Parvovirus B19
IgM Enzyme Immunoassay

An enzyme immunoassay for the qualitative detection of
B19 virus (B19V) IgM antibodies
in human serum and plasma.

Biotrin

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A. INTENDED USE

The Biotrin Parvovirus B19 IgM Enzyme Immunoassay is intended for the qualitative detection of IgM antibodies to B19 virus (B19V, previously known as human parvovirus B19) in human serum and, lithium heparin, EDTA and citrated plasma. This test, in conjunction with the Biotrin Parvovirus B19 IgG Enzyme Immunoassay, may be used for testing women of childbearing age to determine their serological status where there is a suspicion of exposure to B19V. The results of these assays may be used to make a serological determination of past, recent, or current infection with B19V. The clinician should consider the results of these assays as presumptive for risk of fetal infection with B19V. The test may also be used for all patients as an aid in the diagnosis of fifth disease (erythema infectiosum).

Precaution: The performance characteristics of these assays have not been established for other B19V associated diseases or testing neonates.

Caution: Federal law restricts this device to sale by or on the order of a physician.

B. INTRODUCTION

B19 virus (B19V) 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 fifth disease (erythema infectiosum), rash, arthralgia and fetal damage[1,2,3]. B19V, previously known as human parvovirus B19, has been classified in the genus *Erythrovirus* of the family *Parvoviridae.*[4] B19V is an iscosahedral, non-enveloped virus of 18-25nm diameter and comprises a linear single stranded DNA genome (5.5kb) which is encapsulated within an outer capsid[5,6]. The viral capsid is composed of two structural proteins, namely VP1 (83kDa) and VP2 (53kDa). B19V infection is normally acquired by direct contact with respiratory secretions and normally occurs in localised outbreaks during the winter and spring months[6].

In the normal host, the most widely recognised manifestation of B19V is the mild, rash illness erythema infectiosum (EI), also called fifth disease. This is typically the fifth rash disease of childhood and is also seen in adults. EI is usually a mild illness characterised by an intensive erythematous maculopapular facial rash, which gives rise to the use of the term 'slapped cheek disease'. Onset of the rash occurs coincident with production of B19V-specific antibodies suggesting that it is immune mediated.[6]

It is now accepted that seronegative women are susceptible to B19V[7,8]. The majority of pregnancies during which B19V infection occurs result in delivery of a healthy fetus at term[8,9,10]. However, infection during pregnancy presents the risk

of transmission to the fetus that 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%[8,11,12]. It has been suggested that because B19V 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[13,14].

The symptoms associated with B19V infection only become apparent after the viremic (contagious) stage has terminated[8]. Furthermore, it is known that there is an increased risk of transmission in situations where close contact between individuals is likely, such as schools, daycare centers and hospitals. Most infections during pregnancy were attributable to exposure from a woman's own children and much less so to occupational exposure.[15] The Centers for Disease Control and Prevention (CDC) do not recommend that persons exhibiting signs of B19V infection (e.g., erythema infectiosum) be excluded from such environments. However, it is recommended that all relevant individuals are made aware of the possibility of disease transmission[8].

Consequently, it is important to identify the B19V antibody status in individuals who may be at risk of infection from, or who have been infected with, B19V.

C. ASSAY PRINCIPLE

The **Biotrin Parvovirus B19 IgM Enzyme Immunoassay** is a mu-capture sandwich enzyme immunoassay for the detection of IgM class antibodies to B19V in human serum and plasma. The IgM in serum or plasma will bind to the rabbit anti-human IgM coated on the wells. Following a wash step, biotinylated B19V recombinant VP2 protein is added that binds to any human specific anti-B19V IgM if present. After another wash step, streptavidin peroxidase is added which binds to the biotinylated VP2 present. The whole complex is then detected by addition of tetramethylbenzidine substrate (TMB) which turns blue in the presence of peroxidase. A stable yellow end product is achieved by the addition of a stopping reagent.

D. PRECAUTIONS

- **For *in vitro* diagnostic use only.**

Safety

- Reagents marked with ** on the label are considered POTENTIALLY BIOHAZARDOUS MATERIAL. Each donor unit used in the preparation of the calibrator and control sera was tested by an FDA-approved method for HBsAg and antibodies to HIV-1, HIV-2 and HCV and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, all reagents labelled with ** and all patient specimens should be

handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories", 1988.

- Some reagents contain thimerosal that may be toxic if ingested. Stop Solution also contains sulfuric acid that is corrosive. Avoid contact with the skin and eyes. If contact occurs rinse off immediately with water and seek medical advice.
- The substrate contains TMB which may irritate the skin and mucous membranes. Any substrate that comes into contact with the skin should be rinsed off with water.
- The Biotin VP2 concentrate and Biotin VP2 diluent contain sodium azide which may form potentially explosive metal azides with lead and copper plumbing. For disposal, reagent should be flushed with large volumes of water to prevent azide build up.
- 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.
- 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.
- Test performance may be affected by deviation from the procedure, interpretation, or recommended precautions.

Procedural

- **Note: Since the quality control material is prediluted, it is recommended that an additional control material (e.g. a weakly reactive specimen) be used to control Sample Diluent preparation.**
- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- 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 above 4°C for extended periods.
- Ensure Wash Concentrate is mixed thoroughly and no crystals remain before reconstitution.
- High quality distilled or deionised water is required for the Wash Solution. The use of poor quality or contaminated water may lead to background colour in the assay.

- 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.
- Reagent delivery should be aimed at the midpoint of the side of the wells, taking care not to scratch the side with the pipette tip.
- Do not allow the wells to dry at any stage during the assay procedure. Always keep the upper surface of the wells free of droplets. Drops should be gently blotted dry on completion of the procedural step.
- Ensure that the bottom surface of the plate is clean and dry before reading.
- Before commencing the assay an identification and distribution plan should be established.

E. KIT COMPONENTS

Materials Provided

1. Coated ELISA plate
   12 x 8 wells coated with rabbit anti-human IgM contained in a foil pouch

2. Calibrator**
   1 x 2 ml of prediluted positive human plasma in a stabilising buffer with thimerosal (0.01%).

3. Low Positive Control **
   1 x 2ml of prediluted weakly positive human sera in a stabilising buffer with thimerosal (0.01%).

4. Negative Control **
   1 x 2ml of prediluted negative human sera in a stabilising buffer with thimerosal (0.01%).

5. Enzyme Conjugate Concentrate
   1 x 1.4ml of Streptavidin-HRP conjugate (10x) in stabilising buffer with thimerosal (0.01%).

6. Enzyme Conjugate Diluent
   1 x 14ml of a dilution buffer containing stabilisers and thimerosal (0.01%).

7. Sample Diluent Concentrate
   1 x 5ml of concentrated (21 x) PBS buffer containing stabilisers and thimerosal (0.095%).

8. Wash Concentrate
   1 x 55ml of a concentrated buffer (20x) containing surfactant and thimerosal (0.01%).

9. Biotin VP2 Concentrate
   1 x 1.4ml of a concentrated (10x) Biotinylated recombinant VP2 in a buffer solution containing stabilisers and sodium azide (0.01%).

10. Biotin VP2 Diluent
    1 x 14ml of a dilution buffer containing stabilisers and thimerosal (<0.01%) and sodium azide (0.01%)

11. Substrate
    1 x 11 ml of tetramethylbenzidine (TMB) solution.

12. Stop solution
    1 x 11ml of 1N H_2SO_4.

13. Product insert
    Instructions for use.

Additional Materials Required

- High quality distilled or deionised water
- Serum or plasma collection equipment
- Accurate pipettes, micropipettes and disposable tips to deliver 10µl, 100µl, 1ml and 5ml volumes
- Test tubes or equivalent for sample preparation
- Graduated cylinders
- Clean volumetric labware
- Plastic lid or sealing tape for microwell plate
- Paper towels or absorbent paper
- Timer
- Manual or Automatic washing device
- ELISA plate reader with 450 nm filter (additional 630 nm reference filter is optional but recommended)

F. 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.

- 8-well Strips should be stored in the pouch along with the sachet of desiccant.

- All unused components should be returned to 2-8°C storage immediately after use.

- Reconstituted Biotin VP2, Wash Solution and Sample Diluent are stable for 1 month when stored at 2-8°C.

- Reconstituted Enzyme Conjugate is stable for one month when stored at 2-8°C in glass containers.

G. SPECIMEN COLLECTION AND STORAGE

Either serum or plasma can be used in the Biotrin Parvovirus B19 IgM Enzyme Immunoassay. If serum is to be used, once collected by venipuncture, blood should be allowed to clot at room temperature (20-25°C) followed by centrifugation at 1500 x g for 10 minutes. If serum or plasma is not tested within 8 hours, the serum or plasma can be placed at 2-8°C for up to 2-3 days or frozen at –20°C if extended storage or shipment is required (samples are stable at –20°C for at least 1 year). Lithium heparin, EDTA and citrated plasma are compatible with the test procedure. It is recommended that hemolysed, icteric, lipemic or microbially contaminated sera not be used for testing. Test specimens should not be subjected to repeated freeze-thaw cycles.

Note: The overall concentration of immunoglobulins will be slightly reduced in citrated plasma due to the volume of citrate buffer used to prevent coagulation.

Note: See Clinical Interpretation section for issues concerning the collection of specimens for seroconversion.

H. REAGENT AND SPECIMEN PREPARATION

Reagent Preparation

- Reagent volumes are based on duplicate sample testing.

- **Wash Solution**
  For each 8-well Strip add 4ml of Wash Concentrate to 76ml of deionised water.
  Prepared reagent stable for 1 month if stored at 2-8°C.

- **Sample Diluent**
  For each 8-well Strip add 0.25ml of Sample Diluent Concentrate to 5ml of prepared **Wash Solution**.
  Prepared reagent stable for 1 month if stored at 2-8°C.

- **Biotin VP2**
  For each 8-well Strip, add 100µl of Biotin VP2 Concentrate to 900µl of Biotin VP2 Diluent.
  Prepared reagent stable for 1 month if stored at 2-8°C.

- **Enzyme Conjugate**
  For each 8-well Strip add 100µl of Enzyme Conjugate Concentrate to 900µl of Enzyme Conjugate Diluent. Prepared reagent stable for 1 month if stored at 2-8°C.

All remaining reagents are **ready to use** and are at working strength.

Specimen Preparation
For each sample dispense 1ml of prepared Sample Diluent into a labeled test tube or equivalent. Add 10µl of serum or plasma sample and mix.

Since the quality material furnished is prediluted, it is recommended that additional control material (e.g. a weakly reactive specimen) be used to control Sample Diluent preparation.

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

I. ASSAY PROCEDURE

1. Allow all reagents and specimens to equilibrate to room temperature (20 – 25°C) before use.

2. Determine the number of 8-well Strips required. Establish an identification and distribution plan for controls and samples. One strip is suitable for testing 1 patient specimen, each additional strip allows for testing of a further 4 patient specimens.

Figure 1

	Strip 1	
A		**Negative Control**
B		**Negative Control**
C		**Calibrator**
D		**Calibrator**
E		**Low Positive Control**
F		**Low Positive Control**
G		**Patient No. 1**
H		**Patient No. 1**

Reagent volumes are based on duplicate sample testing. Assay performance characteristics have been established using duplicate testing.

3. Remove the desired number of 8-well Strips, place in a plastic frame and cover with a plastic lid/sealant tape. Return the remaining strips to the pouch and reseal along with desiccant.

4. Prepare Wash Solution and Sample Diluent (see "Reagent and Specimen Preparation").

5. Prepare patient sera (see "Reagent and Specimen Preparation").

6. Remove cover from strips and pipette 100µl of the **ready to use** Negative Control, **ready to use** Low Positive Control, **ready to use** Calibrator and prepared patient specimen, in duplicate, to the wells.

7. Cover the wells with a plastic lid/sealing tape and incubate for 1 hour (+/- 5 minutes) at room temperature (20-25°C).

8. Remove cover and wash each well 4 times with Wash Solution (250-300µl). After washing firmly tap the plate against an absorbent paper towel.

9. Prepare Biotin VP2 (see "Specimen and Reagent Preparation").

10. Add 100µl of prepared Biotin VP2 to each well immediately after the wash step is completed.

11. Cover the wells with a plastic lid/sealing tape and incubate for 30 minutes (+/- 2 minutes) at room temperature (20-25°C).

12. Remove cover and wash each well 4 times with Wash Solution (250-300µl). After washing firmly tap the plate against an absorbent paper towel.

13. Prepare Enzyme Conjugate (See "Reagent and Specimen Preparation").

14. Pipette 100µl of IgM Enzyme Conjugate into all wells immediately after the wash step is completed.

15. Cover the wells with a plastic lid/sealing tape and incubate for 30 minutes (+/- 2 minutes) at room temperature (20-25°C).

16. Remove cover and wash each well 4 times with Wash Solution (250-300µl). After washing firmly tap the plate against an absorbent paper towel.

17. Pipette 100µl of Substrate into all wells immediately after the wash step is completed.

18. Incubate for exactly 10 minutes at room temperature.

19. Pipette 100µl of Stop Solution into all wells and mix. Ensure that each addition is in the same sequence and time interval as the addition of Substrate.

20. Read immediately with an ELISA plate reader.

Note: Dual wavelength reading is recommended at 450nm with 630nm as the reference wavelength (use air as a blank for the 630nm filter). If this function is not available on the ELISA plate reader use 450nm only.

J. QUALITY CONTROL CRITERIA

Note: It is recommended that an additional control material (e.g. a weakly reactive specimen) be used to control Sample Diluent preparation.

The calibrator control material furnished is single donor plasma and the negative control material is single donor serum. Equivalence has been demonstrated for serum, heparinised plasma, EDTA plasma and citrated plasma. The user may wish to include additional control material for different matrices.
Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

The Calibrator, Low Positive and Negative Controls must always be included to determine the validity of test results. The calibrator OD is required to calculate the assay cut off, the low positive control is provided to monitor kit functionality close to the assay cut off and the negative control guards against false positive results. Results of an assay are considered valid if the following criteria are met:

1. The mean absorbance of the Calibrator is greater than or equal to 1.2 Optical Density Units.

2. The mean absorbance of the Negative Control is less than or equal to 0.15 Optical Density Units and below the COV x 0.9.

3. The Low Positive Control must have an OD value $\geq$ 0.25 and $\leq$ 0.6 with an Index $\geq$ 1.1 .

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

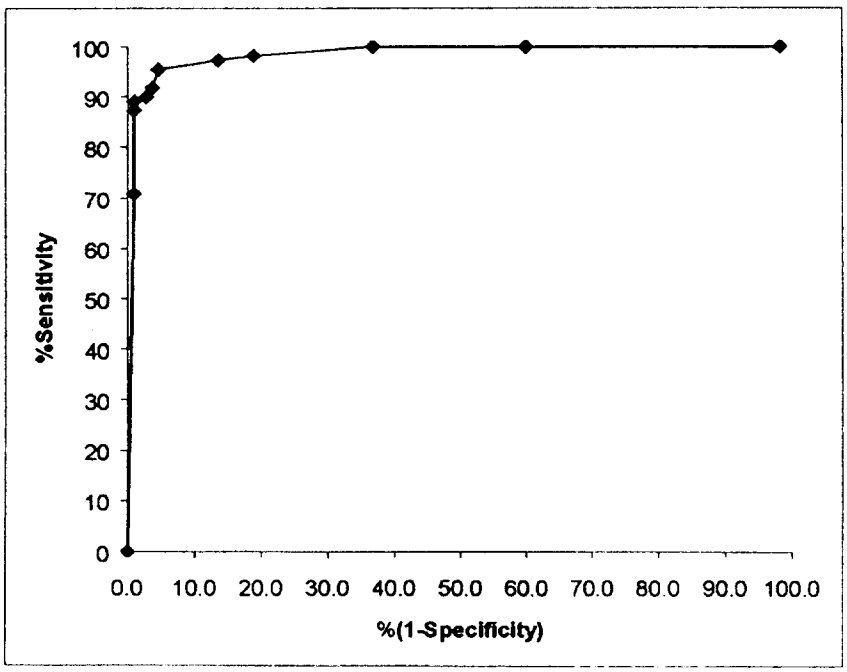
Note: Please refer to NCCLS C24-A2 for further information concerning quality control procedures and practices[16].

K. INTERPRETATION OF RESULTS

The presence or absence of anti-B19V IgM is determined in relation to a calculated Cut Off Value (COV).

To establish the assay cut-off Receiver Operating Characteristic (ROC) analysis* was performed using a panel of 112 specificity specimens and 110 sensitivity specimens to assess the assay sensitivity and specificity. At the determined assay cut-off, the Biotrin Parvovirus B19 IgM Enzyme Immunoassay demonstrated sensitivity and specificity of 89.9% and 99.1%, respectively.

Figure 2: ROC Analysis of the Biotrin Parvovirus B19 IgM Enzyme Immunoassay.



*An immunofluorescence assay was used to classify patient status according to NCCLS guidelines for ROC analysis[16].

Calculation of COV

Calculate the COV by multiplying the mean absorbance of the Calibrator (C) by the Lot Specific Constant (LSC) as indicated on the inside cover of the box,

$$COV = C \times LSC$$

Interpretation (1): Absorbance

Samples with a mean absorbance reading greater than the COV x 1.1 are considered reactive (positive) for detectable levels of anti-B19V IgM.

Samples with a mean absorbance reading less than the COV x 0.9 are considered non-reactive (negative) for anti-B19V IgM.

Samples with a mean absorbance reading greater than or equal to COV x 0.9 and less than or equal to COV x 1.1 are equivocal.

Interpretation (2): Index Value

Data comparison between different assay runs is facilitated by using an index value whereby sample absorbance is expressed relative to the assay cut-off value. In this case, an index value <0.9 or >1.1 indicates sample negativity or positivity, respectively. Equivocality is indicated if the index value is in the range 0.9-1.1.

$$\text{Index} = \frac{\text{Sample absorbance}}{\text{Cut-off Value(COV)}}$$

Equivocal samples should be retested in duplicate to verify the result. The index is calculated from the average OD of the duplicate retest. Samples which are positive on retest are considered to be reactive. Samples which are negative on retest are considered to be unreactive. Samples equivocal on retest should be reported as equivocal and a new specimen requested. Regardless of the repeat test result, subsequent samples should be drawn 1 to 2 weeks later to confirm the reported result or to determine if seroconversion has occurred.

Clinical Interpretation:

Biotrin Parvovirus B19 IgM Serology	Biotrin Parvovirus B19 IgG Serology	Interpretation
IgM Negative	IgG Negative	Implies No Past Infection - Patient May be Susceptible to Infection
IgM Negative	IgG Positive	Implies Past Exposure/ Infection – minimal risk of B19V infection
IgM Equivocal	IgG Positive or Negative	May be indicative of a Current or Recent Infection – resample patient within 1 to 2 weeks and retest.
IgM Positive	IgG Positive	Implies Current or Recent Infection – fetus may be at risk
IgM Positive	IgG Negative or Equivocal	May be indicative of a Current Infection – resample patient within 1 to 2 weeks and retest.

Due to the nature of the B19V IgG and IgM presentation over time, it may be possible that samples are drawn from patients following the decline of the IgM response. Clinicians should therefore be aware of this possibility when evaluating IgG+/IgM- test results.

Interpretation of the serological results must be made in the context of the clinical presentation of each patient. Seroconversion studies have demonstrated that patients infected with B19V may develop detectable levels of B19V specific IgM antibodies 4 to 10 days following infection and tend to decrease over a period of several weeks or months. B19V specific IgG antibodies may develop within 7 to 12 days following infection and tend to remain at detectable levels for a minimum of 1 to 2 years. Specimens taken prior to seroconversion may yield negative IgM and IgG antibody results, while specimens taken after IgM antibody levels have begun to decline may yield negative IgM antibody results. The results of a single assay or combination of the Biotrin Parvovirus B19 IgM and IgG Enzyme Immunoassay's results should not preclude additional testing, subsequent sampling from the patient 1 to 4 weeks following the initial test, or other pertinent medical care.

The magnitude of the measured result, above the cutoff, is not indicative of the total amount of antibody present.

If Index Values are reported it is suggested that results be reported in the following manner: "The following results were obtained with the Biotrin Parvovirus B19 IgM Enzyme Immunoassay. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgM level cannot be correlated to an endpoint titer."

EXPECTED RESULTS

A total of 399 samples from two separate US populations (Age range: 17-75) were tested using the Biotrin Parvovirus B19 IgM ELISA to evaluate the seroprevalence of IgM antibodies to B19V. The data from this seroprevalence study demonstrate that the total rate of B19V IgM positivity is 0.5% (1/200) and 1.5% (3/199). The B19V IgM positivity rate observed is 1% when both populations are combined (n=399). Notably all of the positive samples detected were either in or slightly above the equivocal range of the Biotrin IgM Parvovirus B19 Enzyme Immunoassay. Of the IgM positive samples, one was from a male and 3 were from female donors with an age range of 25 to 45 years. No attempt was made to determine whether the positive results were from recently or acutely infected individuals.

L. LIMITATIONS FOR USE

- Specimens containing anti-nuclear antibodies may produce equivocal or positive test results in the Biotrin Parvovirus B19 IgM Enzyme Immunoassay (See Section M. Performance Characteristics).
- EBV IgM positive specimens may also produce positive or equivocal test results in the Biotrin Parvovirus B19 IgM Enzyme Immunoassay (See Section M. Performance Characteristics).
- Test results of specimens from immunocompromised patients may be difficult to interpret.
- Assay performance characteristics for visual interpretation of results have not been established.
- Testing should not be performed as a screening procedure for the general population.

M. PERFORMANCE CHARACTERISTICS

The clinical utility of the Biotrin Parvovirus B19 IgM and IgG Enzyme Immunoassays were assessed in a population group of pregnant women in a study of 300 specimens from 250 women conducted at the Magee Womens Hospital, Pittsburgh, USA using archival specimens. As there is no standard reference method for B19V serology, samples were analysed for B19V specific IgG and IgM by reference immunofluorescence assays (IFAs). Samples were also analysed for B19V specific IgM by a reference B19V IgM Western blot assay and for B19V specific IgG by a reference B19V IgG Immunoblot assay.

Data from the reference B19V IFAs and blot assays were available for 239 of the initial specimens from the 250 patients. Taking the initial, or presentation, serum sample from each of these 239 patients, the B19V IgG and IgM results from all methods were compared. A serological diagnosis was established

based on the result of any one reference assay. Patients were then grouped according to their serological diagnosis as having an acute or recent infection (IgM+, IgG+ or IgG-), having had a previous B19V infection, (IgM- and IgG+) or not having had a previous B19V infection (IgM- and IgG-).

IFA IgG	IFA IgM	IB IgG	WB IgM	No. Pts.	Patient's Serological Status
+	-/E	+	-	141	Previously infected
-/E	-	+	-	6	
+	+	+	+	13	Acute/Recent Infection
+	+	+	-	3	
+	-	+	+	2	
-	+	+	+	1	
-	-	-	-	73	Not previously infected
			Total =	239	

E = equivocal

Note: Other permutations = 0, not shown.

The clinical grouping was then compared to the Biotrin Parvovirus B19 IgM and IgG Enzyme Immunoassays' results alone.

Figure 3: Comparison of the Biotrin Parvovirus B19 Enzyme Immunoassays' results to the serological diagnosis.

Serological Diagnosis		Acute/Recent Infection IgG+/IgM+ IgG-/IgM+ (n=19)	Previously Infected IgG+/IgM- (n=147)	Not Previously Infected IgG-/IgM- (n=73)
Biotrin Parvovirus B19 EIAs	Agree	15	143	73
	Disagree	4[#]	4[*]	0
	Agreement	78.9% (15/19)	97.3% (143/147)	100% (73/73)
	95% CI	54.9 to 94.0	93.2 to 99.2	95.1 to 100

Note: [#]4 specimens were Biotrin Parvovirus B19 Enzyme Immunoassay IgG+/IgM-

[*] 1 specimen was Biotrin Parvovirus B19 Enzyme Immunoassay IgG+/IgM+, 3 were Biotrin Parvovirus B19 Enzyme Immunoassay IgG-/IgM-

Serological Diagnosis Predictive Value:

		Acute/Recent Infection		
		+	-	
Biotrin Parvovirus B19 EIAs	+	15	1	16

Combined Biotrin Parvovirus B19 Parvovirus B19 Enzyme Immunoassays Positive Predictive Value for the Serological Diagnosis of an Acute/Recent B19V infection = 93.8% (15/16), 95% CI = 69.8 to 99.8.

		Previously Infected		
		+	-	
Biotrin Parvovirus B19 EIAs	+	143	4	147

Combined Biotrin Parvovirus B19 Enzyme Immunoassays Positive Predictive Value for the Serological Diagnosis of a Previously Infected patient = 97.3% (143/147), 95% CI = 93.2 to 99.2.

		Not Previously Infected		
		+	-	
Biotrin Parvovirus B19 EIAs	-	3	73	76

Combined Biotrin Parvovirus B19 Enzyme Immunoassays Negative Predictive Value for the Serological Diagnosis of a Not Previously Infected patient = 96.1% (73/76), 95% CI = 88.9 to 99.2.

An overall "Acute/Recent Infection" and "Previously Infected" Negative Predictive Value" for the Biotrin assays is calculated as 90.1% (73/73+8).

CDC Sera Panel:

The following information is from a serum panel established by the CDC (Centers for Disease Control and Prevention). The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC.

The panel consists of 55% positive and 45% negative anti-B19V IgM samples. The Biotrin Parvovirus B19 IgM Enzyme Immunoassay demonstrated 93.4% total agreement with the CDC results. Of the results obtained by Biotrin International, Limited, there was 86.4% agreement with the positive specimens and 100% agreement with the negative specimens.

Intra-assay Reproducibility:

A series of specimens ranging in B19V IgM levels from weakly reactive to strongly reactive were each assayed a total of twenty two times. Replicates were tested on a single ELISA plate from a single master lot of product. The resultant optical density (OD) values were summed and the mean OD value, standard deviation and percentage coefficient of variation (%CV) calculated, Table 1. These same results are presented in terms of assay index value in Table 2.

Test Specimen	Mean OD	SD	%CV	n
SR-A•	1.460	0.074	5.1	22
SR-B***	1.000	0.020	2.0	22
SR-C*	0.899	0.035	3.9	22
SR-D***	0.896	0.038	4.2	22
MR-E**	0.723	0.030	4.2	22
MR-F•	0.590	0.020	3.4	22
MR-G*	0.529	0.016	3.0	22
WR-H*	0.290	0.010	3.5	22
WR-I•	0.290	0.010	3.5	22
WR-J**	0.280	0.010	3.6	22
UR-K•	0.030	0.002	6.7	22
UR-L•	0.020	0.002	10.0	22

Table 1: Intra-assay reproducibility expressed in terms of OD ranging in B19V IgM levels from weakly reactive to strongly reactive. SR: strong reactive, MR: medium reactive, WR: weak reactive, UR: unreactive. •Serum, * EDTA plasma, **Heparinised plasma, ***Citrated plasma. Studies conducted at Biotrin

Test Specimen	Mean OD	SD	%CV	n
SR-A•	7.710	0.074	1.0	22
SR-B***	6.410	0.070	1.1	22
SR-C*	4.610	0.180	3.9	22
SR-D***	4.600	0.020	0.4	22
MR-E**	2.840	0.120	4.2	22
MR-F•	2.320	0.100	4.3	22
MR-G*	2.080	0.060	2.9	22
WR-H*	1.540	0.050	3.3	22
WR-I•	1.390	0.070	5.0	22
WR-J**	1.490	0.070	4.7	22
UR-K•	0.148	0.006	4.1	22
UR-L•	0.120	0.012	10.0	22

Table 2: Intra-assay reproducibility expressed in terms of index values on 22 replicates of each of 12 different serum specimens ranging in B19V IgM levels from weakly reactive to strongly reactive. SR: strong reactive, MR: medium reactive, WR: weak reactive, UR: unreactive. • Serum, * EDTA plasma, **Heparinised plasma, ***Citrated plasma. Studies conducted at Biotrin.

Inter-laboratory Reproducibility

Inter-laboratory reproducibility was investigated at two independent laboratories and at Biotrin. Each laboratory evaluated three Master Lots of the Biotrin Parvovirus B19 IgM Enzyme Immunoassay against a defined panel of coded samples comprising strongly reactive (serum n=3, heparinised plasma n=1, EDTA plasma n=1), weakly reactive (serum n=5, heparinised plasma n=1, EDTA plasma n=1) and unreactive (serum n=2, heparinised plasma n=1, EDTA plasma n=1) specimens. Inter-laboratory reproducibility data is presented below (Table 3). For each Master Lot, each sample was assayed three times per day (in duplicate) on three different days at each laboratory site. In most cases, each sample was therefore assayed 81 times, with the exception of one strongly reactibe specimen, which was assayed 79 times. Linear regression analysis of inter-laboratory reproducibility demonstrated correlation between results, analysed in terms of OD and index values, at all test sites and across all Master lots ($y=1.0501x +0.0199$ $r^2=0.99$; $y=0.9691x - 0.112$ $r^2=0.99$; $y=1.0107x -0.1644$ $r^2=0.99$; for site 1 versus 2, site 2 versus 3 and 1 versus 3, respectively).

No. of Specimens	Specimen Type	Detection rate (Expected result/Total number of times assayed)
5	Strongly reactive	100% (403/403)
7	Weakly reactive	95.5% (542/567)
4	Unreactive	100% (324/324)

Table 3: Overall detection rate for the Biotrin Parvovirus B19 IgM Immunoassay.
Note: Total No. of times assayed = No. of specimens x No. of assays (79 to 81, as described above).

Inter-assay Reproducibility

When the above data is analysed in terms of interassay reproducibility, the Biotrin Parvovirus B19 IgM enzyme immunoassay demonstrates good correlation in test results between different centers and different Master Lots. Reproducibility data for six test specimens is given in Tables 4 and 5.

Specimen	Mean OD	SD	%CV	n
SRI**	1.201	0.233	19.4	81
SR2•	1.103	0.199	18.0	81
SR3*	1.204	0.229	19.0	79
SR4***	1.539	0.028	1.8	9
WR1•	0.462	0.113	24.5	81
WR2**	0.303	0.090	29.7	81
WR3*	0.407	0.108	26.5	81
WR4***	0.335	0.023	6.9	9

Table 4:Overall interassay reproducibility. Data (OD) accumulated from 3 test sites / 3 Master lots of Biotrin Parvovirus B19 IgM Enzyme Immunoassay. SR: Strong reactive, WR: Weakly reactive. • Serum, * EDTA plasma, **Heparinised plasma, ***Citrated plasma. (***studies conducted at Biotrin only).

Specimen	Mean OD	SD	%CV	n
SRI**	5.493	0.832	15.2	81
SR2•	5.044	0.670	13.3	81
SR3*	5.55	0.653	11.8	79
SR4***	5.666	0.196	3.5	9
WR1•	2.088	0.350	16.8	81
WR2**	1.356	0.234	17.3	81
WR3*	1.834	0.305	16.6	81
WR4***	1.238	0.072	5.8	9

Table 5:Overall interassay reproducibility. Data (Index Values) accumulated from 3 test sites, 3 Master lots of Biotrin Parvovirus B19 IgM Enzyme immunoassay. SR: Strong reactive, WR: Weakly reactive. • Serum, * EDTA plasma, **Heparinised plasma, ***Citrated plasma. (***studies conducted at Biotrin only).

Inter-assay reproducibility of kit controls

	Mean OD	SD	%CV	n
Calibrator	1.579	0.265	16.8	81
Low Positive Control	0.404	0.101	25.0	81
Negative Control	0.036	0.009	25.0	81

Table 6: Inter-laboratory reproducibility of the Biotrin Parvovirus B19 IgM Enzyme Immunoassay controls expressed in OD units, from 3 batches of kits tested at three different sites over a total of 81 assays.

	Mean Index	SD	%CV	n
Low Positive Control	1.812	0.206	11.4	81
Negative Control	0.167	0.039	23.4	81

Table 7: Inter-laboratory reproducibility of the Biotrin Parvovirus B19 IgM Enzyme Immunoassay controls expressed in terms of index values, from 3 batches of kits tested at three different sites over a total of 81 assays.

Assay Specificity

The specificity of the Biotrin Parvovirus B19 IgM Enzyme Immunoassay was assessed by testing specimens from patients with viral infections or disease states which may cause clinical symptoms similar to B19V (Table 7).

Specimens tested	Number of positives
Cytomegalovirus (CMV)	0/21
Epstein-Barr Virus (EBV)	1/25▲
Rubella virus	0/20
Rubeola virus	0/5
Mumps virus	0/5
Varicella Zoster virus (VZV)	0/5
Human Herpes virus -1	0/5
Human Herpes virus - 2	0/5
Toxoplasma gondii	0/14
Lyme disease	0/5
Thyroiditis	0/5
Rheumatoid Arthritis (RA)	0/5
Lupus erythematosus	0/5
Anti-Nuclear Antibodies (ANA)	2/8▲
Rheumatoid Factor (RF)	0/5
Mycoplasma	0/5
Parainfluenza type 3	0/5
Influenza B	0/4

Table 7: Biotrin Parvovirus B19 IgM Enzyme Immunoassay specificity.

▲EBV IgM and ANA specimens which were reactive in the Biotrin Parvovirus B19 IgM Enzyme Immunoassay were also tested by B19V immunoblot and immunofluorescent assays and were found to be reactive in both formats. Hence, the likelihood of genuine B19V IgM reactivity cannot be excluded. Studies conducted at Biotrin.

Summary of Biotrin Parvovirus B19 IgM Enzyme Immunoassay Procedure

Important Note:
Please read the entire product instruction leaflet before starting the assay. This summary is for quick reference only.

Prepare Wash Buffer and Sample Diluent

▼

Dilute samples 1 in 101 in Sample Diluent

▼

Pipette 100µl of **(ready to use)** Negative Control,
100µl **(ready to use)** Low Positive Control,
100µl **(ready to use)** Calibrator
and 100µl of prepared samples, in duplicate, into wells

▼

Incubate for 1 hour @ room temperature

▼

Wash strips 4 times

▼

Prepare Biotin VP2

▼

Add 100µl of prepared Biotin VP2

▼

Incubate for 30 min @ room temperature

▼

Wash strips 4 times

▼

Prepare Enzyme Conjugate

▼

Add 100µl of IgM Conjugate

▼

Incubate for 30 min @ room temperature

▼

Wash strips 4 times

▼

Add 100µl of Substrate

▼

Incubate for 10 min @ room temperature

▼

Add 100µl of Stop Solution

▼

Read at 450nm
With or without 630nm reference

BIBLIOGRAPHY

1. **Cossart, Y.E., Field, A.M., Cant, B. and Widdows, D.**
(1975) "Parvovirus like particles in human sera." Lancet, 1, 72-73

2. **Anderson, M.J., Higgins, P.G., Davis, L.R., Willman, J.S., Jones, S.E., Kidd, I.M., Pattison, JR. and Tyrrell, D.A.J.**
(1985) "Experimental parvoviral infection in humans." J. Infect. Disease, 152, 257-265.

3. **Knott P.D., Welply, G.A.C. and Anderson, M.J.**
(1984) "Serologically proved intrauterine infection with parvovirus." Br. Med. J. 289,1660.

4. **Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A.W., Martilli, G.P., Mayo, M.A., Summers, M.D. (eds.)**
(1995) "Virus Taxonomy: Classification and Nomenclature of Viruses; Sixth report of the international committee on taxonomy of viruses." Archives of Virology. Supplement 10, 586pp. Springer Verlag, Wien, New York.

5. **Ozawa, K., Ayub, J., Yu-Shu, H., Kurtzman, G., Shimada, T., and Young, N.S.**
(1987) "Novel Transcription Map for the B19 (human) Pathogenic Parvovirus." J. Virol., 61 (8), 2395-2406.

6. **Török, T.,**
(1995) "Human Parvovirus B19" in "Infectious Diseases of the Fetus and Newborn Infant." Eds: Remington, J.S. and Klein, J.O. 4th ed. 668702 ISBN: 0-7216-6782-1.

7. **Jordan, J.A.**
(1996) "Identification of human parvovirus B19 infection in idiopathic nonimmune hydrops fetalis." Am. J. Obstet. Gynecol.174 (1), 3742.

8. **Centers for Disease Control and Prevention**
(1989) "Risks Associated with human Parvovinus B19 Infection." MMWR CDC Surveill. Summ., 38, 81-97.

9. **Woernle C.H., Anderson L.J., Tattersall P., et al.**
(1987) "Human Parvovirus B19 during pregnancy." J. Infect. Dis. 156, 17-20.

10. **Gratacos E., Torres P-J., Vidal J., Antolin E., Costa J., Jimenez de Anta M. T., Cararach V., Alonso P.L., Fortuny A.**
(1995) "The incidence of human Parvovirus B19 infection during pregnancy and its impact on perinatal outcome." J. Infect. Dis. 171, 1360-1363.

11. **Public Health Laboratory Service Working Party on Fifth Disease.**
(1990) "Prospective study of human Parvovirus (B19) infection in pregnancy." Br. Med. J. 300 1166-1170.

12. **Rodis J.F., Hovick T.J., Quinn D.L., Rosengren S.S., Tattersall P.**
(1988) "Human Parvovirus infection in pregnancy." Obstet. Gynecol. 72. 733-738.

13. **Brown, K.E., Hibbs, J.R. Gallinella, G., Anderson, S.M., Lehman, E.D., McCarthy P. and Young, N.S.**
(1994) "Resistance to Parvovirus B19 infection due to lack of virus receptor (Erythrocyte P Antigen)." New Eng. J. Med., 330 (17), 1192-1196.

14. **Anderson, M.J., Khousam, M.N. Maxwell, D.J., Gould S.J., Happerfield, L.C. and Smith W.J.**
(1988) "Human Parvovirus B19 and Hydrops Fetalis." Lancet, March 5, 535.

15. **Valeur-Jensen, et al.**
(1999) "Risk Factors for Parvovirus B19 Infection in Pregnancy." JAMA Volume 281(12) 24-31 March, pp 1099-1105.

16. **NCCLS**
(1999) "Statistical Quality Control for Quantitative Measurements: Principles and Definitions, Approved Guideline." C24-A2, 19(5)

17. **NCCLS**
(1995) "Assessment of the clinical accuracy of Laboratory tests using Receiver Operating Characteristic (ROC) plots: Approved guidelines." GP10-A, 15 (19).

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