RIDASCREEN® Parvovirus B19 IgG, IgM

Article no: K6021 (IgG)
K6031 (IgM)
1. Intended use

For in vitro diagnostic use. The RIDASCREEN® Parvovirus B19 Tests are enzyme immunoassays for the quantitative determination of IgG or IgM antibodies against Parvovirus B19 in human serum.

The tests should be used for confirmation purposes when there is a suspected case of infection with Parvovirus B19 or for clarifying the immune status.

2. Summary and explanation of the test

Parvovirus B19 is a small single-stranded DNA virus lacking a lipid envelope and representing the most important pathogen within the family of Parvoviridae. Besides the induction of inflammatory processes the pathogenicity is caused by an suppressed erythropoiesis due to a selective replication of the virus in progenitor erythroid cells. Infections with Parvovirus B19 occur worldwide and the transmission occurs predominantly by droplet infection via the respiratory tract. Acute parvovirus infections occur in all age groups, however are most often observed in children between the age of 6-15 years. The incubation period is 1-3 weeks. The percentage of the adult population infected is about 70%.

Parvovirus B19 infections are often asymptomatic or show weak flu-like symptoms. However parvovirus infections are also associated with different clinical pictures. Erythema infectiosum, also called fifth disease, shows the highest prevalence generally affecting children between the age of 4-10 years. Complications can occur in pregnant women being seronegative due to a 30% chance of foetal transmission. Foetal infections may lead to spontaneous abortions or severe foetal deformities like Hydrops fetalis. Maternal infection during the first and second trimester of pregnancy can lead to an increased risk of pregnancy loss. Overall, fetal death is estimated to occur in 5-10% of cases of foetal infection. Upon infection with parvovirus B19 patients suffering from chronic hemolytic anemia are at high risk of developing aplastic crisis due to a suppressed erythropoiesis.

After infection with paroviruses, specific antibodies are formed against the pathogen as a result of the response from the immune system. By using immunological methods, it is possible to determine those antibodies in the serum. Both the choice of the pathogen-specific antigen and the selected test method have a significant influence on the test result. The ELISA is very suitable as a screening method for drawing conclusions about the immunological status of a patient. Subsequently a positive result can be further differentiated by Line Blot and can be assigned to the different phases of an infection such as virus persistence (see RIDA®LINE Parvovirus B19 IgG, Art.No. LB6023, RIDA®LINE Parvovirus B19 IgM, Art.No. LB6033 (LB6023 and LB6033 worldwide distributed except for USA, Canada, Australia and Israel)).
3. Test principle

Recombinant antigens are coated onto the surface of microtiter plates. In case of IgG detection, a mixture of VP1 and VP2 is used as Parvovirus B19 antigen whereas for IgM detection, VP2 is used. Antibodies in the patient samples bind to the antigens and are determined during the second incubation step by using enzyme-labelled anti-human antibodies (the conjugate). The enzyme converts the colourless substrate (H₂O₂/TMB) to a blue end product. The enzyme reaction is stopped by adding sulphuric acid and the colour of the mixture switches from blue to yellow at the same time. The final measurement is carried out at 450 nm on a photometer using a reference wavelength ≥ 620 nm.
4. Reagents provided

Table 1: Pack contents (there are enough reagents in a pack for 96 determinations)

<table>
<thead>
<tr>
<th>Description</th>
<th>Volume</th>
<th>Contents</th>
<th>K6021 IgG</th>
<th>K6031 IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate</td>
<td>96 det.</td>
<td>Microwell plate; 12 microwell strips (can be divided) in the strip holder; coated with recombinant Parvovirus B19 antigen; IgG- and IgM-test: eukaryotic-expressed antigens</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>SeroPP</td>
<td>110 ml</td>
<td>Sample buffer, ready for use; phosphate-buffered NaCl solution; coloured yellow;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>SeroWP</td>
<td>100 ml</td>
<td>Wash buffer, 10-fold concentrate; tris-buffered NaCl solution;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Control IgG + green lid</td>
<td>2.5 ml</td>
<td>Standard control IgG, ready for use; diluted human serum; coloured green;</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Control IgM + red lid</td>
<td>2.5 ml</td>
<td>Standard control IgM, ready for use; diluted human serum; coloured red;</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Control IgG - colourless lid</td>
<td>1.2 ml</td>
<td>Negative control IgG, ready for use; diluted human serum;</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Control IgM - colourless lid</td>
<td>1.2 ml</td>
<td>Negative control IgM, ready for use; diluted human serum;</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Control IgG A green lid</td>
<td>1.2 ml</td>
<td>Quality control A IgG, ready for use; diluted human serum</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Control IgG B green lid</td>
<td>1.2 ml</td>
<td>Quality control B IgG, ready for use; diluted human serum</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Control IgM A red lid</td>
<td>1.2</td>
<td>Quality control A IgM, ready for use; diluted human serum</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Control IgM B red lid</td>
<td>1.2</td>
<td>Quality control B IgM, ready for use; diluted human serum</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>SeroG HD green lid</td>
<td>12 ml</td>
<td>Anti-human IgG conjugate HD (goat), ready for use; peroxidase conjugated antibodies in stabilised protein solution;</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>SeroM HD red lid</td>
<td>12 ml</td>
<td>Anti-human IgM conjugate HD (goat), ready for use; peroxidase conjugated antibodies in stabilised protein solution;</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>SeroSC</td>
<td>12 ml</td>
<td>Substrate; H$_2$O$_2$/tetramethylbenzidine; ready for use</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Stop</td>
<td>12 ml</td>
<td>Stop reagent 0.5 M sulphuric acid; ready for use</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Details of hazardous substances according to labeling obligations. For more details see Material Safety Data Sheets (MSDS) at www.r-biopharm.com
5. Storage instructions

The test kit must be stored at 2 – 8 °C and can be used until the expiry date printed on the label. The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 – 8 °C or for one week when stored at room temperature (20 – 25 °C). After the expiry date, the quality guarantee is no longer valid.

The aluminium bag containing the microwell plate must be opened in such a way that the clip seal is not torn off. Any microwell strips which are not required must be immediately returned to the aluminium bag and stored at 2 – 8 °C.

The reagents also must not be allowed to become contaminated and the colourless substrate must be protected from exposure to direct light.

6. Additional necessary reagents – and necessary equipment

6.1. Reagents

– distilled or deionised water

6.2. Accessories

– Incubator at 37 °C
– Test tubes
– Vortex mixer
– Micropipettes for 10 - 100 µl and 100 - 1000 µl capacities
– Measuring cylinder (1000 ml)
– Stop clock
– Microplate washer or multichannel pipette
– Microplate reader (450 nm, reference wavelength ≥ 620 nm)
– Filter paper (laboratory towels)
– Waste container containing 0.5 % sodium hypochlorite solution

7. Precautions for users

For in vitro diagnostic use only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used. For more details see Material Safety Data Sheets (MSDS) at www.r-biopharm.com
The control sera (standard control, negative control, quality control A and quality control B) in the kit have been tested for HIV- and HCV-Ab as well as HbsAg with negative results. Nevertheless, they must be treated as potentially infectious in the same way as the patient samples and all other materials with which they come into contact and they must be handled in accordance with the relevant national safety regulations.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

8. Specimen collection and storage

The test has been developed for testing human serum samples. After blood collection, the blood should be separated from blood clots as soon as possible in order to prevent haemolysis. The samples must be stored cold or frozen until they are tested. Repeated freezing and thawing of the samples and microbial contamination must be prevented at all costs. Using heat-inactivated, lipaemic, haemolytic, icteric or turbid samples can lead to false results.

Table 2: Sample storage

<table>
<thead>
<tr>
<th>Undiluted serum</th>
<th>Diluted serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 – 8 °C</td>
<td>-20 °C</td>
</tr>
<tr>
<td>2 – 8 °C</td>
<td>&gt;1 week</td>
</tr>
<tr>
<td>1 week</td>
<td>7 hours</td>
</tr>
</tbody>
</table>

9. Test procedure

9.1. General information

All reagents and the microwell plate must be brought to room temperature (20 – 25 °C) before use. The microwell strips must not be removed from the aluminium bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the kit must be immediately returned to storage at 2 – 8 °C.

Take only the volume of reagents that is needed for test procedure. Do not pour reagents back into vials as reagent contamination may occur. Do not pour reagents back into vials as this may lead to reagent contamination.

The microwell strips cannot be used more than once. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking.

Some of the reagents in the kit are not test specific. The reagents labelled Sero (such as SeroPP) can also be used with other RIDASCREEN® Sero ELISA with the corresponding reagents.

The control sera relate to the lot. Control sera from kits with different lot numbers must not be exchanged.
RIDASCREEN® Sero ELISA controls A and B are provided as additional components to the respective RIDASCREEN® Sero ELISA test kits. These are controls for additional quality control purposes which can be used optional. They contain human control serum with different antibody concentrations.

9.2. Preparing the wash buffer

1 part wash buffer concentrate [SeroWP] is mixed with 9 parts distilled water. In order to do this, place 100 ml of the concentrate in a 1000 ml measuring cylinder and make up the solution to 1000 ml with distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37 °C. The diluted wash buffer can be used for a maximum of 4 weeks when stored at 2 – 8 °C or for one week when stored at room temperature (20 – 25 °C).

9.3. Preparing the samples

Dilute the serum samples to be tested with sample buffer [SeroPP] 1:50 before starting the test.

\[ \text{e.g. } 10 \mu l \text{ Serum } + 490 \mu l \text{ SeroPP} \]

For the IgM determinations, it is recommended to subject the sera to IgG absorption (e.g. with RIDA® RF Absorbent, Article no. Z0202) before testing.

**Note:**
The negative control, standard control, quality control A and quality control B are ready for use and must NOT be diluted or absorbed.

9.4. First incubation

After insertion of a sufficient number of wells into the frame, pipette 100 µl diluted sera and 100 µl ready-to-use control into each of the corresponding wells leaving Position A1 (reagent blank value) empty. Add the negative control [Control IgG -] or [Control IgM -] once and the standard control [Control IgG +] or [Control IgM +] in duplicate. Add the quality controls [Control IgG A] and [Control IgG B] or the quality controls [Control IgM A] and [Control IgM B] once. Cover the plate and incubate at 37 °C for 30 minutes in an incubator. During this process, the bottoms of the wells must not be in contact with heat-conductive materials. The microwell plate must be covered during incubation.

The controls which correspond to the determination (IgG or IgM) must be used.
A1  Reagent blank value  
B1  Negative control  
C1  Standard control  
D1  Standard control  
E1  Quality control A  
F1  Quality control B  
G1, H1  Patient serum 1 and 2 etc.

**Note:**
The microwell plate must not be placed in a cold incubation container reaching 37 °C during incubation. The temperature of the container must be adjusted to 37 °C beforehand.

9.5. Washing

The wells must be emptied into a waste container containing hypochlorite solution for disinfection. Then knock out the plate onto absorbent paper in order to remove the residual moisture. After this, wash the plate 4 times using 300 µl wash buffer each time. Make sure that the wells are emptied completely by knocking them out on an unused part of the absorbent paper after each wash.

When using a microplate washer, make sure that the machine is correctly adjusted to the type of plate being used. After washing, knock out the plate onto clean absorbent paper in order to remove any residual moisture.

9.6. Second incubation

Add 100 µl Anti-human IgG conjugate HD SeroG HD or Anti-human IgM conjugate HD SeroM HD to the corresponding wells (including A1). Next, cover the plate and incubate at 37 °C for 30 minutes in an incubator (see Section 9.4).

9.7. Washing

Wash 4 times in accordance with Section 9.5.

9.8. Third incubation

Add 100 µl substrate SeroSC to each well. Then, cover the plate and incubate at 37 °C for 30 minutes in an incubator. After this, stop the reaction by adding 100 µl stop reagent Stop to each well. After mixing carefully (by lightly tapping the side of the plate), measure the absorbance at 450 nm (reference wavelength ≥ 620 nm) in a plate photometer. Calibrate the reagent blank value (Position A1) to zero.

**10. Quality control and indications of instability or deterioration**
For quality control purposes, the standard control (in duplicate) and the negative control must be used every time the test is carried out. The test has been carried out correctly if the average absorbance for the standard control at 450/620 nm is within the range stated on the enclosed data sheet. If the two individual measurements deviate from the average by more than 20 %, the test must be repeated. The absorbance for the negative control at 450/620 nm must be < 0.3.

RIDASCREEN® Sero ELISA controls A and B are additional controls for quality control purposes which can be used optional.

The target values are stated on the enclosed, lot-specific quality assurance certificate. The obtained values (U/ml, IU/ml or mIU/ml) are recommended as reference values for the quality assurance in accredited laboratories.

If the values differ from those required, if the reagent is turbid or the substrate has turned blue before adding to the wells, it may indicate that the reagents have expired.

If the stipulated values are not met, the following points must be checked before repeating the test:

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks – a substrate solution which has turned blue must not be used.

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

11. Evaluation and interpretation

The IgG test can be evaluated in international units (IU/ml) by adjusting the standard curve to the international WHO standard (NIBSC 93/724).

The test can be evaluated using three different methods:

1. By using the standard curve provided in the kit
2. By using the table of values (see data sheet provided in the kit)
3. Mathematically using the 4-parameter method or the \( \alpha \) method

The reagent blank value must be subtracted from each measured value before the evaluation.
11.1. Evaluation by using the standard curve provided in the kit

In order to carry out the evaluation using the standard curve, a correction must be made to the average value for the standard control first in order to take into account any fluctuations which may occur from one day to the next. The correction factor \( F \) is calculated from the current measured average value for the standard control and its target value. The target value, which depends on the lot, is recorded on the enclosed data sheet.

\[
F = \frac{\text{standard control target value}}{\text{standard control measured average value}}
\]

All OD values for the samples must be multiplied by the factor \( F \). The corresponding IU/ml (IgG) or U/ml (IgM) values are then read off the standard curve using these corrected values.

11.2. Evaluation by using the table of values

The absorbance for the standard control is used to identify the column in the table with the range of values which applies to the current measurement. The measured absorbance for the sample is assigned to the appropriate range of values and then the titer in U/ml is read from the second column to the left in the table.

For example, the absorbance for the standard control for a certain measurement is 1.02. In this case, the column in the table with the range 1.00 - 1.05 is the one to use to determine the results. A patient sample with an absorbance of 1.38 therefore corresponds to the titer range 50.1 - 100.0 IU/ml. (The values cited are merely to be regarded as examples and may differ from the current values on the data sheet.)

The evaluation for the determined results (positive (+), negative (-) or equivocal (?)) must be taken from the first column of the table of values.

<table>
<thead>
<tr>
<th>IU/ml</th>
<th>Range of values for the standard control</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>&lt; 3.0</td>
</tr>
<tr>
<td>?</td>
<td>3.0 - 5.0</td>
</tr>
<tr>
<td>+</td>
<td>5.1 - 10.0</td>
</tr>
<tr>
<td></td>
<td>0.18 - 0.31</td>
</tr>
<tr>
<td></td>
<td>0.32 - 0.68</td>
</tr>
<tr>
<td></td>
<td>0.69 - 0.95</td>
</tr>
<tr>
<td></td>
<td>0.96 - 1.42</td>
</tr>
<tr>
<td></td>
<td>1.43 - 2.09</td>
</tr>
<tr>
<td></td>
<td>&gt; 200.0</td>
</tr>
</tbody>
</table>

Figure 1: Example of an IgG determination (Extract from a lot-specific data sheet)
11.3. Mathematical evaluation

The required values for mathematical evaluation according to the 4-parameter method or the $\alpha$ method are recorded on the enclosed data sheet.

11.4. Test result

Table 3: Evaluation of the determined units

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>&lt; 3 IU/ml</td>
<td>&lt; 10 U/ml</td>
</tr>
<tr>
<td>equivocal</td>
<td>3 - 5 IU/ml</td>
<td>10 - 12 U/ml</td>
</tr>
<tr>
<td>positive</td>
<td>&gt;5 IU/ml</td>
<td>&gt; 12 U/ml</td>
</tr>
</tbody>
</table>

12. Limitations of the method

RIDASCREEN® Parvovirus B19 EIA detects IgG or IgM antibodies against Parvovirus B19. The test cannot be used to derive a relationship between the extinction determined and occurrence of serious clinical symptoms. The results obtained must always be interpreted in combination with the clinical picture.

A negative result does not necessarily mean that there is no infection. During the early stages of the infection, the number of antibodies may still be so small that looking for them may yield a negative result. If there is a suspected clinical case, therefore, a follow up serum should also be tested.

With pregnant women, the IgM test can also sometimes yield positive results due to polyclonal stimulation of the immune system without there being an infection. For this reason, positive results should be confirmed using Line Blot (RIDA®LINE Parvovirus B19 IgG, Art.No. LB6023, RIDA®LINE Parvovirus B19 IgM, Art.No. LB6033 (LB6023 and LB6033 world-wide distributed except for USA, Canada, Australia and Israel)).

With this method, more accurate assertions can be made about an acute or short-term past infection or an immune status by identifying antibodies against individual epitopes of the virus antigen.

Two consecutive samples of sera should always be collected from a patient and subjected to serological testing in order to improve the quality of the diagnostics. The progress of the titre is important for interpreting the findings.

A positive result does not rule out the presence of another infectious pathogen as the cause of the disease.
13. Performance characteristics

Table 4: Inter-assay variation (n = 30)

<table>
<thead>
<tr>
<th>Inter-assay variation</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD</td>
<td>CV</td>
</tr>
<tr>
<td>Serum 1</td>
<td>0.21</td>
<td>9.3%</td>
</tr>
<tr>
<td>Serum 2</td>
<td>0.469</td>
<td>8.6%</td>
</tr>
<tr>
<td>Serum 3</td>
<td>0.718</td>
<td>7.5%</td>
</tr>
</tbody>
</table>

Table 5: Intra-assay variation (n = 24)

<table>
<thead>
<tr>
<th>Intra-assay variation</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD</td>
<td>CV</td>
</tr>
<tr>
<td>0,212</td>
<td>0.212</td>
<td>9.3%</td>
</tr>
<tr>
<td>Serum 2</td>
<td>0.442</td>
<td>8.2%</td>
</tr>
<tr>
<td>Serum 3</td>
<td>1.098</td>
<td>8.5%</td>
</tr>
</tbody>
</table>

Table 6: Sensitivity and specificity in comparison with two other commercial ELISAs

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100.0%</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100.0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 7: Results from testing 200 blood-donor sera taken from a blood donor center in Germany

<table>
<thead>
<tr>
<th>200 blood donor sera</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>22.5%</td>
<td>99.5%</td>
</tr>
<tr>
<td>equivocal</td>
<td>4.5%</td>
<td>0%</td>
</tr>
<tr>
<td>positive</td>
<td>73.0%</td>
<td>0.5%</td>
</tr>
</tbody>
</table>
References