Parvovirus B19 IgM Test System

IVD

REF 9Z7701M/SM9Z7701M Σ96
9Z7701MB Σ480

For Prescription Use Only

INTENDED USE

The ZEUS ELISA Parvovirus B19 IgM Test System is intended for the qualitative detection of IgM class antibodies to human parvovirus B19 in human serum including women of childbearing age where there is a suspicion of exposure to human parvovirus B19. The test is also for all symptomatic patients as an aid in the diagnosis of fifth disease (erythema infectiosum). This test is intended for *in vitro* diagnostic use only.

SUMMARY AND EXPLANATION

Human parvovirus B19 is a simple single-stranded DNA virus (1) that can only infect humans. The non-enveloped mature viral particles are composed of only two structural proteins; VP1 and VP2. The VP2 capsomer proteins comprise approximately 96% of the total capsid (2). The human parvovirus B19 is different from the parvovirus that infects dogs and cats. A human cannot acquire parvovirus B19 from dogs and cats, nor can a human infect dogs and cats with human parvovirus B19.

Human parvovirus B19 can be found globally and commonly infects people. Infected humans have varying symptoms depending on their age and general health. Up to 20% of infected adults or children are essentially asymptomatic, or have a very mild, nonspecific, cold-like illness (3). One symptomatic form of the human parvovirus B19 infection is erythema infectiosum. This is commonly referred to as "fifth disease" or "slapped cheek" disease and is frequently the outcome of exposure of the virus to children (4, 5). The illness may start with symptoms such as fever, runny nose and/or headache and then may progress into the classic red rash on the face (slapped cheek rash). The rash may spread to other parts of the body, may become itchy and may vary in intensity before dissipating typically in 7 - 10 days (3). People with fifth disease can also develop pain and swelling in joints (polyarthropathy syndrome). This is more typical in adults (especially women) than in children. The joint pain may last for weeks to months and will usually go away with no long term problems (3).

Infected people are generally contagious before they develop overt symptoms and when contagious, the virus spreads through respiratory secretions when an infected person coughs or sneezes. The virus can also spread through blood or blood products and therefore a pregnant woman can pass the virus to the fetus (6). Infection of the fetus may lead to serious complications. The infant may develop severe anemia or myocarditis as a result of infection of the heart. These disorders may ultimately lead to congestive heart failure and hydrops fetalis(4). Hydrops fetalis is a serious condition resulting from fluid build-up as a result of severe anemia. The fluid buildup may result in organ failure. Sometimes the fetus may be born without symptoms, but there is also a 2-6% chance of fetal loss (4). Parvovirus B19 infection during the first trimester pregnancy is low risk, whereas the second trimester pregnancies are most vulnerable (7). If a pregnant woman is exposed to human parvovirus B19, acute infection should be confirmed by the presence of IgM antibody or seroconversion of IgG or both. If an acute infection is confirmed the fetus should be monitored frequently to prevent risk of hydrops fetalis.

PRINCIPLE OF THE ASSAY

The ZEUS ELISA Parvovirus B19 IgM Test System is designed to detect IgM class antibodies to parvovirus B19 in human sera. The wells of the plastic microwell strips are coated with recombinant parvovirus B19 viral proteins as antigen. The test procedure involves three incubation steps:

1. Test sera are diluted with the Sample Diluent provided. The Sample Diluent contains anti-human IgG that precipitates and removes IgG and rheumatoid factor from the sample leaving IgM free to react with the immobilized antigen on the surface of the wells. During sample incubation any antigen specific IgM antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgM is added to the wells and the plate is incubated. The Conjugate will react with IgM antibody immobilized on the solid phase in step 1. The wells are washed to remove unbound Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator and Sample Diluent.**

Component	Σ96	Σ480	Description
PLATE	1	5	Plate: 96 wells configured in twelve, 1x8-well, strips coated with recombinant parvovirus B19 viral proteins as antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	1	5	Conjugate: Conjugated (horseradish peroxidase) anti-human IgM (μ chain specific) in 15mL, white-capped bottle(s). Ready to use.
CONTROL +	1	2	Positive Control (Human Serum): 0.35mL, red-capped vial(s).
CAL	1	4	Calibrator (Human Serum): 0.5mL, blue-capped vial(s).
CONTROL -	1	2	Negative Control (Human Serum): 0.35mL, green-capped vial(s).
DIL SPE	1	4	Sample Diluent: 30mL, green-capped, bottle(s) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Purple Solution. Ready to use.
SOLN TMB	1	5	TMB: 15mL, amber-capped, amber bottle(s) containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN STOP	1	3	Stop Solution: 15mL, red-capped, bottle(s) containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF 10X	1	5	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped, bottle(s) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably within the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. The Sample Diluent may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 006M.**
2. **Test System also contains:**
   a. **Component Label containing lot specific information inside the Test System box.**

b. Package Insert providing instructions for use.

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (10).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The SAVe Diluent®, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).

STORAGE CONDITIONS

2°C – 8°C	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening - strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, SAVe Diluent®
2°C – 25°C	Stop Solution: 2 - 25°C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days. Wash Buffer (10X): 2 - 25°C

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated serum obtained by approved aseptic venipuncture procedures in this assay (8,9). Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. They are stable at –20°C or lower for a minumum of 12 months. Avoid multiple

freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (9).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Negative Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Positive Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient specimen.
4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of Sample Diluent to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1. and 2. for a total of 5 washes.
      4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.
   b. **Automated Wash Procedure**:
      If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. ⟶ *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. ⟶ *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. ⟶ *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

	OD Range
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥0.500

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
   c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay Cutoff.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations:**
   a. *Correction Factor:* The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.

b. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.

c. *Cutoff OD Value:* To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above. *(CF x Mean OD of Calibrator = Cutoff OD Value)*

d. *Index Values/OD Ratios:* Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step c.

Example:	Mean OD of Calibrator	=	0.793
	Correction Factor (CF)	=	0.25
	Cutoff OD	=	0.793 x 0.25 = 0.198
	Unknown Specimen OD	=	0.432
	Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

	Index Value/OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 - 1.09
Positive Specimens	≥1.10

a. An OD ratio ≤0.90 indicates no significant amount of IgM antibodies to parvovirus detected.

b. An OD ratio ≥1.10 indicates that IgM antibodies specific to parvovirus were detected.

c. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimen using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

Cutoff Determination: The cutoff of the ZEUS ELISA Parvovirus B19 IgM assay was determined by initially choosing a negative, borderline, and medium positive samples from a comparison assay. The amount of antigen coated onto the plate, as well as the HRP-conjugate concentration were both titrated, and the assay was run in a matrix fashion using the 3 samples. The antigen and conjugate concentration combination, along with a cut off value was identified by choosing those that yielded data most closely in-line with the chosen index values for each respective sample. These values were subsequently used as a guideline to create an assay prototype. An expanded comparative testing of 245 serum samples was performed with the prototype ZEUS IgM ELISA kits in an effort to verify the established cutoff.

LIMITATIONS OF THE ASSAY

1. To make a serological determination of past, recent or current infection, specimens must be tested on both the IgG and IgM versions of the ELISA test.
2. Avoid the use of hemolytic, lipemic, bacterially contaminated or heat inactivated specimens. Otherwise erroneous results may occur.
3. The magnitude of the measured result above the cut-off is not indicative of the total amount of antibody present and cannot be correlated to IFA titers.
4. Assay performance characteristics have not been established for visual result determinations.
5. Use caution when evaluating samples obtained from immunosuppressed patients.

EXPECTED RESULTS

A total of 3210 specimens were tested at two different sites during the clinical studies. 210 of the specimens were from expectant women; 70 from each of all three trimesters of pregnancy. The remaining 3000 specimens were collected from three different geographical locations (1000 per location) within the US. The demographic characteristics representative of each cohort is shown in the table below:

	Pregnancy Samples	North Eastern US		Mid Atlantic US		Western US	
n =	210	1000		1000		1000	
Percent Male	0%	15.2%		18.8%		21.7%	
Percent Female	100%	84.8%		78.7%		77.8%	
Percent Unknown	0%	0.5%		2.5%		0.4%	
		Male	Female	Male	Female	Male	Female
Lowest age	18	<1	<1	<1	<1	<1	<1
Highest age	43	88	86	89	88	87	88
median age	27	40	33	47	32	40	32
mean age (years)	27.6	39.5	34.8	44.0	35.3	39.3	35.2

For the 210 pregnancy specimens tested, the incidence of parvovirus IgM antibody is 3.3% with the ZEUS ELISA. For the 3000 prospective specimens, the incidence of parvovirus B19 IgM antibody is 3.8% with the ZEUS ELISA.

PERFORMANCE CHARACTERISTICS

1. Comparative Study

A comparative study was conducted using 3210 specimens consisting of pregnancy samples, 70 from each of the three trimesters and 3000 prospective specimens. The prospective specimens were routine submits for parvovirus B19 serology collected from three different geographical locations (1000 per location) within the US. The outcome of the comparative testing is summarized below.

PREGNANCY SAMPLES:

Correlation between the ZEUS ELISA and a composite reference method for the Specimens from Pregnant Women.

The table below shows the correlation of the ZEUS IgM ELISA Test System with a composite reference method. The latter is based on results from an FDA approved comparator ELISA in combination with a validated Parvovirus B19 IgM Immunoblot results using the following criteria:
Composite reference method positive = Comparator ELISA positive/immunoblot positive
Composite reference method negative = All Comparator ELISA negatives
Composite reference method negative = Comparator ELISA positive/immunoblot negative
Composite reference method equivocal = Comparator ELISA positive/immunoblot uncertain

		Composite Reference Method			
		Positive	Negative	Equivocal	Total
ZEUS IgM ELISA Test System	Positive	0	7	0	7
	Negative	0	203	0	203
	Equivocal	0	0	0	0
	Total	0	210	0	210

Positive Agreement = N/A
Negative Agreement = 96.7% (203/210) (95% CI = 94.2% to 99.1%).

PROSPECTIVE SAMPLES:

Correlation between the ZEUS ELISA and a composite reference method for the 3000 prospective specimens

The table below shows the correlation of the ZEUS IgM ELISA Test System with the composite reference method described above.

		Composite Reference Method			
		Positive	Negative	Equivocal	Total
ZEUS IgM ELISA Test System	Positive	41	66	5	112
	Negative	0	2870	3	2873
	Equivocal	1	12	2	15
	Total	42	2948	10	3000

Positive Agreement = 91.1% (41/45) (95% CI = 82.8% to 99.4%)
Negative Agreement = 97.2% (2870/2953) (95% CI = 96.6% to 97.8%).

NOTE: In the table above, for calculations of Positive Agreement and Negative Agreement, specimens that were equivocal by either ZEUS ELISA or the composite reference method were counted against the ZEUS ELISA.

PERFORMANCE PANEL:

A commercially available 21 member parvovirus B19 serology panel was tested. ZEUS ELISA Parvovirus B19 IgM Test System performance was compared relative to a referernce parvovirus B19 IgM ELISA. Ninteen of the twenty-one specimens (90.5%) were in agreement between the two devices. The two discordant specimens when tested on an immunoblot agreed with the ZEUS ELISA Parvovirus B19 IgM Test System.

2. Precision/Reproducibility

Two studies were conducted to assess the precision of the ZEUS ELISA Parvovirus B19 IgM Test System. The Precision Study was conducted in-house at ZEUS Scientific. The Reproducibility Study was conducted at three locations (two outside locations and one at ZEUS Scientific).

Precision Study: Six specimens were included in the study. Parvovirus B19 IgM ELISA results for these six specimens could be characterized as follows: negative, high negative, borderline, low positive, moderate positive and high positive. These six specimens were tested in duplicate, two times per day, for 20 days by the same technician. Percent coefficient of variation (%CV) for Repeatability, Between Run, Between Day and Total %CV are shown in the table below:

			Repeatability		Between Run		Between Day		Total	
Sample	N	Mean	Standard Deviation	%CV	Standard Deviation	%CV	Standard Deviation	%CV	Standard Deviation	%CV
Borderline	80	1.12	0.08	7.46	0.04	4.01	0.08	7.16	0.12	11.09
High Negative	80	0.68	0.04	6.60	0.01	2.02	0.03	3.79	0.05	7.87
High Positive	80	3.54	0.13	3.79	0.05	1.32	0.16	4.58	0.22	6.09
Low Positive	80	1.49	0.09	6.01	0.03	2.32	0.10	6.71	0.14	9.30
Moderate Pos.	80	2.09	0.09	4.55	0.00	0.00	0.07	3.22	0.12	5.57
Negative	80	0.30	0.06	N/A	0.01	4.28	0.03	11.39	0.07	N/A

Reproducibility Study: Six specimens were included in the study. Parvovirus B19 IgM ELISA results for these six specimens could be characterized as follows: negative, high negative, borderline, low positive, moderate positive and high positive. These six specimens were tested in triplicate, twice a day, for 5 days by two separate technicians, using two lots of ZEUS ELISA Parvovirus IgM Test Systems. Percent coefficient of variation (%CV) for the Repeatability, Between Run, Between Day, Between Sites, Between Lots, Between Operators and Total %CV are shown in the table below:

			Repeatability		Between Run		Between Day		Between Site		Between Lot		Between Operator		Total	
Sample	N	Mean	Standard Deviation	%CV	Standard Deviation	%CV	Standard Deviation	%CV	Standard Deviation	%CV	Standard Deviation	%CV	Standard Deviation	%CV	Standard Deviation	%CV
Borderline	360	1.24	0.11	9.20	0.00	0.00	0.00	0.00	0.02	1.74	0.07	6.00	0.03	2.08	0.14	11.31
High Neg	360	0.68	0.06	9.32	0.01	1.14	0.01	1.13	0.02	3.38	0.03	4.57	0.04	5.51	0.08	12.33
High Pos	360	4.47	0.27	6.04	0.05	1.12	0.00	0.00	0.07	1.65	0.09	2.08	0.10	2.21	0.32	7.05
Low Pos	360	1.63	0.10	6.42	0.00	0.00	0.00	0.00	0.02	1.20	0.05	3.18	0.07	4.22	0.14	8.40
Mid Pos	360	2.46	0.18	7.16	0.00	0.00	0.03	1.26	0.02	0.75	0.00	0.00	0.03	1.16	0.18	7.40
Neg	360	0.17	0.07	N/A	0.00	0.00	0.01	7.73	0.02	12.93	0.06	N/A	0.00	0.00	0.10	N/A

3. Cross Reactivity

Samples that were IgM positive to different infectious agents or associated with other disease conditions were tested with ZEUS ELISA Parvovirus B19 IgM Test System and found to be non-reactive as shown in the table below.

Analyte	Number of Specimens	Number Positive on ZEUS Parvovirus IgM	Percent Cross Reactivity
Rubella IgM	10	0	0%
CMV IgM	10	0	0%
HSV1 IgM	10	0	0%
HSV2 IgM	10	0	0%
Mycoplasma IgM	10	0	0%
VZV IgM	10	0	0%
EBV VCA IgM*	5	0	0%
Influenza A IgM	10	0	0%
Influenza B IgM	10	0	0%
Measles IgM *	2	0	0%
RF IgM *	5	0	0%
ANA *	5	0	0%
Parvovirus IgG *	5	0	0%

*The results may not be conclusive due to the low number of specimens tested

4. Interference

An investigation was performed to assess the potential impact of commonly encountered interfering substances on the ZEUS ELISA Parvovirus B19 IgM Test System. Briefly, three serum samples were obtained. The samples could be characterized as follows: negative for parvovirus B19 IgM, low positive and high positive for parvovirus B19 IgM. Interfering substances were spiked into each of the three serum samples at two (high and low) different concentrations. Matrix controls were prepared to account for the spiking process. The interferents used and the amout spiked is shown below.

Interferent	High	Low	Matrix
Albumin (Human)	50 mg/mL	35 mg/mL	Serum
Bilirubin	0.15 mg/mL	0.01 mg/mL	Serum-10% PBS
Cholesterol	2.5 mg/mL	1.5 mg/mL	Serum-10% Ethanol
Hemoglobin	200 mg/mL	100 mg/mL	Serum
Intralipids	7.5mg/mL	3 mg/mL	Serum
Triglycerides	5mg/mL	1.5 mg/mL	Serum-10% Ethanol

The results of the testing are shown below:

Interferent	High Concentration Interferent			Low Concentration Interferent		
	High Positive (% Recovery)	Low Positive (% Recovery)	Negative (Still Negative?)	High Positive (% Recovery)	Low Positive (% Recovery)	Negative (Still Negative?)
Albumin (Human)	112	100	Yes	114	109	Yes
Bilirubin	104	120	Yes	102	100	Yes
Cholesterol	107	110	Yes	114	103	Yes
Hemoglobin	97	86	Yes	94	84	Yes
Intralipids	86	83	Yes	89	94	Yes
Triglycerides	100	92	Yes	105	91	Yes

The percent recovery ranged from 83% to 120%. These results show that the qualitative results remain the same at the concentrations of the interferent tested.

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