Parvovirus B19 IgM DxSelect™ (OUS)

Enzyme-linked Immunosorbent Assay (ELISA)  
REF  EL0100M

Rev. I.

Enzyme-linked immunosorbent assay for the qualitative detection of human IgM class antibodies to Parvovirus B19.

This package insert is for export only and not for distribution in the United States.

Outside of the United States:  For in vitro Diagnostic Use.

INTENDED USE
Focus Diagnostics’ Parvovirus B19 IgM DxSelect™ test is intended for the qualitative detection of human IgM 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 it 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 fetal death in an infected pregnancy estimated to be 9%.

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 reach a peak then began to fall 30 to 60 days after the illness. IgM levels may reach undetectable levels after 60 to 90 days. High levels of IgG antibodies may persist for years. Thus acute infection can be diagnosed by clinical symptoms together with the presence of B19 IgM antibodies. A recent infection can be diagnosed by the presence of B19 IgM antibodies and/or a rising titer of IgG antibodies and a past infection by the presence of IgG antibodies.

The Focus Diagnostics Parvovirus B19 IgM DxSelect™ assay utilizes recombinant VP1 B19 protein.

TEST PRINCIPLE
In the Focus Diagnostics’ Parvovirus B19 IgM DxSelect™ assay, the polystyrene microwells are coated with Parvovirus B19 antigen. Patient 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. Non-specific 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 Focus Diagnostics Parvovirus B19 IgM DxSelect™ Test kit contains sufficient materials to perform 96 determinations. All 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  
REF  EL0121  Ag

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  
REF  EL0102  CONJ  IgM

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

IgM Detectable Control, 0.14 mL  
REF  EL0115  CONTROL >

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  
REF  EL0112  CONTROL <

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 Cut-Off Calibrator, 0.25 mL  
REF  EL0103  CONTROL CAL

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 Sample Diluent, 100 mL  
REF  EL1613  DIL  SPE

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

10X Wash Buffer, 100 mL  
REF  EL0405  BUF  WASH

One vial of surfactant in PBS with 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.

Substrate Reagent, 16 mL  
REF  EL0009  SUBS  TMB

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  
REF  EL0105  SOLN  STOP

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

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. 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 decontaminated or disposed of using proper biohazard precautions. 
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 patient specimens can cause erroneous results. Add patient 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

1. 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 IgM into an immune complex and preventing its interference in the IgM assay. The precipitate will not interfere with the assay.

TEST PROCEDURE

1. 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.)
2. Dispense 100 µL of IgM DxSelect™ to all wells, using a 100 µL 8- or 12-channel pipettor.
3. Dispense 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.)
4. Incubate for 10 ± 1 minutes at room temperature (20 to 25°C).
5. 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.
6. 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.)
7. 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.

QUALITY CONTROL

Each plate run (or strips or wells from a single plate) must include the Cut-off Calibrator and two controls. 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...
FOCUS Diagnostics


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.

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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 the range of 0.150 to 0.700 OD units. All replicate Cut-off Calibrator ODs should be within 0.10 absorbance units from the mean value.

Index Values, divide specimen optical density (OD) values by the mean of the Cut-off Calibrator absorbance values.

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. Patient specimens which exhibit an index value of > 1.20 indicate the presence of IgM antibodies to Parvovirus B19. This is indicative of an early response to infection.

< 0.80 Negative. Patient specimens which exhibit an index value of < 0.80 indicate no detectable IgM antibodies to Parvovirus B19.

≥ 0.80 but ≤ 1.20 Equivocal. Patient specimens which exhibit an index value of ≥ 0.80 but ≤ 1.20 are considered doubtful or equivocal results. All equivocal results should be retested. If, on retesting, the first sample remains equivocal, a second sample should be drawn several weeks later to identify a rise in IgM antibody titer. If the second sample is either negative or equivocal this indicates no IgM antibody to Parvovirus B19 are detectable.

LIMITATIONS

1. All results from this and other serologies must be correlated with the clinical history, epidemiological data, and other data available to the attending physician in making the diagnosis of Parvovirus B19 infection.
2. Timing of the sample is critical in the serological demonstration of an IgM response. Patients with early Parvovirus may test IgM-negative. A negative result does not rule out Parvovirus. If a negative test is obtained on a patient with signs and symptoms of Parvovirus, repeat testing on a second sample two weeks later.
3. Patients with high titers of IgM antibody to both viral and non-viral diseases occasionally show crossreactivity on Focus's Parvovirus B19 IgM DxSelect.

EXPECTED VALUES

The timing of the sample is critical in the demonstration of an IgM response. IgM antibodies generally appear 10 to 13 days after infection and continue to increase until 30 to 60 days after the illness. IgM levels may reach undetectable levels after 60 to 90 days. The likelihood of a positive result therefore depends on the stage of the disease at which the sample was collected. A negative result does not rule out Parvovirus B19 infection.

A positive result may indicate early disease or a persistent IgM antibody response at a later disease stage when other cross-reactive conditions have been ruled out.

PERFORMANCE CHARACTERISTICS

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

REFERENCES


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