# DRG® Parvovirus B19 IgG ELISA (EIA-4328)

**As 28 Sept. 2010 rm (Vers. 1.1)** **USA: RUO**

## INTENDED USE

The DRG Parvovirus B19 IgG test is intended for detection of human IgG class antibodies to Parvovirus B19 in human sera.

## TEST PRINCIPLE

In the DRG Parvovirus B19 IgG 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. Nonspecific 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 DRG Parvovirus B19 IgG test kit contains sufficient materials to perform 96 determinations. Allow the supplied reagents to warm to room temperature before use. All un-opened materials are stable at 2 to 8°C until the expiration date stated on the reagent label.

**IgG Antigen Wells, 96 wells**

12 Eight-well polystyrene microwell strips on a frame. Each well is coated with recombinant VP1 B19 protein. Each strip may be broken down into individual wells for cost effective use. To avoid condensation, allow the antigen strips to warm to room temperature before opening the sealed packets.

**IgG Conjugate, 12 mL**

One vial of affinity-purified and peroxidase-conjugated goat anti-human IgG (Fc fragment specific). Contains protein, buffer and non-azide preservatives.

**IgG Detectable Control, 0.14 mL**

One vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see **Specimen, Controls and Calibrator Preparation**, below).

**Non-Detectable Control, 0.14 mL**

One vial of human serum. Contains 0.1% sodium azide as a preservative. Requri4es dilution beore use (see **Specimen, Controls and Calibrator Preparation**, below).

**IgG Cut-Off Calibrator, 0.25 mL**

One vial of human serum. Contains 0.1% sodium azide as a preservative. Requri4es dilution beore use (see **Specimen, Controls and Calibrator Preparation**, below).

**Sample Diluent, 100 mL**

One vial of protein, surfactant, and non-azide preservatives in PBS.

**10X Wash Buffer, 100 mL**

One vial of surfactant in PBS with non-azide preservatives. Prepare a 1X wash buffer solution before use.

To prepare a 1X wash buffer solution**,** mix 100 mL **10X Wash Buffer** with 900 mL **distilled (or deionized) water** and rinse out any crystals. Use only the highest grade purified water for reconstitution of the wash buffer. It has been observed that some sources of deionized water contain materials which can interfere in the assay. Swirl until well mixed and all crystals are dissolved.

**As 28 Sept. 2010 rm (Vers. 1.1)** **USA: RUO**

**Substrate Reagent, 16 mL**

One vial of tetramethylbenzidine (TMB) and organic peroxide in buffer. A dark blue color indicates contamination with peroxidase; and, if this occurs, use a fresh bottle.

**Stop Reagent, 16 mL**

One vial 1 M sulfuric acid.

**Sealing Tape**

Two sheets of sealing tape.

**As 28 Sept. 2010 rm (Vers. 1.1)** **USA: RUO**

## 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 Product regulatory status within the United States is for investigational use only: the performance characteristics of this product have not been established.
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 the drains with 10% sodium hydroxide (CAUTION: caustic), allow to stand for 10 minutes, then flush with large volumes of water.
5 The stop reagent contains sulfuric acid. Do not allow to contact skin or eyes. If exposed, flush with copious amounts of water.
6 Do not substitute or mix reagents from different kit lots or from other manufacturers.
7 Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
8 Cross-contamination of donor specimens can cause erroneous results. Add donor specimens and handle strips

carefully to avoid mixing of sera from adjoining wells. Avoid contamination of the substrate reagent with traces of the enzyme conjugate.

9 Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.

10 Perform the assay at room temperature (approximate range 20 to 25°C).

11 Use proper pipetting techniques, maintaining the pipetting pattern throughout the procedure to ensure optimal and reproducible values.

## SPECIMEN COLLECTION AND PREPARATION

Serum is the preferred specimen source. No attempt has been made to assess the assay's compatibility with other specimens. Hyperlipemic, heat-inactivated, hemolyzed, or contaminated sera may cause erroneous results; therefore, their use should be avoided.

**Specimen Collection and Handling** Collect blood samples aseptically using approved venipuncture techniques by qualified personnel. Allow blood samples to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage at 2 to 8°C. If testing is to be delayed longer than 5 days, the sample should be frozen at –20°C or colder. Thaw and mix samples well prior to use.

**Specimen, Controls and Calibrator Preparation** Dilute each specimen, control and calibrator 1:101 as follows: label tubes and dispense 1mL of **Sample Diluent** into each labeled tube. Add **10 µL of specimen, control or calibrator** to each appropriate tube containing the 1mL Sample Diluent and mix well by vortex mixing.

## 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 reseal 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 **1X 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. Dispense 100 µL of the **Sample Diluent** into the "blank" wells and 100 µL of each **diluted specimen, control or calibrator** (see **Specimen, Controls, and Calibrator Preparation**, above) 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 8- or 12-channel pipettor.)
4. Cover plates with sealing tape (or place in a humid chamber), and incubate for 60 ± 1 minute at room temperature (20 to 25°C).
5. Remove sealing tape (or remove wells from the humid chamber), and empty the contents of the wells into a sink or a discard basin.
6. Fill each well with a gentle stream of **1X Wash Buffer** solution from a wash bottle then empty contents into a sink or a discard basin.
7. Repeat wash (step 6) an additional 2 times.
8. Tap the antigen wells vigorously to remove 1X Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbant paper to remove residual 1X Wash Buffer.
9. Dispense **100 µL Conjugate** to all wells, using a 100 µL 8-or 12-channel pipettor.
10. Cover plates with sealing tape (or place in a humid chamber) and incubate for 30 ± 1 minutes at room temperature (20 to 25°C).

11. Repeat wash steps 5 through 8.
12. 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.)
13. Incubate for 10 ± 1 minutes at room temperature (20 to 25°C).
14. Stop the reaction by adding 100 µL of **Stop Reagent** to all wells using a 100 µL 8-or 12-channel pipettor. Add the Stop Reagent in the same sequence and at the same pace as the Substrate was added. In antibody-positive wells, color should change from blue to yellow.
15. Gently blot the outside bottom of wells with a paper towel to remove droplets that may interfere with reading by the spectrophotometer. Do not rub with the paper towel as it may scratch the optical surface of the well. (**Note**: Large bubbles on the surface of the liquid may affect the OD readings.)
16. Measure the absorbance of each well within 1 hour of stopping the assay. Set the microwell spectrophotometer at a wavelength of 450 nm. Zero the instrument on the blank wells.

## QUALITY CONTROL

Each plate run (or strips or wells from a single plate) must include the **Cutoff Calibrator** and all three controls. If multiple plates are run, include the Cut-off Calibrator and all three 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 Cutoff 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 0.150 to 0.450 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, donor test results should be considered invalid and the assay repeated.**

## REFERENCES

1. Anderson, L.J. 1987. Role of Parvovirus B19 in human disease. Pediatr. Infect. Dis. J. 6:711-718.
2. Young N.S., Mortimer P.P., Moore J.G., et al. 1984. Characterization of a virus that causes transient aplastic crisis. J. Clin. Invest. 73:224-230.
3. Anderson, L.J. 1990. Human Parvoviruses. J. Infect. Dis. 161:603-608.
4. Prospective study of Human Parvovirus (B19) infection in pregnancy. Public Health Laboratory Service Working party on Fifth Disease. B.M.J. 1990 300 (6733) 1166-1170.
5. Anderson M.J., Higgins P.G., Davis L.R. et al. 1985. Experimental parvoviral infection in humans. J. Infect. Dis. 152:257-265.
6. Fridell, E., Cohen, B.J., and Wahren, B. 1991. Evaluation of a synthetic-peptide Enzyme-linked immunosorbent assay for immunoglobulin M to Human Parvovirus B19. J. Clin. Microbiol. 29:1376-1381.