

## **Parvovirus B19 IgG Enzyme Immunoassay**

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



**Biotrin**

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## **A. INTENDED USE**

The Biotrin Parvovirus B19 IgG Enzyme Immunoassay is intended for the qualitative detection of IgG antibodies to B19 virus (B19V, previously known as human parvovirus B19) in human serum, lithium heparin, EDTA, and citrated plasma. This test, in conjunction with the Biotrin Parvovirus B19 IgM 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<sup>1,2,3</sup>. B19V, previously known as human parvovirus B19, has been classified in the genus *Erythrovirus* of the family *Parvoviridae*.<sup>4</sup> B19V 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<sup>5,6</sup>. 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<sup>6</sup>.

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.<sup>6</sup>

It is now accepted that seronegative women are susceptible to B19V<sup>7,8</sup>. The majority of pregnancies during which B19V infection occurs result in delivery of a healthy fetus at term<sup>8,9,10</sup>. 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%<sup>8,11,12</sup>. 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<sup>13,14</sup>.

The symptoms associated with B19V infection only become apparent after the viremic (contagious) stage has terminated<sup>8</sup>. 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.<sup>15</sup> 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.

It is, however, recommended that all relevant individuals are made aware of the possibility of disease transmission<sup>8</sup>.

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 IgG Enzyme Immunoassay** is a sandwich enzyme immunoassay for the detection of IgG class antibodies to B19V in human serum and plasma. Specific B19V IgG antibodies if present will bind to the wells coated with B19V recombinant VP2 protein. Following a wash step, peroxidase-labelled rabbit anti-human IgG is added which binds to the human B19V IgG 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.
- 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 a specimen identification and distribution plan should be established.

## **E. KIT COMPONENTS**

### ***Materials Provided***

1. Coated ELISA plate  
12 x 8 wells coated with purified recombinant VP2 protein contained in a resealable pouch.
2. Calibrator \*\*  
1 x 2ml of prediluted positive human sera 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  
1 x 11 ml of rabbit anti-human IgG HRP conjugate in a stabilising buffer with thimerosal (0.01%).
6. Sample Diluent Concentrate  
1 x 5ml of concentrated PBS buffer (21x) containing stabilisers and thimerosal (0.095%).
7. Wash Concentrate  
1 x 55ml of a concentrated PBS buffer (20x) containing surfactant and thimerosal (0.01%).
8. Substrate  
1 x 11ml of tetramethylbenzidine (TMB) solution.
9. Stop solution  
1 x 11ml of 1N H<sub>2</sub>SO<sub>4</sub>.
10. Product insert: Instructions for use.

### ***Additional Materials Required***

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

### **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 Wash Solution and Sample Diluent are stable for 1 month when stored at 2-8°C.

### **G. SPECIMEN COLLECTION AND STORAGE**

Either serum or plasma can be used in the Biotrin Parvovirus B19 IgG Enzyme Immunoassay. 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 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.

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

### ***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 components 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 as indicated in Figure 1 (below). The first strip is suitable for testing 1 patient specimen, each subsequent 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 specimen (see "Reagent and Specimen Preparation").
6. Remove cover from strips and pipette 100 $\mu$ l, in duplicate, of the **ready to use** Negative Control, **ready to use** Low Positive Control, **ready to use** Calibrator and prepared patient specimen 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 (250-300 $\mu$ l). After washing firmly tap the plate against an absorbent paper towel.
9. Pipette 100 $\mu$ l of IgG Enzyme Conjugate into all wells immediately after the wash step is completed.
10. Cover the wells with a plastic lid/sealing tape and incubate for 30 minutes (+/- 2 minutes) at room temperature (20-25°C).

11. Remove cover and wash each well 4 times with Wash Solution (250-300 $\mu$ l). After washing firmly tap the plate against an absorbent paper towel.
12. Pipette 100 $\mu$ l of Substrate into all wells immediately after the wash step is completed.
13. Incubate for exactly 10 minutes at room temperature (20-25°C).
14. Pipette 100 $\mu$ l 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.
15. 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 quality control material furnished is in a serum matrix. 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 also 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<sup>16</sup>.**

## **K. INTERPRETATION OF RESULTS**

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

### **Establishment of the assay cut off**

The Biotrin Parvovirus B19 IgG Enzyme Immunoassay cutoff has been calibrated against the WHO Parvovirus B19 IgG International Standard - 1995 (IS). The enzyme immunoassay cut-off value equates to an IS level of 3-5 IU/mL<sup>17</sup>.

### **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,

$$\text{COV} = C \times \text{LSC}$$

### **Interpretation (1): Absorbance**

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

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

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:**

<b>Biotrin Parvovirus B19 IgM Serology</b>	<b>Biotrin Parvovirus B19 IgG Serology</b>	<b>Interpretation</b>
IgM Negative	IgG Negative	Implies No Past Infection - Patient May be Susceptible to B19V 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 B19V Infection – resample patient within 1 to 2 weeks and retest.
IgM Positive	IgG Positive	Implies Current or Recent B19V Infection – fetus may be at risk
IgM Positive	IgG Negative or Equivocal	May be indicative of a Current B19V 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 Biotrin Parvovirus B19 IgM and IgG Enzyme Immunoassays' 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 IgG Enzyme Immunoassay. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgG 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 IgG Enzyme Immunoassay. The total seroprevalence was found to be 73.2% in a healthy US blood donor population. B19V IgG positivity was essentially unaffected by sex and the geographical location from which the samples were derived. The only factor which appears to affect the rate of positivity is age this is in agreement with previous studies relating to seroprevalence and age.

### **L. LIMITATIONS FOR USE**

- 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 IFAs. Samples were also analysed for B19V specific IgM by the a reference B19V IgM Western blot assay and for B19V specific IgG by a reference 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-). The clinical grouping was then compared to the Biotrin Parvovirus B19 IgM and IgG Enzyme Immunoassay result alone.

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.

**Figure 2: 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 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 "Previous 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 73% positive and 27% negative anti-B19V IgG samples. The Biotrin Parvovirus B19 IgG Enzyme Immunoassay demonstrated 99.0% total agreement with the CDC results (note one specimen QNS for Biotrin testing). Of the results obtained by Biotrin International, Limited, there was 98.6% agreement with the positive specimens and 100% agreement with the negative specimens.

**Intra-assay Reproducibility:**

A series of specimens, ranging in B19V IgG levels from weakly 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 summated and the mean OD value, standard deviation and percentage coefficient of variation (% CV) in terms of assay index value are given in Table 2.

Test Specimen	Mean OD	SD	%CV	n
SR-A•	1.590	0.012	0.8	22
SR-B•	1.261	0.025	2.0	22
SR-C*	1.853	0.045	2.4	22
SR-D**	1.862	0.050	2.7	22
SR-E***	1.837	0.031	1.7	22
WR-A•	0.380	0.012	3.2	22
WR-B•	0.310	0.010	3.2	22
WR-C*	1.305	0.034	2.6	22
WR-D**	1.183	0.019	1.6	22
WR-E***	1.104	0.018	1.6	22
UR-A•	0.190	0.010	5.3	22
UR-B•	0.070	0.006	8.6	22
UR-C*	0.062	0.005	8.1	22
UR-D**	0.068	0.007	10.3	22
UR-E***	0.058	0.004	6.9	22

Table 1: Intra-assay reproducibility expressed in terms of optical density (OD). SR: strong reactive, WR: weak reactive, UR: unreactive, • Serum, \* EDTA plasma, \*\*Heparinised plasma, \*\*\*Citratd plasma. Studies conducted at Biotrin.

Test Specimen	Mean Index	SD	%CV	n
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SR-A•	5.560	0.120	2.2	22
SR-B•	4.510	0.091	2.0	22
SR-C*	6.464	0.156	2.4	22
SR-D**	6.497	0.176	2.7	22
SR-E***	6.408	0.110	1.7	22
WR-A•	1.280	0.040	3.1	22
WR-B•	0.950	0.040	4.2	22
WR-C*	4.467	0.117	2.6	22
WR-D**	4.049	0.064	1.6	22
WR-E***	3.781	0.061	1.6	22
UR-A•	0.580	0.020	3.5	22
UR-B•	0.220	0.017	7.7	22
UR-C*	0.194	0.015	7.7	22
UR-D**	0.214	0.023	10.8	22
UR-E***	0.180	0.013	7.2	22

Table 2: Intra-assay reproducibility. SR: strong 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 in-house (3 sites). Each laboratory evaluated three Master Lots of the Biotrin Parvovirus B19 IgG Enzyme Immunoassay against a defined panel of coded specimens 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, except for one strongly reactive specimen, which was assayed 72 times, and one weakly reactive specimen, which was assayed 80 times. Linear regression analysis of interlaboratory reproducibility demonstrated correlation between results, analysed in terms of OD and index values, at all test sites and across all Master lots ( $y = 0.9691x - 0.112$   $R^2 = 0.9932$ ,  $y = 1.0678x + 0.0652$   $R^2 = 0.99$ , and  $y = 0.9575x + 0.0728$   $R^2 = 0.99$  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% (396/396)
7	Weakly reactive	99.8% (565/566)
4	Unreactive	100% (324/324)

Table 3: Overall detection rate for the Biotrin Parvovirus B19 IgG Immunoassay.

Note: Total No. of times assayed = No. of specimens x No. of assays (72 to 81, as described above).

### Inter assay Reproducibility

When this data is analysed in terms of interassay reproducibility, the Biotrin Parvovirus B19 IgG 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*	1.445	0.225	15.6	81
SR2**	1.362	0.251	18.4	81
SR3•	1.104	0.201	18.2	81
SR***	1.373	0.117	8.5	9
WR1•	0.537	0.108	20.1	81
WR2•	0.518	0.085	16.4	81
WR3•	0.466	0.110	23.6	81
WR***	0.439	0.039	8.9	9

Table 4: Overall interassay reproducibility. Data (OD) accumulated from 3 test sites/3 Master lots of the Biotrin Parvovirus B19 IgG Enzyme Immunoassay. SR: strong reactive, WR: weak reactive. • Serum, \* EDTA plasma, \*\*Heparinised plasma, \*\*\*Citratd plasma. (\*\*\*Conducted at Biotrin only.)

Specimen	Index	SD	%CV	n
SRI*	5.081	0.583	11.5	81
SR2**	4.778	0.610	12.8	81
SR3•	3.872	0.487	12.6	81
SR4***	4.423	0.262	5.9	9
WR1•	1.883	0.293	15.6	81
WR2•	1.814	0.264	14.6	81
WR3•	1.550	0.234	15.1	81
WR4***	1.413	0.101	7.1	9

Table 5: Overall interassay reproducibility. Data (Index Values) accumulated from 3 test sites/3 Master lots of the Biotrin Parvovirus B19 IgG Enzyme Immunoassay. SR: strong reactive, WR: weak reactive. • Serum, \* EDTA plasma, \*\*Heparinised plasma, \*\*\*Citratd plasma. (\*\*\*Conducted at Biotrin only.)

### Inter-assay reproducibility of the kit controls

	Mean OD	SD	%CV	n
Calibrator	1.971	0.218	11.1	81
Low Positive Control	0.509	0.092	18.1	81
Negative Control	0.034	0.018	52.9	81

Table 6: Inter-laboratory reproducibility of the Parvovirus B19 IgG 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	1.785	0.194	10.9	81
Negative Control	0.118	0.059	50.0	81

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

### WHO International Standard

The Biotrin Parvovirus B19 IgG enzyme immunoassay detects the WHO Parvovirus B19 IgG International Standard (IS) whereby the enzyme immunoassay cut-off value equates to an IS level of 3-5 IU/ml. No claims for immunoassay quantitation should be implied from this statement which is solely intended to indicate that the Biotrin Parvovirus B19 IgG enzyme immunoassay is compatible with the WHO International Standard.

### **Assay Specificity**

The majority of people have been exposed to B19V upon reaching adulthood, hence a relatively high IgG seroprevalence rate is observed in the adult population.<sup>18</sup> It would be expected that samples containing potentially cross-reactive antibodies would yield the same level of positivity (approx. 70%) as a blood donor group if the assay was specific for B19V IgG. A total of 45 sera, all testing IgG positive for variety of conditions (Table 8), were included in this study. All samples were tested on the Biotrin Parvovirus B19 IgG Enzyme Immunoassay.

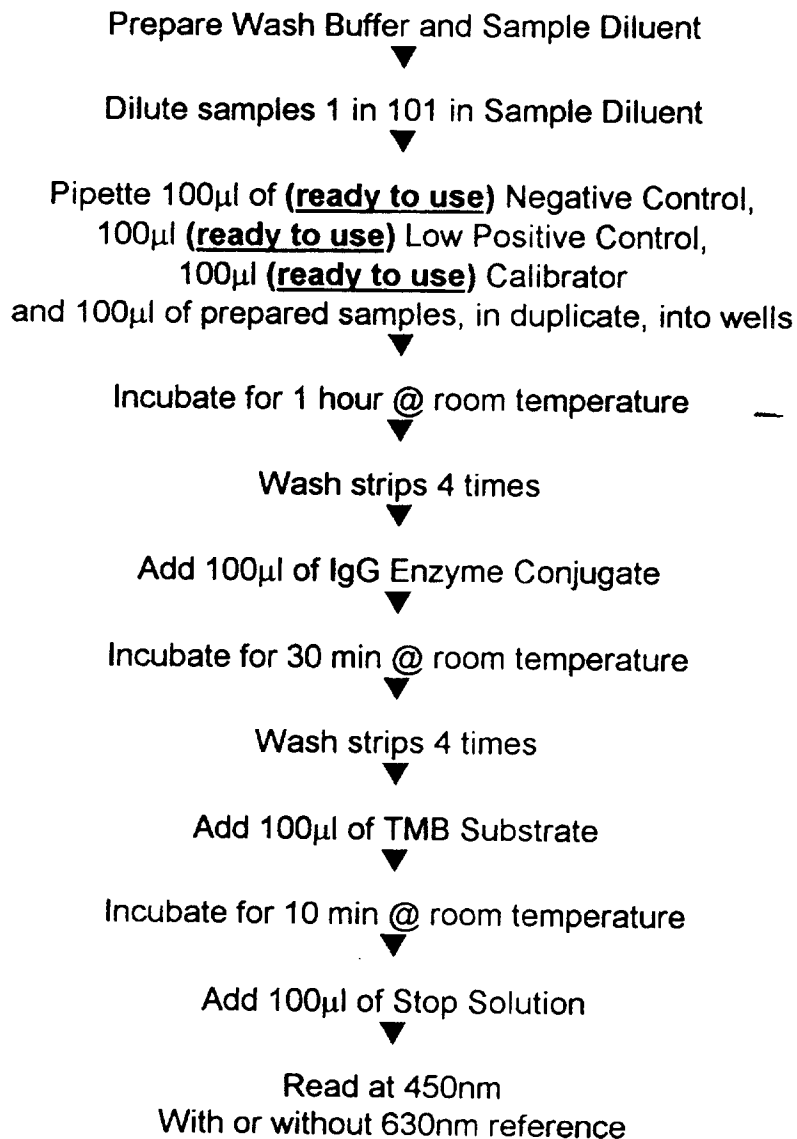
<b>Clinical Diagnosis (IgG+)</b>	<b>Total Number</b>	<b>Number Positive</b>	<b>Number Negative</b>	<b>Number Equivocal</b>	<b>% Positive</b>
Rubella	5	5	0	0	100
Varicella	5	3	2	0	60
Mumps	5	2	3	0	40
Herpes I, II	5	5	0	0	100
Rubeola	5	3	2	0	60
CMV	5	4	1	0	80
EBV	5	5	0	0	100
Lyme disease	5	4	1	0	80
Toxoplasmosis	5	5	0	0	100
Overall Numbers	45	36	9	0	
Overall %		80	20	0	80

**Table 8:** Overall Summary of Biotrin Parvovirus B19 IgG Enzyme Immunoassay specificity results. Studies conducted at Biotrin.

The overall rate of positivity observed is 80%, which is consistent with published literature.<sup>16</sup>

## Summary of Biotrin Parvovirus B19 IgG Enzyme Immunoassay Procedure

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



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**Biotrin International Ltd.**  
93 The Rise, Mount Merrion,  
Co. Dublin  
IRELAND  
Tel: (01) 283 1166  
Int: + 353 1 283 1166  
Fax: (01) 283 1232  
Int: + 353 1 283 1232  
email: info@biotrin.i.e.

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**Biotrin International GmbH**  
Wiesenstraße 16,  
74889 Sinsheim-Reihen,  
GERMANY  
Tel: (07261) 62122  
Fax: (07261) 62624

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**Biotrin International SARL**  
14 rue Gorge de Loup,  
69009 Lyon,  
FRANCE  
Tel: 72.53.04.61  
Fax: 72.53.04.76

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