



Biotrin

**Parvovirus B19
Immunofluorescent Assay**

(FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES)

An immunofluorescence assay for the detection of
anti-Parvovirus B19 IgG and IgM antibodies.

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Intended Use

The Parvovirus B19 Immunofluorescent Assay is intended for the qualitative and semi-quantitative detection of anti-Parvovirus B19 IgG and IgM antibodies in human serum.

Introduction

Parvovirus B19 was first identified as a human pathogen in 1975 and has subsequently been shown to be the causative agent of a number of clinical conditions such as rash, arthralgia and fetal damage.^{1,2,3} Parvovirus B19 infection in adults, especially women, may cause acute arthritis which can persist for some time.⁴ Infection can lead to life threatening anemia in immunocompromised patients and individuals with underlying hemolytic disorders such as sickle cell disease.^{5,6} The virus is an icosahedral, non-enveloped virus of 18 – 25nm diameter and comprises a linear single stranded DNA genome (5.5kb) which is

encapsulated within an outer capsid.^{7,8} The viral capsid is composed of two structural proteins, namely VP1 (83kDA) and VP2 (53kDA). Parvovirus B19 infection is normally acquired by direct contact with respiratory secretions and normally occurs in localised outbreaks during the winter and spring months.⁸

It is now accepted that seronegative women are susceptible to Parvovirus B19 infection.^{9,10} The majority of pregnancies during which Parvovirus B19 infection occurs result in delivery of a healthy fetus at term.^{10,11,12} However, infection during pregnancy presents the risk of transmission to the fetus which may result in hydrops fetalis or intrauterine death. Estimates in the literature, for the rate of fetal death following maternal infection range between 1 and 9%.^{10,13,14} It has been suggested that because Parvovirus B19 replicates predominantly in red blood cell precursors, infection during pregnancy can lead to fetal death due to severe fetal anemia. It is thought that this severe anemia, whereby hemoglobin

levels fall to less than 2g/dL, is the primary cause of fetal hydrops.^{15,16}

The symptoms associated with Parvovirus B19 infection only become apparent after the viremic (contagious) stage has terminated.¹⁰ Furthermore, it is known that there is an increased risk of transmission in situations where close contact between individuals is likely, such as schools, day care centers and hospitals. Centers for Disease Control (CDC) do not recommend that persons exhibiting signs of Parvoviral infection (e.g. erythema infectiosum) be excluded from such environments. It is, however, recommended that all relevant individuals are made aware of the possibility of disease transmission.¹⁰

Consequently, it is important to identify the Parvovirus B19 antibody status in individuals who may be at risk of infection from, or who have been infected with, Parvovirus B19.

Serological assays are the mainstay of B19 diagnosis and Biotrin International offers a panel of Parvovirus B19 assays suitable for routine laboratory diagnosis.

- Parvovirus B19 IgG EIA
Catalogue No. V519IGUS
- Parvovirus B19 IgM EIA
Catalogue No. V619IMUS

Assay Principle

The Biotrin Parvovirus B19 Immunofluorescent Assay utilises the indirect immunofluorescence antibody technique first described by Coons *et al.*¹⁷ Serum is incubated with Parvovirus B19 recombinant VP1 antigen in insect cells stabilised on a glass slide.^{18,19} If anti-Parvovirus B19 antibodies are present in the sample, a stable complex is formed with the antigen. Bound antibody is then reacted with a fluorescent-labelled anti-human IgG or IgM antibody and this three-part complex is visualised with the aid of a fluorescence microscope. To prevent interference from rheumatoid factors (Rf) and to reduce IgG competition in the IgM test, samples should be pre-treated with a suitable adsorbent reagent.

Kit Components

Materials Provided

1. Slides – 6 x 10 well slides

SLIDE

On which recombinant Parvovirus B19 antigen expressed in insect cells has been stabilised. Foil pouch.

2. IgM Positive Control**

CONTROL + IgM

1 x 250µL positive sera containing specific anti-Parvovirus B19 antibodies. Contains thiomersal (0.01%). Green Cap.

3. IgG Positive Control**

CONTROL + IgG

1 x 250µL positive sera containing specific anti-Parvovirus B19 antibodies. Contains thiomersal (0.01%). Blue cap.

4. Negative Control**

CONTROL -

1 x 250 μ L negative control sera containing no detectable anti-Parvovirus B19 antibodies. Contains thiomersal (0.01%). Red cap.

5. Fluorescent anti-IgM Conjugate

CONJ IgM

1 x 2mL Rabbit anti-human IgM antibody conjugated to fluorescein isothiocyanate (FITC). Contains Evans Blue counterstain and thiomersal (0.01%). Purple cap.

6. Fluorescent anti-IgG Conjugate

CONJ IgG

1 x 2mL Rabbit anti-human IgG antibody conjugated to fluorescein isothiocyanate (FITC). Contains Evans Blue counterstain and thiomersal (0.01%). Yellow cap.

7. Wash Buffer Concentrate

BUF WASH 25X

1 x 45mL of concentrated (25x) Tris buffered saline with Tween 20 (0.25%) and thiomersal (0.01%). Clear cap.

8. Mounting Media

MM

1 x 2mL Tris – glycerol buffer. Contains thiomersal (0.001%). Orange cap.

9. Product Insert

INS

Instructions for use.

** Potentially Biohazardous Material

Additional Materials Required

- High quality distilled or deionised water.
- Accurate pipettes, micropipettes and disposable tips to deliver 5 μ L to 50 μ L, 50 μ L to 200 μ L.
- Serum collection equipment.
- Timer.
- Clean volumetric laboratory glassware.
- A suitable Rf and IgG adsorbent reagent with a minimum IgG binding capacity of 18mg/mL.
- 1L beaker.
- Dilution tubes and minifuge tubes (0.5mL).
- Benchtop minifuge.
- Incubation tray containing moistened tissue paper.
- Wash bottles and wash tray.
- 37°C incubator.
- No. 1 coverslips (22 x 50mm).
- Fluorescence microscope with appropriate filter combination for FITC (excitation filter 495nm, barrier filter 515nm), a halogen light source is recommended.
- Wax pencil.
- Fan.

Precautions

Safety

- For *in vitro* use only.
 - The kit is intended for use by qualified laboratory staff only.
 - All reagents derived from human origin are considered POTENTIALLY BIOHAZARDOUS MATERIAL. The Positive and Negative Control sera were tested by FDA-cleared methods for HBsAg and antibodies to HIV 1/2 and HCV and found to be negative. However, since no test can provide complete assurance of the absence of virus, treat all controls as potentially infectious.
 - Some reagents contain thiomersal which may be toxic if ingested.
 - Avoid contact with Evans Blue (in FITC IgM and IgG conjugates) as it is a potential carcinogen.
- If skin contact occurs, flush with large volumes of water.
- Dispose of all clinical specimens, infected or potentially infected material in accordance with good laboratory practice. All such materials should be handled and disposed of as though potentially infectious.
 - Residues of chemicals, preparations and kit components are generally considered as hazardous waste. All such materials should be handled and disposed of as though potentially infectious.
 - Wear protective clothing, disposable latex gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
 - Do not pipette materials by the mouth and never eat or drink at the laboratory workbench.

Procedural

- Do not use kit or individual reagents past their expiry date.
- Do not mix or substitute reagents from different kit lot numbers.
- Deviation from the protocol provided may cause erroneous results.
- Allow all reagents to come to room temperature (20 - 25°C) and mix well prior to use.
- Avoid leaving reagents in direct sunlight and/or outside 2 - 8°C for extended periods.
- When staining multiple samples on a slide avoid cross contamination between samples by marking between wells with a wax pencil.
- Application of excess mounting media may cause blurred fluorescence.
- Always use clean, preferably disposable, glassware for all reagent preparation.

- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component.
- Do not scratch the well with the pipette tip or dropper.
- Before commencing the assay an identification and distribution plan should be established.

Storage and Stability

- The kit is stable until the expiry date indicated on the outer box label provided it is stored between 2 - 8°C.
- Diluted Wash Buffer is stable for 6 months when stored at 2 - 8°C. Discard immediately if microbial contamination occurs.
- All unused components should be returned to 2 - 8°C storage immediately after use.

Specimen Collection and Storage

Serum samples should be obtained using aseptic laboratory techniques. These samples can be stored for up to 24 hours at 2 - 8°C and at -20°C or lower for longer periods.

Specimen and Reagent Preparation

Reagent Preparation

The Wash Buffer is supplied as liquid concentrate (25x). Prepare Wash Buffer for use by diluting 40mL of Wash Buffer Concentrate in 960mL of distilled water. Store in a clean closed container at 2 - 8°C for up to 6 months.

All remaining reagents are supplied ready to use and are at working dilution.

Specimen Preparation and Adsorption

- Qualitative Test:

For an IgG test dilute the serum sample 1:64 in Wash Buffer.

For IgM tests, samples should be adsorbed with suitable adsorbent for IgG and Rheumatoid factor. A final serum dilution of 1:16 is optimal for human serum.

- Semi Quantitative Test:

For IgG tests, the sample 'titer' can be determined by serially diluting the sample 1:64, 1:256, 1:1024 in wash buffer or until a '+ 1' grade of fluorescence is achieved (see "Interpretation of Results").

For IgM tests, the sample 'titer' can be determined by serially diluting the 1:16 adsorbed sample to 1:64, 1:256 in Wash Buffer

or until a '+1' grade of fluorescence is achieved (see "Interpretation of Results").

Note: The 'titer' of the sample is the dilution used to achieve a '+1' grade of fluorescence.

Note: Diluted samples should not be stored. If a repeat test is needed a fresh preparation should be used.

Assay Procedure

1. Allow all components to equilibrate to room temperature (20 - 25°C) before use.
2. Remove the desired number of slides from their sachets.
3. Mark between wells with a wax pencil to avoid cross contamination.
4. Dispense 20µL of each diluted sample and 20µL of the ready to use positive and negative controls onto numbered wells.
5. Incubate slides in an incubation tray for 3 hours at 35 - 39°C and 100% humidity.
6. Rinse slides briefly along the edge with Wash Buffer using a wash bottle. Place slides in a wash tray containing Wash Buffer (20mL/slide) for 10 minutes at room temperature (20 - 25°C). Rinse with Wash Buffer from wash bottle and fan dry slides.
7. For an IgM test dispense one drop (40µL) of anti-IgM FITC conjugate to each well. For an IgG test dispense one drop (40µL) of anti-IgG FITC conjugate to each well.
8. Incubate slides in the incubation tray in the dark for 30 minutes at 35 - 39°C and 100% humidity.

9. Repeat the washing procedure described in step 6 above.
10. Add one small drop of mounting media to the centre of each well and apply a coverslip.
11. Examine under a fluorescence microscope using 400 x magnification.

Interpretation of results

Negative: A sample is considered to be negative for Parvovirus B19 IgM and IgG antibodies if there are no visible fluorescence of the VP1 aggregates ('Bunch of Grapes' morphology).

Positive: A sample can be considered positive for Parvovirus B19 IgM and IgG antibodies if a positive fluorescent result is obtained at a dilution of $\geq 1:16$ and $\geq 1:64$, respectively. Positive fluorescence is indicated by staining of the distinct VP1 protein aggregates ('Bunch of Grapes' morphology) and can be graded as follows:

+4 = Brilliant green fluorescence indicating very high titer Parvovirus B19 antibody presence.

+3 = Bright green fluorescence indicating high titer Parvovirus B19 antibody presence.

+2 = Green fluorescence indicating medium titer Parvovirus B19 antibody presence.

+1 = Dull green fluorescence indicating weak titer Parvovirus B19 antibody presence. This also indicates the end point dilution or 'titer' of the sample.

+/- = Faint green fluorescence indicating possible Parvovirus B19 antibody presence – confirm using other detection methods.

Cells which do not contain VP1 protein are included in each well to allow comparison of positive and negative cells. These stain blood red with Evans Blue counterstain.

Quality Control Criteria

The Calibrator (Positive Control) and Negative Control must always be included to determine the validity of test results. Results of an assay are considered valid if the following criteria are met.

1. The IgG and/or IgM Positive Control yields a fluorescence greater than or equal to +2.
2. The Negative Control yields no visible fluorescence of the VP1 aggregates ('Bunch of Grapes' morphology).

If the above criteria are not met the assay is considered invalid and must be repeated.

Limitations of Use

- For research use only - Not to be used in Diagnostic Procedures.
- Test performance may be affected by deviation from the procedure, interpretation, sample type or recommended precautions.

Summary of Parvovirus B19 IFA IgG and IgM Procedure

Important Note:

Please read the entire product instruction leaflet before starting the assay.

This summary is for quick reference only.

- For an IgG test dilute serum samples 1 in 64 in Wash Buffer.
- For an IgM test adsorbed samples should be diluted to final dilution of 1 in 16.
- Pipette 20 μ L of Positive Control (ready to use), 20 μ L Negative Control (ready to use) and 20 μ L of prepared samples onto wells.
- Incubate for 3 hours @ 35 - 39°C, 100% humidity.
- Wash slides with Wash Buffer.
- For IgG test add 40 μ L IgG conjugate to each well.

- For IgM test add 40 μ L IgM conjugate to each well.
- Incubate for 30 min in the dark @ 35 - 39°C, 100% humidity.
- Wash slides with Wash Buffer.
- Add mounting media to each well.
- Examine under a fluorescence microscope.

Interpretation of Symbols



- Temperature limitation



- Use by end of



- Manufacturer



- Batch code



- Catalogue Number

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