strip is taken out of the vial with a pair of tweezers (touch the strips at the end with the numeration only). Dip the strips into the buffer. Record the number of the strip vial and the strip numbers on the evaluation sheet.

**The strips must be completely wetted with buffer. The strip numeration must show upwards.**

20 μl of the undiluted sample (serum or plasma) are added to the corresponding wells (dilution 1:101). Record the sample number and the immunoglobulin class to be detected on the evaluation sheet. Please be sure to add the sample at one end of the immersed strips into the wash buffer and mix as soon as possible by shaking the tray carefully. It must be taken care that the neighboring wells are not contaminated since this could lead to false positive results. During the incubation, the trays should be covered up with the enclosed lids. Be particularly careful to avoid splashing when opening and closing the lid.

The blot strips with the samples are placed on a horizontal shaker and are incubated for 1 hour at room temperature (18 – 25 °C).

9.5. Washing

Remove the solution (via suction), preferably with an attached disinfection trap. When the solutions have been aspirated from a well, the pipette tip has to be changed or rinsed well with deionised water after each aspiration procedure to prevent cross-contamination. In the case of machine processing the references of the equipment manufacturer have to be considered.

Then place 2 ml of the ready to use wash buffer in each well and wash on the shaker while shaking gently for 5 minutes. The wash buffer is aspirated after the washing procedure. Carry out this washing step a total of three times.

9.6. Second incubation

Add 2 ml of the corresponding ready to use conjugate solution to each strip. Incubate the strips for 45 minutes on a horizontal shaker at room temperature (18 – 25 °C).

9.7. Washing

Wash sequence: see 9.5.
9.8. Third incubation

Add 1.5 ml of the ready to use Substrate solution to each strip. Incubate the strips for 5 - 10 minutes at room temperature (18 – 25 °C), until the cut off band becomes visible. Thereafter, the substrate is removed via suction. Still in their wells, the strips should be rinsed shortly three times with deionized water to stop the color reaction. The strips are carefully taken out of the wells with tweezers. To dry the strips, they are placed between filter paper for about 2 hours. After drying the strips can be adhesively attached to the enclosed evaluation sheet and the results can be recorded.

Summary of the test procedure

1. Bring all reagents to room temperature
2. Pipet 2 ml wash/dilution buffer into each well
3. Place one strip into each well
4. Add 20 µl sample
5. 1 hour incubation at room temperature
6. Wash 3 times with 2 ml wash/dilution buffer; 5 min each time
7. Add 2 ml freshly prepared conjugate solution
8. 45 min incubation at room temperature
9. Wash 3 times with 2 ml wash/dilution buffer; 5 min each time
10. Add 1.5 ml substrate
11. 5 - 10 min incubation at room temperature
12. Rinse strips with deionized water
13. Dry on filter paper
14. Evaluation
10. Quality control – indications of instability or deterioration

The packages have an expiry date after which a quality warranty cannot be given. Additionally, control bands are applied to each strip: reaction control, immunoglobulin control (IgG, IgM), cut off control.

The incubation control band can be found below the numeration. It must show a clear reaction with each serum sample.

The immunoglobulin bands serve as controls for the antibody class detected in each case. For instance, if the test strip is used to detect IgG antibodies, the IgG control band shows a clearly defined band. The other conjugate control band may develop a weak, non-specific colouring. This applies analogously to the IgM band.

The cut off control serves as control of the coloration process and evaluation of the test strip. The intensity of this band provides a basis for evaluation of the antibody reactivity as positive, borderline or negative. It should be of low intensity but nevertheless clearly visible.

A missing reaction of the applied control bands may indicate that the reagents have expired. If the expected conditions are not fulfilled please check the following before repeating the test:

– expiration date of the reagents
– calibration of the used instruments
– exact test procedure
– visual examination of kit components for signs of contamination or leakage

If the conditions are not fulfilled after repeating, please contact your local distributor of R-Biopharm.

11. Evaluation and interpretation

Attach the corresponding test strips with a glue stick into the corresponding fields of the evaluation sheet. To do this, place the test strips with the reaction control band on the marking lines. Then use clear adhesive tape to attach the test strips left from the marking line. Complete sticking of the test strip with glue or adhesive tape may lead to aberrations of the colouring. Record the date and lot number on the evaluation sheet.

Now identify the bands of the developed test strips based on the printed control strip on the evaluation sheet and record them in the protocol sheet (table 4). Identify the bands on the test strips separately for the respective immunoglobulin classes.
Table 4: Analysis of the band intensity

<table>
<thead>
<tr>
<th>Band intensity</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>invisible</td>
<td>-</td>
</tr>
<tr>
<td>very weak intensity</td>
<td>+/-</td>
</tr>
<tr>
<td>(weaker than the cut off band)</td>
<td></td>
</tr>
<tr>
<td>weak intensity</td>
<td>+</td>
</tr>
<tr>
<td>(comparable to the cut off band)</td>
<td></td>
</tr>
<tr>
<td>strong intensity</td>
<td>++</td>
</tr>
<tr>
<td>(stronger than the cut off band)</td>
<td></td>
</tr>
<tr>
<td>very strong intensity</td>
<td>+++</td>
</tr>
</tbody>
</table>

The band patterns in RIDA® LINE Parvovirus B19 IgG and IgM detection tests may show varying intensities. It is possible that the IgG test shows more intense and darker bands than the IgM. The intensity of the protein bands depends on the concentration of the Parvovirus B19-specific antibodies.

For the positive/negative analysis, a possibly existing reactivity of antibodies against the NS-1 protein is not taken into consideration.

Table 5: IgM-Analysis

<table>
<thead>
<tr>
<th>IgM positive</th>
<th>IgM questionable</th>
<th>IgM negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>two of the following bands at least + :</td>
<td>one of the following bands at least + :</td>
<td>no reaction or other constellations</td>
</tr>
<tr>
<td>VP-2p</td>
<td>VP-N</td>
<td></td>
</tr>
<tr>
<td>VP-N</td>
<td>VP-1s</td>
<td></td>
</tr>
<tr>
<td>VP-1s</td>
<td>VP-2r</td>
<td></td>
</tr>
<tr>
<td>VP-2r</td>
<td>VP-C</td>
<td></td>
</tr>
<tr>
<td>VP-C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6: IgG-Analysis

<table>
<thead>
<tr>
<th>IgG positive</th>
<th>IgG questionable</th>
<th>IgG negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>two of the following bands at least +:</td>
<td>one of the following bands at least +:</td>
<td>no reaction or other constellations</td>
</tr>
<tr>
<td>VP-N</td>
<td>VP-N</td>
<td></td>
</tr>
<tr>
<td>VP-1s</td>
<td>VP-2r</td>
<td></td>
</tr>
<tr>
<td>VP-2r</td>
<td>VP-C</td>
<td></td>
</tr>
<tr>
<td>VP-C</td>
<td>or</td>
<td></td>
</tr>
<tr>
<td>VP-2p at least +</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12. Limitations of the method

12.1. Diagnostic significance of recombinant B19 antigens

The parovirus B19 genome with a size of approximately 5.4 kb contains the genetic information of two structural proteins and one non-structure protein (see fig.1). The capsid which envelops the DNA is formed with the two structural proteins VP1 and VP2. Both structure proteins with a size of 84 kD and 58 kD are encoded on the common reading frame of the viral genome, whereby the smaller VP2 has a downstream transcription start on the reading frame of VP1. This means that both proteins are identical except for an additional N-terminal sequence on VP1 which has a size of 26 kD. The non-structure protein (NS-1) with a size of approximately 77 kD is necessary for the virus replication and multiplication.

The RIDA® LINE blot uses Parovirus B19 antigens made with the help of genetic engineering. They are produced as nonfusin proteins in E. coli alternatively in eukaryotic cell cultures.
Structure of the Parvovirus genome:

```
5'  NS-1  VP1  VP2  3'
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Antigens used in the RIDA® LINE verwendete:

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NS-1  VP-N  VP-1S  VP-C  VP-2p; VP-2r
```

Figure 1: Structure of the Parvovirus genome and recombinant antigens

Serological interpretation based on division of the structural protein VP1 into two segments, and separate presentation of linear and conformational epitopes, reveals the possible time of infection. Generally speaking, IgG antibodies to linear epitopes on the entire structural protein are produced at the onset of the immune response subsequent to primary infection; that is to say a patient sample with this immune status recognizes both the N-and C-terminal regions (VP-N and VP-C, as well as VP-2r and VP-1S). In the course of a period of approx. 6-9 months, the VP-C-specific IgG antibodies directed against linear epitopes are replaced by antibodies that show a preference for recognition of conformational epitopes. These epitopes are presented by the protein VP-2p. The corresponding antibodies normally show lifelong persistence. With increasing duration of the primary infection, the disappearance of the reaction to VP-C (usually after 6 months) and VP-2r (usually after a few years), becomes visible on the strips, whereas the IgG antibodies to VP-N (and partly antibodies to VP-1S as well) remain detectable for many years; antibodies to VP-2p show lifelong persistence in most cases.

In addition, the non-structural protein NS-1 of Parvovirus B19 is also present on the test strip. According to the literature, IgG antibodies against NS-1 are to be found in patients with Parvovirus B19 induced arthritis and other chronic forms of the disease, but not in patients suffering from acute infections.
As a result of the infection titer in the population, a diagnosis can never be made solely on the basis of anti-NS-1, but must always be supported by a positive PCR result. If a persistent Parvovirus B19 infection is suspected, the presence of a NS-1 titer can only be regarded as an indication.

Table 7: Description and diagnostic significance of the used antigens

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Description and diagnostic significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP2 (VP-2p, VP-2r)</td>
<td>main capsid antigen; IgG antibodies to the conformation epitopes (VP-2p) normally show lifelong detectability, those to the linear epitopes (VP-2r) are detectable for months to a few years. Both antigens show good reactivity in the early phase of the infection.</td>
</tr>
<tr>
<td>VP-N</td>
<td>N-terminal fragment of the capsid antigen VP1; IgG antibodies often detectable for years. Taken into account in avidity testing.</td>
</tr>
<tr>
<td>VP-C</td>
<td>C-terminal fragment of capsid antigens VP1 resp. VP2; the reactivity (IgG and IgM) of the linear epitopes appears early in the course of a Parvovirus B19 infection, then disappears soon after (IgG: usually after about 6 months, IgM: usually after about 12 weeks at the latest).</td>
</tr>
<tr>
<td>VP-1S</td>
<td>N-terminal, specific fragment of the capsid antigen VP1 (the fragment in which VP-1 and VP-2 differ), IgG antibodies remain detectable long after an infection. Taken into account in avidity testing.</td>
</tr>
<tr>
<td>NS-1</td>
<td>Non-structural protein 1, appears in IgG at the earliest 6-8 weeks after infection in at least 20% of ill persons, may be indicative of virus persistence.</td>
</tr>
</tbody>
</table>

12.2. Interpretation

When evaluating the test results, IgG and IgM findings should always be considered together.

In all test interpretations, especially of weak positive results, it is important to include any other clinical record data available. Close co-operation between laboratory and the physician in charge of treatment is recommended here as well. Samples with unclear or borderline results should be rechecked after 2 - 3 weeks in keeping with the clinical situation.

A negative result in the immunoblot does not exclude an infection with parvovirus B19. Very early, at the beginning of the infection, the immune response can be so weak, that a serological detection is not possible. In this case, if a clinical suspicion persists or if the result of the immunoblot is questionable, a consecutive serum should be examined. If a distinct seroconversion is not visible, a parvovirus B19 infection is not likely.
Isolated positive IgM reactivities (no IgG antibodies) should be carefully interpreted, it may concern an acute infection in the early phase of infection.

Weak IgM reactivity can be caused by persistent IgM antibodies from an infection in the more distant past, by reactivated IgM antibodies, e.g., in the case of an Epstein Barr virus infection, or by non-specifically attached antibodies.

If the result in the IgM detection is positive or weakly positive, the IgG result should also be taken into consideration. In an **acute infection**, a pronounced IgG reactivity to VP-C and VP-2r is observed in addition to VP-2p, VP-N and VP-1S. This pronounced reactivity to VP-C is normally found in an incipient or recent-past infection (duration usually no longer than 6 months). If no IgG reactivity to VP-C occurs, an acute infection can be excluded with a high level of probability; if suspicion of infection persists, the course should be monitored.

In cases of **long-past infections**, an IgG reactivity to VP-2p and/or VP-N (usually with VP-1S) is observed in most cases.

**Avidity testing:** In most cases, determination of the avidity of the IgG antibodies to the specific individual antigens allows for a more exact definition of the actual infection status, in particular facilitating differentiation of an acute Parvovirus B19 infection from a subacute infection with persisting IgM antibodies (see 12.5.).

Use of icteric sera may lead to an increased number of positive results.

Some patient sera may cause a dark, continuous or patterned coloration on the entire nitrocellulose strip (e.g. sera from patients with milk protein allergies). Various different factors in each patient serum are responsible for this effect. Evaluation of these strips is usually feasible in a restricted sense only. For instance, "inverse" bands (white bands on a dark background) must be evaluated as negative. The corresponding serum should in any case be tested using other serological methods.

12.3. Typical reaction profiles in the RIDA® LINE Parvovirus B19

Due to evaluations that have been carried out so far, the following IgM/IgG reaction patterns can be an indication of a specific parvovirus B19 status. It must be pointed out that this involves typical reaction patterns, but they can also show deviations.

The analysis of titers against the NS-1 was not considered in table 8 for the clarification of persistent parvovirus B19 infections, reactive arthropathies, chronic anaemias or other symptoms with suspicion of a B19 persistence, since depending on the duration, the clinic and the immunocompetence, numerous combinations are possible.
<table>
<thead>
<tr>
<th>IgG</th>
<th>IgM</th>
<th>possible B19 status</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP-2p, VP-N, VP-2r, VP-C (reactivities mostly ≤ +)</td>
<td>VP-2p, VP-N (reactivities mostly ≥ ++)</td>
<td>acute infection, IgG titer still weak or absent</td>
</tr>
<tr>
<td></td>
<td>VP-2r, VP-C (reactivities mostly + or ++)</td>
<td></td>
</tr>
<tr>
<td>VP-2p, VP-N, VP-1S, VP-2r, VP-C (reactivities mostly ≥ ++)</td>
<td>VP-2p, VP-N and VP-2r (reactivities mostly ≥ +)</td>
<td>status after infection (weeks to months) with IgM and IgG response</td>
</tr>
<tr>
<td>VP-2p, VP-N, VP-2r and VP-C mostly with VP-1S (reactivities mostly ≥ ++)</td>
<td>negative or questionable (isolated reactivities with ≤ +)</td>
<td>status after infection (months)</td>
</tr>
<tr>
<td>VP-2p and/or VP-N with VP-1S (reactivities mostly ≥ ++) negative: VP-2r and VP-C</td>
<td>negative</td>
<td>long past infection (years)</td>
</tr>
<tr>
<td>VP-2p, VP-N and VP-2r, (reactivities mostly just +)</td>
<td>VP-2p, VP-N and/or VP-2r (possibly VP-1S) (reactivities mostly just +)</td>
<td>unclear status, possibly caused by boosting, test should be repeated at time intervals</td>
</tr>
<tr>
<td>VP-2p, VP-N, VP-1S (reactivities mostly ≥ +) negative: VP-2r and VP-C</td>
<td>VP-N, VP-1S (reactivities mostly just +)</td>
<td>past infection by IgG response and atypical IgM reactivity, constellation was founded in some rheumatic factor positive samples, test should be repeated at time intervals</td>
</tr>
</tbody>
</table>

12.4. Analysis of reactivity against NS-1 (IgG)

The NS-1 response should only be considered in term of IgG. In evaluation of the result for anti-Parvovirus B19 – positive/negative – the NS-1 response should not be taken into consideration in normal cases.

When determining the possible time of infection, positive NS-1 reactivity in the IgG will generally mean that the infection is at least 6 weeks old. It must, however, also be remembered that NS-1 antibodies are only produced in about 20 % of cases.
In the context of relevant symptoms and preliminary clinical findings, an NS-1 titer may be an indication of a possible persistence of this virus.

However, the diagnosis “persistent Parvovirus B19 infection“ must never be made on the basis of serological findings alone. The detection of virus antigen or virus DNA must always be demanded. It must also be taken into account that NS-1 IgG antibodies do not develop until after a certain amount of time, so that a PCR DNA would not necessarily be positive at every point in time in a case of persistence. For this reason, a number of tests should always be carried out at intervals during the course of such cases.

12.5. Extension of diagnosis with avidity determination

Parvovirus B19 diagnosis can be advanced by determining the avidity of the IgG antibodies. IgG antibodies undergo a maturation process whereby early-phase antibodies show a low avidity level and post-infection antibodies show a high level of avidity. Low-avidity antibodies can be washed off the strip binding location with an avidity reagent, whereas high-avidity antibodies are not removed by the same reagent. Parallel processing of two IgG charges, one of which must be treated with the avidity reagent, can determine whether the IgG antibodies are of low-avidity (acute infection) or high-avidity (past infection).

The avidity determination of Parvovirus B19 IgG can be done with the RIDA® Aviditatsreakenz (Art. No. LB0023). The test procedure is described in detail in the avidity reagent’s insert.

The avidity is analysed by IgG antibodies against VP-N and VP-1S. High-avide antibodies against the linear epitopes of VP-2r and VP-C present in the RIDA®LINE Parvovirus B19 are not developed in many cases during the course of infection. The conformational epitopes of VP-2 antigen are modified by using the avidity reagent, therefore the avidity determination of VP2 is not reliable.

Analysis and interpretation of avidity in the RIDA® LINE Parvovirus B19 IgG

Avidity determination is done by IgG antibodies against VP-N and VP-1S only.

Reactivities on the IgG strip below the cut off control (very weak intensities) should not be considered.

Compare the intensities of the corresponding bands on the two test strips incubated with the same patient sample (IgG strip and avidity strip). Check whether the intensities have changed.

At low avidity the intensity should be reduced by at least 50 %, at intermediate avidity the intensity is reduced by approx. 50 %.

At high avidity levels, the band intensity on the avidity strip is reduced either not at all or practically not at all.
The IgG antibodies to VP-N and VP-1S reach a high avidity level at the earliest in the course of about 4 weeks and at the latest 6 to 8 weeks after infection. A low resp. intermediate avidity of the VP-N and VP-1S band is therefore to be interpreted as a clear indication of an acute infection. If highly avid IgG antibodies to VP-N and VP-1S are present, an infection within the past 4 weeks can usually be excluded. If the avidity levels of VP-N and VP-1S deviate from one another, the avidity of VP-N is then decisive.

Generally speaking, no absolute rules apply to the assessment of avidity. In individual cases, it must be kept in mind that low avidity levels are possible in past infections as well, since the maturation of avidity may be delayed. The interpretation of avidity must always be assessed within the context of all test results.

13. Performance characteristics

Patient samples from different sources were tested to assess the performance capability of RIDA®LINE Parvovirus B19 IgG and IgM. This study considered a total of 298 IgG res. 287 IgM determinations. Additional 70 potentially falsifying samples and 10 serum-plasma comparisons were tested. 67 serum samples were tested for avidity determination of the IgG antibodies.

13.1. Patients samples from acute infection with Parvovirus B19

A panel of 52 human sera samples from patients supposed to be recently infected with Parvovirus B19 was analysed, included 12 courses of an infection (2 to 5 samples of each patient).
In all 12 courses of an infection specific Parvovirus B19 antibodies were found. In some cases, the first patient sample in the course of an infection was positive; in cases in which the samples were first negative, a seroconversion was observed in the following samples. IgM antibodies remained detectable in eleven of these twelve courses of infection until at least the 42nd day after initial occurrence of symptoms.

13.2. Blood donors

187 (IgG) and 176 (IgM) non-selected blood donors plasma were tested. These samples were tested with a reference ELISA Parvovirus B19 testing system (see 13.3.), also.
IgG result: 66 % of patient samples had IgG antibodies, a lot of these positive samples showed a typical reaction pattern as long past infections (see table 8).
IgM result: 3 % of patient samples had IgM antibodies.

13.3. Comparison of the RIDA®LINE Parvovirus B19 with a reference B19 ELISA

The agreement between the two Parvovirus B19 testing systems was good; the level of agreement of the results for detection of IgG antibodies was approx. 98 %; in detection of IgM antibodies, the determined agreement levels for the results was between 93.1 % and 95.5 % in
different patient collectives. In the calculations of the “agreement of the two testing systems,” borderline results were assessed to be positive results.

Table 9: results of 101 serum samples of patients with acute or recent Parvovirus B19 infection

<table>
<thead>
<tr>
<th></th>
<th>reference ELISA IgG Parvovirus B19 IgG/IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>RIDA® LINE Parvovirus B19 IgG</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>questionable</td>
</tr>
<tr>
<td></td>
<td>negative</td>
</tr>
<tr>
<td>RIDA® LINE Parvovirus B19 IgM</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>questionable</td>
</tr>
<tr>
<td></td>
<td>negative</td>
</tr>
</tbody>
</table>

sum IgG resp. IgM 101
agreement between the two IgG testing systems 98.0%
agreement between the two IgM testing systems 93.1%

Table 10: Results of blood donor testing

<table>
<thead>
<tr>
<th></th>
<th>reference ELISA IgG Parvovirus B19 IgG/IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>RIDA® LINE Parvovirus B19 IgG</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>questionable</td>
</tr>
<tr>
<td></td>
<td>negative</td>
</tr>
<tr>
<td>RIDA® LINE Parvovirus B19 IgM</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>questionable</td>
</tr>
<tr>
<td></td>
<td>negative</td>
</tr>
</tbody>
</table>

sum IgG 187
sum IgM 176
agreement between the two IgG testing systems 97.8%
agreement between the two IgM testing systems 95.5%
13.4. Determination of sensitivity by WHO standard IgG

The WHO standard IgG (100 IU/ml) was tested in dilutions derived from that standard in RIDA® LINE Parvovirus B19 IgG and in a reference ELISA IgG. The WHO standard IgG still showed a borderline result (isolated positive VP-N reactivity) at a dilution of 1.5 IU/ml. The reference ELISA IgG also showed a borderline result at this level.

13.5. Potentially falsifying samples

Investigations of potential interference factors revealed no evidence that test results were affected in cases of lipaemic patient samples, CRP-positive or in ANA-positive patient samples. Haemolytic or icteric samples or samples from patients that were positive for rheumatism factor showed a slightly increased number of positive IgM results (see table 8).

In cases of infectious mononucleosis (Pfeiffer’s disease, EBV infection), a polyclonal stimulation of B lymphocytes may occur. This may lead to positive reactions in IgM class antibody detection tests.

13.6. Serum-plasma comparison

Analysis of 10 samples from patients revealed no differences between serum and plasma analyses.

13.7. Avidity evaluation data (IgG)

67 serum samples from cases of acute, recent-past and long-past Parvovirus B19 infections were tested in the RIDA® LINE Parvovirus B19 IgG for avidity of the IgG antibodies to VP-N and VP-1S.

The results of the avidity test correlated reliably with the clinical data and the interpretation of the reactivities of the individual antigens with IgG and IgM antibodies. The data demonstrate that the IgG antibodies to VP-N and VP-1S had matured to a high level of avidity at the earliest after 4 weeks, but in most cases 6 to 8 weeks, after the infection. After that point, only highly avid IgG antibodies were found.
References