Parvovirus B19 IgG DxSelect™ (OUS)

Enzyme-linked Immunosorbent Assay (ELISA)

**INTENDED USE**
Focus Diagnostics’ Parvovirus B19 IgG DxSelect™ test is intended for the qualitative detection of human IgG class antibodies to Parvovirus B19 in human sera.

**SUMMARY AND EXPLANATION OF TEST**
Infection with Parvovirus B19 is a common phenomenon and at least 50% of adults show evidence of past infection. The most consistent symptom of a current Parvovirus B19 infection is erythema infectiosum, a characteristic rash seen mainly in children. In adults, the main feature of the disease is an acute arthropathy which may persist for several weeks or in a few cases even months.

Parvovirus B19 replicates in the nucleus of erythroid precursor cells and, because the infection is lytic, causes a transient cessation of red cell production. For the otherwise healthy person there may be no major consequences. However, when a pre-existing abnormality of red cell production such as sickle cell anemia exists, a life-threatening condition (transient aplastic crisis) may develop. Serious consequences may also arise in immunodeficient patients (acute leukemia, congenital immunodeficiencies, AIDS) where Parvovirus B19 infection can become chronic, resulting in persistent severe anemia. The fetus also appears to be at risk from infection with Parvovirus B19 where it has been associated with fetal hydrops and spontaneous abortion or stillbirth. A recent prospective study in the United Kingdom indicated a transplacental transmission rate of 33% with a risk of spontaneous abortion or stillbirth. A recent prospective study in the United Kingdom indicated a transplacental transmission rate of 33% with a risk of spontaneous abortion or stillbirth.

From the results of a study involving the intranasal inoculation of 9 human volunteers with live Parvovirus B19 it appears that appreciable levels of pre-existing IgG antibody confer immunity, though one individual with low levels of IgG antibody developed a brief low level viremia. Antibody negative volunteers developed a high titered viremia about 1 week post infection which persisted for about 1 week. IgM antibodies were first detected 10 to 13 days after infection and IgG antibodies were detected very soon after that. IgM levels increased to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of optical density (OD) which is directly proportional to the amount of antigen-specific IgG present in the sample. Sample optical density readings are compared with reference cut-off OD readings to determine results.

**TEST PRINCIPLE**
In the Focus Diagnostics Parvovirus B19 IgG DxSelect™ assay, the polystyrene microwells are coated with Parvovirus B19 antigen. Diluted serum samples and controls are incubated in the wells to allow specific antibody present in the samples to react with the antigen. Non-specific reactants are removed by washing and peroxidase-conjugated anti-human IgG is added and reacts with specific IgG. Excess conjugate is removed by washing. Enzyme substrate and chromogen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of optical density (OD) which is directly proportional to the amount of antigen-specific IgG present in the sample. Sample optical density readings are compared with reference cut-off OD readings to determine results.

**MATERIALS SUPPLIED**
The Focus Diagnostics Parvovirus B19 IgG DxSelect™ Test kit contains sufficient materials to perform 96 determinations. All unopened materials are stable at 2 to 8°C until the expiration date stated on the reagent label.

- **IgG Antigen Wells, 96 wells**
- **IgG Conjugate, 12 mL**
- **IgG Cut-Off Calibrator, 0.25 mL**
- **Non-Detectable Control, 0.14 mL**

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MATERIALS REQUIRED, BUT NOT SUPPLIED
1. Distilled water
2. 250 or 500mL wash bottle or automated EIA plate washing device
3. 1L graduated cylinder
4. 12 x 75 mm borosilicate glass test tubes or equivalent
5. 10 µL and 100 µL pipettors with disposable tips (100 µL eight-channel pipettor recommended for runs over 48 wells)
6. 1 mL pipet or dispenser
7. 5 mL pipet
8. Timer
9. Paper towels or absorbant paper
10. Sink
11. Vortex mixer or equivalent
12. ELISA plate spectrophotometer, wavelength = 450 nm

SHELF LIFE AND HANDLING
1. Kits and kit reagents are stable through the end of the month indicated by the label expiration date when stored at 2 to 8°C.
2. Do not use test kit or reagents beyond their expiration dates.
3. Do not expose reagents to strong light during storage or incubation.
4. Allow reagents to warm to room temperature before use.

WARNINGS AND PRECAUTIONS
1. This package insert is for export only and not for distribution in the United States. Outside of the United States, product regulatory status is for in vitro diagnostic use.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of using proper biohazard precautions.
3. The Parvovirus B19 antigen plates are produced with inactivated VP1 B19 antigens; however, the plates should be considered potentially infectious and handled accordingly.
4. Sodium azide at a concentration of 0.1% has been added to the controls as an antibacterial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, controls should be discarded into sewerage only if diluted and flushed with large volumes of water. Use copper-free and lead-free drain systems where possible. Occasionally decontaminate lead and copper plumbing, controls should be discarded into sewerage. Note: Always dilute controls in 100 µL of Sample Diluent and mix well by vortex mixing. For runs with more than 48 wells it is recommended that 250 µL of each diluted sample first be added to a blank microtiter plate in the location corresponding to that in the ELISA wells. The samples can then be efficiently transferred into the Antigen Wells with a 100 µL- or 12-channel pipettor.
5. Dispense 100 µL of the Sample Diluent into the "blank" wells and 100 µL of each diluted specimen, control or calibrator into the appropriate wells. (Note: For runs with more than 48 wells it is recommended that 250 µL of each diluted sample first be added to a blank microtiter plate in the location corresponding to that in the ELISA wells. The samples can then be efficiently transferred into the Antigen Wells with a 100 µL- or 12-channel pipettor.)
6. Stop the reaction by adding 100 µL of Stop Reagent solution from a wash bottle then empty contents into a sink or a discard basin.
7. Repeat wash steps 6 through 8.
8. Tap the antigen wells vigorously to remove IX Wash Buffer solution from a wash bottle then empty contents into a sink or a discard basin.
9. Dispense 100 µL of Calibrator and two controls on each plate. If multiple plates are run, include the Cut-off Calibrator and two controls on each plate. It is recommended that until the user becomes familiar with the kit performance, all specimens, controls and the Cut-off Calibrator should be run in duplicate with the Cut-off Calibrator run twice for a total of four wells. If single wells are used, the Cut-off Calibrator should be run in triplicate. Include a minimum of 1 blank well (containing sample diluent only) for instrument calibration purposes.

TEST PROCEDURE
1. Bring all reagents to room temperature before use. Remove the Antigen Well packet from cold storage. To avoid condensation, allow micro-well strips to reach room temperature before opening the foil packet. If less than a full plate is to be used, return unused strips to the foil packet with desiccant and resal completely. Store unused antigen wells at 2 to 8°C. (Note: At the end of the assay, retain the frame for use with the remaining strips.)
2. Fill wells with IX Wash Buffer solution (see Materials Supplied, above) and allow to soak for 5 minutes. Decant (or aspirate) the antigen wells and tap vigorously to remove Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbant paper to remove residual Wash Buffer.
3. Cover plates with sealing tape (or place in a humid chamber), and incubate for 60 ± 1 minute at room temperature (20 to 25°C).
4. Remove sealing tape (or remove wells from the humid chamber), and empty the contents of the wells into a sink or a discard basin.
5. Fill each well with a gentle stream of IX Wash Buffer solution from a wash bottle then empty contents into a sink or a discard basin.
6. Repeat wash steps 5 through 8.
7. Incubate for 60 ± 1 minutes at room temperature (20 to 25°C).
8. Pipet 100 µL of Substrate Reagent to all wells, using a 100 µL 8- or 12-channel pipettor.
9. Pipet 100 µL of Substrate Reagent to all wells, using a 100 µL 8- or 12-channel pipettor. Begin incubation timing with the addition of Substrate Reagent to the first well. (Note: Never pour the substrate reagent into the same trough as was used for the conjugate.)
10. Incubate for 10 ± 1 minutes at room temperature (20 to 25°C).
11. Add patient specimens to the assay's compatibility with other specimens. Hyperlipemic, heat-inactivated, hemolyzed, or contaminated sera may cause erroneous results; therefore, their use should be avoided.

Quality Control
Each plate run (or strips or wells from a single plate) must include the Cut-off Calibrator and two controls. If multiple plates are run, include the Cut-off Calibrator and two controls on each plate. It is recommended that until the user becomes familiar with the kit performance, all specimens, controls and the Cut-off Calibrator should be run in duplicate with the Cut-off Calibrator run twice for a total of four wells. If single wells are used, the Cut-off Calibrator should be run in triplicate. Include a minimum of 1 blank well (containing sample diluent only) for instrument calibration purposes.
The Cut-off Calibrator has been formulated to give the optimum differentiation between negative and positive sera. Although the absorbance value may vary between runs and between laboratories, the mean value for the Cut-off Calibrator wells must be within 0.100 to 0.700 OD units. All replicate Cut-off Calibrator ODs should be within 0.10 absorbance units from the mean value.

Report results as **Index Values** relative to the Cut-off Calibrator. To calculate Index Values, divide specimen optical density (OD) values by the mean of the Cut-off Calibrator absorbance values.

1. **The Detectable Control** Index Values should be between 1.5 and 3.5.
2. **The Non-Detectable Control** Index values should be less than 0.8.

**If the Calibrator or controls are not within these parameters, patient test results should be considered invalid and the assay repeated.**

**INTERPRETATION OF TEST RESULTS**

Report all patient results as **Index Values** relative to the Cut-off Calibrator: to calculate Index Values, divide specimen optical density (OD) values by the mean of the Cut-off Calibrator absorbance values.

- **> 1.20 Positive.** An Index Value of > 1.20 is presumptive for the presence of IgG antibodies to Parvovirus B19.
- **< 0.80 Negative.** An Index Value of < 0.80 indicates no IgG antibodies to Parvovirus B19 were detected.
- **≥ 0.80 and ≤ 1.20 Equivocal.** An Index Value of ≥ 0.80 but ≤ 1.20 is considered an equivocal result. These samples should be retested. If, on retesting, the result remains equivocal, a second sample should be drawn several weeks later and tested to identify a rise in IgG antibody titer. If the second sample is either negative or equivocal, report results as negative. Alternatively, the specimen may be tested using a different methodology such as IFA or Western Blot.

**LIMITATIONS**

1. All results from this and other serologies must be correlated with clinical history, epidemiological data, and other data available to the attending physician in making the diagnosis of Parvovirus B19 infection.
2. Patients with early Parvovirus B19 infection may test negative for IgG antibodies, since the IgG response may be undetectable until 5 weeks post-onset. Therefore, testing for IgM class antibody is recommended. If a negative test result is reported on a patient with signs and symptoms of Parvovirus B19 infection, repeat testing for IgG and IgM antibodies on a second sample obtained 2 to 4 weeks later is recommended.
3. On rare occasions, false positive IgG result may occur in the early stages of an immune response to other infectious agents.

**EXPECTED VALUES**

At least 50% of adults show evidence of past infection. High levels of IgG antibodies may persist for years. A recent infection can be diagnosed by the presence of IgM antibodies and/or a rising titer of IgG antibodies and a past infection by the presence of IgG antibodies.

**PERFORMANCE CHARACTERISTICS**

For customers outside the United States, the product performance characteristics are supplied as a separate sheet.

**REFERENCES**


This package insert is available in French, German, Italian, and Spanish at www.focusdx.com, and is available in other languages from your local distributor.