SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

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

Premarket Approval Application (PMA) Number: P150042

Date of FDA Notice of Approval: September 19, 2017

II. INDICATIONS FOR 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 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 IgM Test System.

V. DEVICE DESCRIPTION

The ZEUS ELISA Parvovirus B19 IgM Test System is designed to detect IgM class antibodies to parvovirus B19 in human sera. The test was developed following a typical indirect, enzyme-linked immunosorbent assay (ELISA) format and includes the following key components; ELISA microwell plate, Sample Diluent, Conjugate, Substrate Solution, and Control/Calibrator. The 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 containing anti-human IgG that precipitates and removes IgG and Rheumatoid Factor from the sample leaving IgM free to react with the immobilized antigen. The diluted test sera are added to individual wells of the ELISA microwell plate. During sample incubation in the ELISA microwell plate, antigen specific IgM antibody in the diluted sample binds to the immobilized antigen. The plate is then washed to remove unbound antibody and other serum components.
2. Peroxidase conjugated goat anti-human IgM (Conjugate) is added to the wells and the plate is incubated. The Conjugate binds with IgM antibody immobilized on the solid phase in step 1. The wells are then washed to remove unreacted Conjugate.
3. The microwells containing immobilized Conjugate are incubated with the peroxidase Substrate Solution which contains 3, 3', 5, 5'- tetramethylbenzidine (TMB). Peroxidase catalyzes the oxidation of TMB producing 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

The ZEUS ELISA Parvovirus B19 IgM Test System has not been marketed in the United States or any foreign country.

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. IgM Class Specificity of the Assay
   In order to test the IgM specificity of the device, five samples that had significant amounts of both anti-parvovirus B19 IgG and anti-parvovirus B19 IgM antibody were tested with Sample Diluent containing 1% beta-mercaptoethanol. The treatment was shown to be effective at reducing the IgM signal from the five samples. The results demonstrated that the ELISA ZEUS Parvovirus B19 IgM Test System specifically measures IgM antibody.

2. Functional Removal of IgG
   In indirect immunoassays designed to measure IgM antibody, antigen-specific IgG may pose multiple problems. Antigen-specific IgG may out-compete with antigen-specific IgM resulting in false negative results. Additionally, antigen-specific IgG in the presence of Rheumatoid Factor can result in an IgM-like antibody binding to the antigen thereby generating a false positive result. Both of these potential scenarios can be eliminated by functionally removing IgG from the human sample. In the ZEUS ELISA Parvovirus B19 IgM Test System, the Sample Diluent contains goat anti-human IgG (Fc) that was experimentally shown to remove all human IgG from the test serum.

3. Potential Interfering Substances
   A study was performed to assess the potential impact of commonly encountered interfering substances on the ZEUS ELISA Parvovirus B19 IgM Test System. Three serum samples, negative, low positive, and high positive for parvovirus B19 IgM 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

   The samples were tested on the ZEUS Parvovirus B19 IgM ELISA Test System and percent recovery was determined relative to a control. The percent recovery ranged from 83% to 120%. The results of the testing showed that the qualitative results remain unchanged regardless of the interferent at the concentrations tested. This study indicates that the ZEUS ELISA Parvovirus B19 IgM 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 IgM antibody to other infectious agents or associated with other disease states might cross react with the ZEUS ELISA Parvovirus B19 IgM Test System. Of the 107 specimens tested, none were positive on the ZEUS ELISA Parvovirus B19 IgM Test System.

Table 2: Cross Reactivity

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

5. Precison Study
   The study was conducted with six specimens: negative, high negative, borderline, low positive, moderate positive and high positive for parvovirus B19 IgM antibodies. 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

			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

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 for parvovirus B19 IgM antibodies. 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 IgM Test System.

Table 4: Results of the Reproducibility Study

			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

7. Transportation Study

Two ZEUS ELISA Parvovirus B19 IgM 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 did not leave the facility in Branchburg, 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 IgM 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 the ZEUS ELISA Parvovirus B19 IgM Test System was performed at t = 0, 4 months, 6 months, 9 months and 15.5 months. The data from all time points met the acceptance criteria of +/- 20% recovery from t = 0 and supports a shelf life claim of 15 months at 2 - 8^0C for the ZEUS ELISA Parvovirus B19 IgM Test System.

9. Specimen Stability

   a. Specimen Stability Study A

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

   b. Specimen Stability Study B

      A three member sample panel was selected for testing, and consisted of a high positive, low positive, and 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 IgM Test System at multiple time points over a 14 day storage period. The positive samples remained positive and the negative samples remained negative. Index values did not deviate beyond 5% from their expected outcome for the positives. The results showed that the parvovirus B19 IgM antibody is stable when tested by ZEUS ELISA Parvovirus B19 IgM 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 IgM 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 IgM 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 3210 patients. There were three collection sites and two testing sites. The two studies described below evaluated performance of the ZEUS ELISA Parvovirus B19 IgM 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. The specimens were tested with the ZEUS ELISA Parvovirus B19 IgM Test System and 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 using the following criteria:
   - Composite reference method positive = Comparator ELISA positive/immunoblot positive
   - Composite reference method negative = Comparator ELISA negative/immunoblot N/A
   - Composite reference method negative = Comparator ELISA positive/immunoblot negative
   - Composite reference method equivocal = Comparator ELISA positive/immunoblot uncertain

   Key effectiveness outcomes are presented in Tables 5 and 6.

   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 positive and negative percent agreements (PPA and NPA) are summarized in the table below.

Table 5: Comparative Performance of the ZEUS ELISA Parvovirus B19 IgM Test System with Specimens from Pregnant Women

		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%)

b. Prospective Study

Three-thousand (3,000) specimens were collected in an all-comer prospective study from three different geographical locations (1,000 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 samples were tested to determine the correlation between the ZEUS ELISA Parvovirus B19 IgM Test System and the composite reference method described above.

Table 6: Correlation between the ZEUS ELISA and a Composite Reference Method

		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**:** Specimens that were equivocal by either ZEUS ELISA or the composite reference method were counted against the ZEUS ELISA.

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 IgM Test System performance was compared relative to an FDA-approved 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 a parvovirus B19 IgM immunoblot, agreed with the ZEUS ELISA Parvovirus B19 IgM Test System.

3. Subgroup Analyses

No additional subgroup analyses were performed.

4. 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 two 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 IgM Test System specifically measures IgM antibody.
- The Parvovirus B19 IgM ELISA Test System does not present a significant risk for positive or negative influence from the interferents tested.
- The ZEUS ELISA Parvovirus B19 IgM Test System is not susceptible to cross reactivity from IgM antibodies specific to other infectious agents that were tested, as well as from samples that are positive for autoimmune antibodies.
- The results of both the Precision and Reproducibility studies indicate that the ZEUS ELISA Parvovirus B19 IgM 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 15 months when stored at 2-8°C.
- The parvovirus B19 IgM 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 IgM Test System.
- The ZEUS ELISA Parvovirus B19 IgM Test System is not subject to altered performance due to the various factors associated with shipping conditions.
- The ZEUS ELISA Parvovirus B19 IgM Test System demonstrated good 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 IgM Test System relative to a composite reference method 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 IgM Test System, when used according to the directions should be safe and pose minimal risk to the patient due to false test results.

C. Benefit-Risk Determinations

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 IgM Test System serves as an aid to the diagnosis of parvovirus B19 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 IgM 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, or failure to correctly interpret test results, are the primary risks associated with use of the ZEUS ELISA Parvovirus B19 IgM Test System.

- False positive results may result in patient anxiety and unnecessary monitoring. However, treatment is largely supportive and only provided to patients with clinical signs and symptoms of severe disease.
- False negative results may result in less frequent monitoring of pregnant women, however, most pregnant women undergo routine ultrasound monitoring during their pregnancy.

Analysis: Do the probable benefits outweigh the probable risks?
The probable benefits of the ZEUS ELISA Parvovirus B19 IgM 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 IgM 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 IgM 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 IgM 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 IgM Test System, when compared to reference clinical laboratory procedures, has a similar ability to detect the presence of IgM 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 [September 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.