SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Parvovirus B19 IgG Enzyme Immunoassay
Device Trade Name:	ZEUS ELISA Parvovirus B19 IgG Test System
Device Procode:	MYM
Applicant Name and Address:	Zeus Scientific, Inc. PO Box 38 Raritan, NJ 08869
Date of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P150045

Date of FDA Notice of Approval: September 19, 2017

II. INDICATIONS FOR USE

The ZEUS ELISA Parvovirus B19 IgG Test System is intended for the qualitative detection of IgG 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 for *in vitro* diagnostic use only.

III. CONTRAINDICATIONS

None

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the labeling for the ZEUS ELISA Parvovirus B19 IgG Test System.

V. DEVICE DESCRIPTION

The ZEUS ELISA Parvovirus B19 IgG Test System is designed to detect IgG class antibodies to parvovirus B19 in human sera. The test has been developed following a typical indirect, enzyme-linked immunosorbent assay (ELISA) format and includes the key components; the ELISA microwell plate, the conjugate and the controls/calibrator. The microwell strips are coated with recombinant parvovirus B19 viral proteins as capture antigen. The test procedure involves three incubation steps:

1. Test sera are diluted with the Sample Diluent provided in the kit. During sample incubation, any antigen specific IgG 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 IgG (Conjugate) is added to the wells and the plate is then incubated. The Conjugate reacts with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized 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.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

For childhood infections, diagnosis can be largely made based upon the clinical presentation. For pregnant women, serology tests such as ELISA, indirect fluorescent assays (IFA) or western blot are the primary means of determining the mother's serological status. In the case of fetal infections, ultrasound; more specifically, Middle Cerebral Artery Peak Systolic Velocity (MCA-PSV) is a non-invasive means for determining the degree of fetal anemia.

VII. MARKETING HISTORY

There has been no prior commercial distribution of the ZEUS ELISA Parvovirus B19 IgG Test System.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the product to perform as intended, or errors in the use of the product, may lead to a false result and improper patient management. A false negative result may cause spreading of the virus to other individuals through contact and thus present a public health risk. Parvovirus B19 infection is generally self-limiting and benign for most healthy children but may pose a threat for high-risk populations such as immunocompromised patients or those with hemolytic anemia. The virus can also spread through blood and, therefore, a pregnant woman is at risk of passing the virus to the fetus causing serious complications. A false reactive result is not considered a public health risk except that the person may be isolated and monitored for a short period of time.

IX. SUMMARY OF NONCLINICAL STUDIES

1. IgG Class Specificity of the Assay
   The objective of this study was to demonstrate that the assay is designed to detect only human IgG antibody. Sample Diluent that was spiked with 10% goat anti-human IgG was used to remove IgG from five different IgG positive samples. The anti-

human IgG was effective in reducing the signal by at least 90% from the samples showing that the ZEUS IgG ELISA specifically detects IgG antibody.

2. Limits of Detection
   Testing with serial dilutions of the Second WHO Parvovirus B19 International Standard revealed that the ZEUS ELISA Parvovirus IgG Test System is able to detect 5-6 IU/mL at the assay cutoff, and can detect 97% of replicates above the assay cutoff when assayed at 7 IU/mL. Thus, the LOD for this assay is determined to be 5-7 IU/mL of the WHO International Standard.

3. Potential Interfering Substances
   A study was performed to assess the potential impact of commonly encountered interfering substances on the ZEUS ELISA Parvovirus B19 IgG Test System. Three serum samples, negative, low positive, and high positive for parvovirus B19 IgG antibodies were spiked with the interferents as shown below.

   Table 1: Interferent Concentrations Tested

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

   For positive samples, the recovery values ranged from 80-110% for all interfering substances tested. All positive samples remained positive, and all negative samples remained negative, regardless of the interferent or the concentration of the interferent. This study indicates that the ZEUS ELISA Parvovirus B19 IgG ELISA Test System is not at significant risk for positive or negative influence from the interferents tested.

4. Potential Cross Reactive Antibodies
   A study was performed to investigate if IgG antibody to other infectious agents or associated with other disease states might cross react with the ZEUS ELISA Parvovirus B19 IgG Test System. Of the 107 specimens tested, none tested positive with the ZEUS ELISA Parvovirus B19 IgG Test System.

Table 2: Summary of Cross Reactivity Results

Analyte	Number of Specimens	Number Positive on ZEUS Parvovirus IgG	Percent Cross Reactivity
Rubella IgG	10	0	0%
CMV IgG	10	0	0%
HSV1 IgG	10	0	0%
HSV2 IgG	10	0	0%
Mycoplasma IgG	10	0	0%
VZV IgG	10	0	0%
EBV VCA IgG	10	0	0%
Influenza A IgG	10	0	0%
Influenza B IgG	10	0	0%
Measles IgG	10	0	0%
Mumps IgG	10	0	0%
Parainfluenza	10	0	0%

5. Precision Study

The study was conducted with six specimens: 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.

Table 3: Results of the Precision Study

%CV IgG										
			Repeatability		Between Run		Between Day		Total	
Sample	N	Mean	Standard Deviation	%CV	Standard Deviation	%CV	Standard Deviation	%CV	Standard Deviation	%CV
Borderline	80	1.250	0.09	7.16	0.03	2.27	0.04	3.39	0.10	8.24
High Negative	80	0.733	0.04	6.10	0.02	2.65	0.04	5.68	0.06	8.75
High Positive	80	7.981	0.41	5.11	0.26	3.26	0.34	4.22	0.59	7.38
Low Positive	80	1.835	0.13	7.31	0.05	2.64	0.06	3.44	0.16	8.50
Moderate Pos.	80	3.077	0.21	6.89	0.09	2.87	0.11	3.47	0.25	8.24
Negative	80	0.210	0.04	N/A	0.02	8.39	0.02	11.44	0.05	N/A

6. Reproducibility Study

The study was conducted at three locations. Six specimens were included in the study: negative, high negative, borderline, low positive, moderate positive and high positive. These six specimens were tested in triplicate, twice a day, for five days by two separate technicians, using two lots of the ZEUS ELISA Parvovirus B19 IgG Test System.

Table 4: Results of the Reproducibility Study

Parvovirus IgG %CV by Sample																
			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	1.41	0.15	10.54	0.02	1.43	0.01	0.83	0.14	9.73	0.02	1.28	0.07	5.17	0.20	14.50
High Neg	360	0.84	0.09	10.69	0.01	1.21	0.01	1.27	0.08	10.07	0.03	4.04	0.05	6.36	0.13	15.33
High Pos	360	6.90	0.54	7.80	0.00	0.00	0.07	1.06	0.62	9.02	0.85	12.36	0.11	1.56	1.19	17.21
Low Pos	360	2.15	0.24	11.02	0.01	0.53	0.00	0.00	0.22	10.29	0.02	1.06	0.16	7.44	0.32	15.13
Mid Pos	360	3.42	0.30	8.93	0.05	1.59	0.04	1.27	0.21	6.12	0.00	0.00	0.15	4.51	0.38	11.01
Neg	360	0.16	0.04	N/A	0.00	0.00	0.01	8.74	0.05	N/A	0.00	0.00	0.01	4.18	0.07	N/A

7. Transportation Study

Two ZEUS ELISA Parvovirus B19 IgG Test System kits each were shipped from the Zeus Scientific facility in Branchburg, NJ to Austin TX, San Diego CA, and Paris, France. These shipments were returned to Zeus Scientific and analyzed. The shipping packages contained data recorders that logged the time, temperature and relative humidity of the contents during the shipping.

To evaluate the performance of the shipped kits versus the controls that never left the facility, twenty samples, 10 positive and 10 negative were tested. The qualitative results for all 20 samples tested using kits shipped to all three locations, were in complete agreement with data obtained from their respective non-shipped kit controls. These data demonstrated that the ZEUS ELISA Parvovirus B19 IgG Test System is not subject to altered performance due to the various factors associated with shipping conditions.

8. Product Shelf Life

Real time stability testing on different lots of ZEUS ELISA Parvovirus B19 IgG Test System demonstrated that the kits are stable for at least 18 months when stored at 2-8°C.

Table 5: Reagent Stability

	t = 0		t= 3 months			t= 12 months			t= 19 months		
Sample ID	**OD-blk**	**Index**	**OD-blk**	**Index**	**%Recovery**	**OD-blk**	**Index**	**%Recovery**	**OD-blk**	**Index**	**%Recovery**
NC	0.047	0.215	0.038	0.193	NA	0.031	0.240	NA	0.024	0.017	NA
Cal	0.620	2.835	0.554	2.81	99	0.338	2.620	92	0.410	2.890	102
Cal	0.616	2.817	0.525	2.66	94	0.342	2.650	94	0.369	2.600	92
Cal	0.537	2.456	0.521	2.64	108	0.368	2.850	116	0.371	2.610	106
PC	1.021	4.669	0.912	4.63	99	0.680	5.270	113	0.721	5.080	109
Positive	0.743	3.398	ND	ND	ND	ND	ND	ND	0.483	3.400	100
Negative	0.167	0.764	ND	ND	ND	ND	ND	ND	0.106	0.750	NA

ND = No Data exists for these samples at the indicated time points

NA = Not applicable; blk = Blank

9. Specimen Stability:

   a. Specimen Stability Study A
   A three member sample panel that consisted of a high positive, a low positive, and a negative sample was tested over five cycles of freezing and thawing. Both positive samples remained positive and the negative sample remained negative when tested by ZEUS ELISA Parvovirus B19 IgG Test System. The results of this study showed that the parvovirus B19 IgG antibody is stable and not subject to significant change after five freeze/thaw cycles and remained stable for a minimum of 12 months.

   b. Specimen Stability Study B
   A three member sample panel was selected for testing, and consisted of a high positive, a low positive, and a negative sample. The panel was used to assess the impact of storing samples at either room temperature or at 2-8°C. Samples were tested by the ZEUS ELISA Parvovirus B19 IgG Test System at multiple time points over a 14 day storage period. Both positive samples remained positive, and the negative sample remained negative. Index values did not deviate beyond 10% from their expected outcome for the positive samples. The results showed that the parvovirus B19 IgG antibody is stable when tested by the ZEUS ELISA Parvovirus B19 IgG Test System for up to 14 days at either room temperature or at 2-8°C.

10. Antimicrobial Effectiveness Study
    Antimicrobial effectiveness testing (by USP 51) of the reagent components of the ZEUS ELISA Parvovirus B19 IgG Test System demonstrated that the majority of the components meet Category 3 acceptance criteria and all of the components meet

Category 4 acceptance criteria. Those few reagent/bacteria combinations that did not meet Category 3 criteria were tested by Zeus for functionality with and without bacterial spikes. It was demonstrated that even with significant amounts of bacteria spiked into those components, the results of the ZEUS ELISA Parvovirus B19 IgG Test System were similar to non-spiked control results.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

A. Study Design

The samples were collected between July 1, 2015 and August 31, 2015. The database for this PMA reflected data collected through July 1, 2016 and included 3,210 patients. There were three collection sites and two testing sites. The two studies described below evaluated performance of the ZEUS ELISA Parvovirus B19 IgG Test System on clinical samples by comparing to another FDA approved test. One study evaluated samples from pregnant women. The other evaluated routine leftover specimens.

1. Clinical Inclusion and Exclusion Criteria
   Enrollment in the pregnant women study was limited to patients who met the following inclusion criteria.
   - Being pregnant

   Enrollment in the second clinical study was limited to patients who met the following inclusion criteria.
   - Samples submitted for routine Parvovirus B19 serology. All specimens were "leftover" specimens from routine submits and were not prescreened or preselected based upon any criteria.

   No exclusion criteria were applied to the samples used.

2. Follow-up Schedule

   The studies were performed on leftover specimens. Patient follow up was not applicable.

3. Clinical Endpoints
   With regards to both safety and effectiveness, assay-negative results compared to the results from an FDA approved assay are considered. With regards to effectiveness, the assay-positive results are compared to the results from an FDA approved assay. With regard to success/failure criteria, a point agreement result of 95% with the comparator and a lower bound 95% confidence interval of 90% were applied.

B. Accountability of PMA Cohort

There were 3,210 patient samples collected and the studies included 100% of these samples.

C. Study Population Demographics and Baseline Parameters

The demographics of the study population are typical for a clinical study performed in the US. Two hundred ten (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 specimens were routine submits for parvovirus B19 serology consisting of approximately 80% female and 20% male with an age range of <1-89 years. The median age for males was 42.3 years and for female 32.3 years and for the pregnant women cohort the median age was 27 years.

D. Safety and Effectiveness Results

1. Safety Results
   No adverse effects were reported during the study as the study evaluated performance using left over specimens. (For potential adverse effects, see Section XIII.

2. Effectiveness Results
   The analysis of effectiveness was based on the 3,210 evaluable patient samples. Key effectiveness outcomes are presented in Tables 6 and 7.

   a. Pregnant Women Testing
      A total of 210 samples from expectant women, consisting of 70 specimens from each of the three trimesters of pregnancy, was tested at two different locations. The specimens were tested with the ZEUS ELISA Parvovirus B19 IgG Test System and an FDA-approved parvovirus B19 IgG assay. The positive and negative percent agreements (PPA and NPA) are summarized in the table below.

Table 6: Clinical Performance of the ZEUS ELISA Parvovirus B19 IgG Test System

		FDA Approved IgG ELISA Test System			
		Positive	Negative	Equivocal	Total
ZEUS IgG ELISA Test System	Positive	111	3	0	114
	Negative	1	95	0	96
	Equivocal	0	0	0	0
	Total	112	98	0	210
Positive Agreement = 99.1% (111/112) (95% CI = 97.4% to 100%) Negative Agreement = 96.9% (95/98) (95% CI = 93.5% to 100%)					

Parvovirus B19 IgG immunoblotting was performed with a line blot immunoassay with specimens that were not in agreement between the ZEUS ELISA Parvovirus B19 IgG Test System and the FDA-approved assay. Of the four discrepant specimens above, all four were negative on the parvovirus B19 IgG line blot agreeing with the FDA-approved assay for 3 of the specimens.

b. Prospective Study

Three-thousand (3000) specimens were collected from three different geographical locations (1000 per location) within the US. They were specimens routinely submitted for parvovirus B19 serology. The samples were tested with an FDA-approved parvovirus B19 IgG to determine their clinical status. Testing with the ZEUS ELISA Parvovirus B19 IgG Test System was carried out with all specimens to determine PPA and NPA. Parvovirus B19 IgG line blots were performed on the 48 specimens that were not in agreement between ZEUS ELISA Parvovirus B19 IgG Test System and the FDA-approved assay. Those results are shown as footnotes.

Table 7: Clinical Performance of the ZEUS ELISA Parvovirus B19 IgG Test System

		FDA Approved IgG ELISA Test System			
		Positive	Negative	Equivocal	Total
ZEUS IgG ELISA Test System	Positive	1989	22[2]	3[3]	2014
	Negative	10[1]	963	2[4]	975
	Equivocal	2[5]	9[6]	0	11
	Total	2001	994	5	3000

Positive Agreement = 99.3% (1989/2003) (95% CI = 98.9% to 99.7%)
Negative Agreement = 96.6% (963/997) (95% CI = 95.5% to 97.7%).

[1]One specimen was immunoblot positive and nine were immunoblot negative.
[2]Four specimens were immunoblot positive and eighteen were immunoblot negative.
[3]All three specimens were immunoblot negative.
[4]One specimen was immunoblot positive and one was immunoblot negative.
[5]One specimen was immunoblot positive and one was immunoblot negative.
[6]One specimen was immunoblot positive and eight were immunoblot negative

c. Testing of a Parvovirus B19 Performance Panel

A commercially available 21 member parvovirus B19 serology panel was tested to determine accuracy. The ZEUS ELISA Parvovirus B19 IgG Test System performance was compared relative to an FDA-approved parvovirus B19 IgG enzyme immunoassay. Seventeen of the twenty-one specimens were in agreement (81%). Three of the four specimens that were not in agreement were equivocal on either the ZEUS ELISA Parvovirus B19 IgG Test System or the FDA-approved assay. The one true discrepant specimen (ZEUS assay positive) was also positive by a validated parvovirus B19 IgG line blot.

3. Subgroup Analyses

No additional subgroup analyses were performed.

d. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 3 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

- The ZEUS ELISA Parvovirus B19 IgG Test System is specifically measuring IgG antibody.
- The LOD for this assay is determined to be 5-7 IU/mL.
- The ZEUS ELISA Parvovirus B19 IgG Test System does not present a significant risk for positive or negative influence from the interferents tested.
- The ZEUS ELISA Parvovirus B19 IgG Test System is not susceptible to cross reactivity from IgG antibodies specific to other infectious agents tested, as well as from samples that are positive for autoimmune antibodies.
- The results of both Precison and Reproducibility studies indicate that the ZEUS ELISA Parvovirus B19 IgG Test System demonstrates good correlation from well to well, from run to run, from day to day, from site to site, from technician to technician and from lot to lot.
- The kits are stable for a minimum of 18 months when stored at 2-8^{0}C.

- The parvovirus B19 IgG antibody is stable in samples and not subject to significant change after at least five freeze/thaw cycles, and up to 14 days of storage at either room temperature or 2-8°C when tested with the ZEUS ELISA Parvovirus B19 IgG Test System.
- The ZEUS ELISA Parvovirus B19 IgG Test System is not subject to altered performance due to various factors associated with shipping conditions.
- The ZEUS ELISA Parvovirus B19 IgG Test System demonstrated significant agreement with the expected outcome of a commercial parvovirus B19 performance panel.
- In the clinical study, the positive and negative values obtained for the ZEUS ELISA Parvovirus B19 IgG Test System relative to an FDA-approved device assay are comparable.

B. Safety Conclusions

The risks of the device are based on nonclinical laboratory as well as data collected in clinical studies conducted to support PMA approval as described above. False positive and false negative results are discussed in Section VIII. Based on the results of the analytical and clinical studies, the ZEUS ELISA Parvovirus B19 IgG Test System, when used according to the directions should be safe and pose minimal risk to the patient due to false test results.

C. Risk/Benefit Analysis

The probable benefits of the device are based on data collected in the analytical and clinical studies conducted to support PMA approval as described above.

Benefits

- The ZEUS ELISA Parvovirus B19 IgG Test System serves as an aid to the diagnosis of parvovirus infections and may assist clinicians in determining the etiology of febrile illnesses, arthritis and transient aplastic crises.
- Some pregnant women are at risk for severe fetal outcomes, and the ZEUS ELISA Parvovirus B19 IgG Test System may be used to determine the need for more frequent monitoring during pregnancy.

Additional factors to be considered in determining probable risks and benefits for the ZEUS ELISA Parvovirus B19 IgM Test System device included:

Risks

- False positive results, false negative results, failure to correctly interpret test results, or failure to correctly operate the instrument are the primary risks associated with use of the ZEUS ELISA Parvovirus B19 IgG Test System.
- False negative IgG results in the setting of positive IgM results may result in patient anxiety and unnecessary monitoring. However, treatment is largely supportive and only provided to patients with other clinical signs and symptoms of severe disease.

- False positive results may result in less frequent monitoring of pregnant woman, however, most pregnant woman undergo routine ultrasound monitoring during their pregnancy. The ZEUS ELISA Parvovirus B19 IgG Test System should be interpreted with the results of a parvovirus B19 IgM assay, which further mitigates the risks of false positive results.

Do the probable benefits outweigh the probable risks?
The probable benefits of the ZEUS ELISA Parvovirus B19 IgG Test System outweigh the potential risks. The clinical and analytical testing that has been completed is sufficient to mitigate risks associated with the use of the assay. At present time, decisions regarding supportive treatments are made after assessing the severity of the patient's clinical manifestation. The ZEUS ELISA Parvovirus B19 IgG Test System will not be the sole determination to provide or withhold treatment, but it may provide insight into disease etiology and indicate which patients require more careful follow-up and monitoring. Therefore, the proposed mitigations, including the package insert, are sufficient to support safe and efficacious use of the ZEUS ELISA Parvovirus B19 IgG Test System in clinical practice.

1. Patient Perspectives: This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that, for detection of parvovirus B19 IgG antibodies in a patient, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the instructions for use. The submitted clinical studies have shown that the ZEUS ELISA Parvovirus B19 IgG Test System, when compared to reference clinical laboratory procedures, has a similar ability to detect the presence of IgG antibodies against parvovirus B19 in specimens from individuals. The rate of false positivity and false negativity are within acceptable limits. It has been shown that the device has no demonstrable cross reactivity with the many of the infectious agents or disease conditions tested. Therefore, this device should benefit the physician in the diagnosis and management of parvovirus B19 infected patients.

XIII. CDRH DECISION

CDRH issued an approval order on Septemeber 19, 2017.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for Use: See device labeling.

Hazards to Health from Use of the Device: See Warnings, Precautions, and Limitations in the device labeling.

Post-approval Requirements and Restrictions: See approval order.