DRG® Parvovirus B19 IgM ELISA (EIA-4329)

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

INTENDED USE

The DRG Parvovirus B19 IgM test is intended for detection of human IgM class antibodies to Parvovirus B19 in human sera. This kit is intended for Research Use Only.

EXPLANATION OF TEST

The DRG Parvovirus B19 IgM assay utilizes recombinant VP1 B19 protein.

TEST PRINCIPLE

In the DRG Parvovirus B19 IgM assay, the polystyrene microwells are coated with Parvovirus B19 antigen. Donor sera and controls are diluted in a solution containing hyper-immune anti-human IgG precipitating immunoglobulin to remove both free and complexed IgG from the sample. The diluted serum samples and controls are incubated in the wells to allow any specific antibody present in the samples to react with the antigen. Nonspecific reactants are removed by washing and peroxidase-conjugated anti-human IgM is added to react with the IgM present. 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 IgM present in the sample. Sample OD readings are compared with reference cut-off OD readings to determine results.

MATERIALS SUPPLIED

The DRG Parvovirus B19 IgM 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.

IgM Antigen Wells, 96-wells

12 eight-well polystyrene microwell strips on a frame. Each well is coated with a Parvovirus B19 antigen. 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.

IgM Conjugate, 12 mL

One vial of affinity-purified and peroxidase-conjugated goat anti-human IgM (μ chain specific). Contains protein, buffer, and non-azide preservatives.

IgM Detectable Control, 0.14 mL

One vial of human serum per each control level. 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. Requires dilution before use (see **Specimen, Controls and Calibrator Preparation,** below).

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IgM Cut Off Calibrator, 0.25 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).

IgM Sampole Dileunt, 100 mL

One vial of goat anti-human IgG precipitating antibody, protein, surfactant, and non-azide preservatives in PBS.

10X Wash Buffer, 100 mL

One vial of surfactant in PBS with non-azide preservatives.

To prepare a 1X wash buffer solution**,** mix 100mL **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.

Substrate Reagent, 16 mL

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

Stop Reagent, 16 mL

One vial of 1 M sulfuric acid.

Sealing Tape

Two sheets of sealing tape.

MATERIALS REQUIRED, BUT NOT SUPPLIED

1. Distilled water
2. 250 or 500 mL 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- or twelve-channel pipettor recommended for runs over 48 wells)
6. 1mL pipet or dispenser
7. 5mL pipet
8. Timer
9. Paper towels or absorbant paper
10. Sink
11. Vortex mixer or equivalent
12. ELISA plate spectrophotometer, wavelength = 450nm

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SHELF LIFE AND HANDLING

1. Kits and kit reagents are stable through the end of the month indicated in the 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 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.

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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 **IgM Sample Diluent** into each labeled tube. Add **10 µL of specimen, control or calibrator** to each appropriate tube containing the 1mL IgM Sample Diluent and mix well by vortex mixing. Wait 10 minutes during which time a fine precipitate will form in the tubes, sequestering IgG into an immune complex and preventing its interference in the IgM assay. The precipitate will not interfere with the assay.

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 **IgM 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 100µL 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 100µL 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 450nm. Zero the instrument on the blank wells.

REFERENCES

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