510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K072732

B. Purpose for Submission:

To obtain device clearance

C. Measurand:

Trypanosoma cruzi (T. cruzi)

D. Type of Test:

ELISA

E. Applicant:

Ortho Clinical Diagnostics

F. Proprietary and Established Names:

ORTHO® *T. Cruzi* ELISA Test System

G. Regulatory Information:

1. Regulation section:

   866.3870

2. Classification:

   1

3. Product code:

   MIU

4. Panel:

   Microbiology

H. Intended Use:

1. Intended use(s):

   ORTHO *T. cruzi* ELISA Test System is an enzyme-linked immunosorbent assay for the *in vitro* qualitative detection of antibodies (Immunoglobulin G) to *Trypanosoma cruzi (T. cruzi)* in human adult serum (glass, plastic, or serum separator tubes) and plasma (EDTA, lithium heparin or citrate) using whole-cell lysate antigens. Reactive assay results are presumptive evidence of past infection, and in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with Chagas' disease.

   Definitive diagnosis of an acute phase of infection (including acute congenital infection) must be made by alternate methods, e.g., hemoculture, blood smear.

This test is not intended for use on samples of cord blood or screening blood or plasma donors.

2. Indication(s) for use:

ORTHO *T. cruzi* ELISA Test System is an enzyme-linked immunosorbent assay for the *in vitro* qualitative detection of antibodies (Immunoglobulin G) to *Trypanosoma cruzi (T. cruzi)* in human adult serum (glass, plastic, or serum separator tubes) and plasma (EDTA, lithium heparin or citrate) using whole-cell lysate antigens. Reactive assay results are presumptive evidence of past infection, and in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with Chagas' disease.

Definitive diagnosis of an acute phase of infection (including acute congenital infection) must be made by alternate methods, e.g., hemoculture, blood smear.

This test is not intended for use on samples of cord blood or screening blood or plasma donors.

3. Special conditions for use statement(s):

NA

4. Special instrument requirements:

NA

I. Device Description:

The assay procedure is a three-stage test carried out in a microwell coated with lysate (antigens) prepared from *T. cruzi*. In the first stage, test specimen, Negative Control, and Positive Calibrator are diluted directly in the test well containing Specimen Diluent, and incubated for a specified length of time. If antibodies to *T. cruz*i are present, antigen-antibody complexes will form on the microwell surface. If antibodies to *T. cruzi* are absent, complexes will not form. Unbound antibodies in the sample will be removed during the subsequent wash step.

In the second stage, murine monoclonal antibody conjugated with Horseradish Peroxidase (Conjugate) is added to the test well. The Conjugate binds specifically to the antibody portion of the antigen-antibody complex. If complexes are not present, the unbound Conjugate is removed by the subsequent wash step.

In the third stage, an enzyme detection system composed of *o*-phenylenediamine (OPD) and hydrogen peroxide is added to the test well. If bound Conjugate is present, the OPD will be oxidized, resulting in a colored end product. Sulfuric acid is then added to stop the reaction. The color intensity depends on the amount of bound Conjugate and, therefore, is a function of the concentration of antibodies to *T. cruzi* present in the specimen. The intensity of color in the substrate solution is then determined with a microwell reader (spectrophotometer) designed to measure light absorbance in a microwell.

J. Substantial Equivalence Information:

1. Predicate device names:

   Hemagen Chagas Kit (EIA Method)

   Wiener Laboratories *T. cruzi* – Enzyme Linked Immunosorbent Assay,

2. Predicate 510(k) numbers:

   K930272
   K023889

3. Comparison with predicate:

	New Device	*Predicate Device*	*Predicate Device*
Device Characteristic	ORTHO T. cruzi ELISA Test System	K930272 Hemagen Chagas' Kit (EIA Method) – Hemagen Diagnostics, Inc.	K023889 Enzyme Linked Immunosorbent Assay, *T. cruzi* – Wiener Laboratories
Intended Use	… for the *in vitro* qualitative detection of antibodies to *Trypanosoma cruzi (T. cruzi)*	… for the detection of circulating antibodies to *Trypanosoma cruzi*, the causative agent of Chagas' disease	Qualitative detection of antibody to *Trypanosoma cruzi*, the causative agent for Chagas' disease in human serum or plasma.
Indications for Use	Assay results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with Chagas' disease.	When used according to instructions, the kit is useful in exhibiting prior exposure to T. cruzi and as an aid in the diagnosis of Chagas' disease.	When using according to instructions, the kit is useful in establishing prior exposure to *T. cruzi* and as an aid in the diagnosis of Chagas' disease.
Basic Principle	Enzyme-linked immunosorbent assay, ELISA	Enzyme-linked immunosorbent assay, ELISA	Enzyme-linked immunosorbent assay, ELISA
Where used	CLIA Certified Clinical Laboratory	CLIA Certified Clinical Laboratory	CLIA Certified Clinical Laboratory
Sample Type	Serum or Plasma (EDTA, lithium heparin or citrate)	Serum	Serum or Plasma (heparin, EDTA, and citrate based anticoagulants)
Antigen	Trypanosoma spp. (*T. cruzi* Tulahuen)	Trypanosoma spp.	Recombinant *T. cruzi* antigens from the trypomastigote parasite stage: #1, #2, #13, #30, and #36)
Antigen Prep	Whole cell lysate coated onto plastic microwells	Purified antigens from cultured *T. cruzi* organisms	Recombinant technology
Sample Volume	20 µL	10 µL	10 µL
Procedure	Diluted sample is incubated with the antigen prep. After an appropriate time the serum dilution in removed, and the antigen prep is washed. The antigen prep is overlaid with antibody labeled with an chromogenic substrate	Diluted sample is incubated with the antigen prep. After an appropriate time the serum dilution in removed, and the antigen prep is washed. The antigen prep is overlaid with antibody labeled with an chromogenic substrate	Diluted sample is incubated with the antigen prep. After an appropriate time the serum dilution in removed, and the antigen prep is washed. The antigen prep is overlaid with antibody labeled with an chromogenic substrate
Conjugate Antibody	Anti-human IgG	Anti-human IgG	Anti-human IgG
Tracer	Horseradish peroxidase with a Substrate Solution made from Substrate Buffer and OPD Tablets	Horseradish peroxidase with substrate 3, 3', 5, 5' – tetramethylbenzidine (TMB)	Horseradish peroxidase with substrate 3, 3', 5, 5' – tetramethylbenzidine (TMB)
Antibodies Detection	The antibody-HRP bound to the whole cell lysate-antibody complex reacts with the OPD producing a colored end product. The OD is read spectrophotometrically	The antibody-HRP bound to the whole cell lysate-antibody complex reacts with the TMB producing a colored end product. The OD is read spectrophotometrically	The antibody-HRP bound to the recombinant antigens-antibody complex reacts with the TMB producing a colored end product. The OD is read spectrophotometrically

N.B.: Shaded areas show differences between the device and the predicates

K. Standard/Guidance Document Referenced (if applicable):

NA

L. Test Principle:

See device description

M. Performance Characteristics (if/when applicable):

1. Analytical performance: NA

a. Precision/Reproducibility:

The intra-assay (within plate) and inter-assay (between plates) precision of the ORTHO *T. cruzi* ELISA Test System was evaluated using an eight-member precision panel. The precision panel consisted of three moderate to strongly reactive samples, three reactive samples near the assay cutoff (approximately 1.5 – 2.0 S/C), and two nonreactive samples. The panel was tested at three external sites using three different kit lots by the semi-automated processing method. Ten replicates each of the eight-member panel were assayed on a single occasion per day on nine different days by two technologists for a total of 4319 observations (one observation for R7 was a statistical outlier). Mean signal to cutoff (S/C), standard deviation (SD), and coefficient of variation (CV %) results are presented in the table below.

Panel Member	Number Tested	Mean ORTHO *T. cruzi* ELISA S/C	Inter-assay[1]		Intra-assay[2]		Total[3]	
			SD	CV(%)	SD	CV(%)	SD	CV(%)
R1	540	5.954	0.258	4.3	0.324	5.4	0.492	8.3
R2	540	6.424	0.306	4.8	0.324	5.0	0.501	7.8
R3	540	6.647	0.338	5.1	0.345	5.2	0.554	8.3
R4	540	1.946	0.089	4.6	0.143	7.3	0.189	9.7
R5	540	1.909	0.097	5.1	0.128	6.7	0.180	9.4
R6	540	2.173	0.113	5.2	0.134	6.2	0.207	9.5
R7	539	0.084	0.011	N/A[4]	0.025	N/A[4]	0.031	N/A[4]
R8	540	0.101	0.013	N/A[4]	0.029	N/A[4]	0.035	N/A[4]

[1] Between Plate (Between Run (Lot x Site x Technologist)): Variability of the assay performance from plate to plate
[2] Within Plate (Between Replicate): Variability of the assay performance from replicate to replicate
[3] Total: Inter-assay and Intra-assay variability
[4] % CVs are not meaningful when S/C approaches zero

b. Linearity/assay reportable range:

NA

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

NA

d. Detection limit:

NA

e. Analytical specificity:

The specificity of the ORTHO *T. cruzi* ELISA Test System was evaluated using 616 samples from individuals with infections or clinical conditions that might potentially exhibit cross reactivity when tested with the assay. The table below shows the numbers and types of samples tested.

Potentially Cross Reacting Condition or Disease State	Number of Specimens	Nonreactive (%)	Repeatedly Reactive (%)
Leishmania[1]	100	21 (21.0)	79 (79.0)
Malaria	96	94 (97.9)	2 (2.1)
Schistosomiasis	30	30 (100.0)	0 (0)
Syphilis	30	29 (96.7)	1 (3.3)
Pre-Vaccination with Influenza Vaccine	35	35 (100.0)	0 (0)
Post-Vaccination with Influenza Vaccine	35	35 (100.0)	0 (0)
Lupus Erythematosus (ANA titer > 1:640)	30	30 (100.0)	0 (0)
Rheumatoid Arthritis (RF > 30 IU or titer > 1:320)	30	30 (100.0)	0 (0)
Polyclonal Gammopathies	15	15 (100.0)	0 (0)
Monoclonal Gammopathies	15	15 (100.0)	0 (0)
Multiple Leukocyte Alloantibodies	15	15 (100.0)	0 (0)
Multiple Red Cell Alloantibodies	15	15 (100.0)	0 (0)
Cytomegalovirus	20	20 (100.0)	0 (0)
Epstein-Barr Virus	20	20 (100.0)	0 (0)
Herpes Simplex Virus Type 1	20	20 (100.0)	0 (0)
Rubella	20	20 (100.0)	0 (0)
Hepatitis B	20	20 (100.0)	0 (0)
Hepatitis C	20	20 (100.0)	0 (0)
Human Immunodeficiency Virus	20	20 (100.0)	0 (0)
Human T-Cell Lymphotropic Virus	20	20 (100.0)	0 (0)
Toxoplasmosis gondii	5	5 (100.0)	0 (0)
Paracoccidioides brasiliensis	5	3 (60.0)	2 (40.0)[2]
Total	**616**	**532 (86.4)**	**84 (13.6)**

[1] *Leishmania* specimens were collected in India where *T. cruzi* is not endemic and these specimens are presumed to be *T. cruzi* antibody negative

[2] These two specimens were obtained from Argentina, where *T. cruzi* infection is endemic. Both specimens were RIPA positive.

Among the 100 subjects with *Leishmania* infection, 21 (21.0%) were nonreactive and 79 (79.0%) were repeatedly reactive. The specimens were obtained in India where *T. cruzi* is not endemic and, therefore, the most probable *T. cruzi* antibody status of the 100 *Leishmania* specimens is negative. The ORTHO *T. cruzi* ELISA Test System may yield falsely reactive results among test subjects with no *Leishmania* infection.

Of the 516 non-*Leishmania* specimens, 511 (99.0%) were nonreactive and five (1.0%) were repeatedly reactive. Three of the five repeatedly reactive specimens (1 syphilis, and 2 malaria, *P. falciparum*) were RIPA negative. Two of the five repeatedly reactive specimens were obtained

from among the five test subjects with *Paracoccidioides brasiliensis* infection. These two specimens were RIPA positive and were obtained from a *T. cruzi* endemic area. Whether these represent false positive for *T. cruzi* infection due to cross reactivity in both ELISA and RIPA or co-infection with *P. brasiliensis* and *T. cruzi* is not known.

f. Assay cut-off:

NA

2. Comparison studies:

a. Method comparison with predicate device:

See 3c.

b. Matrix comparison:

NA

3. Clinical studies:

a. Clinical Sensitivity:

See 3. c.

b. Clinical specificity:

See 3. c.

c. Other clinical supportive data (when a. and b. are not applicable):

Clinical Performance

A multi-center study was conducted to establish the clinical performance of the ORTHO *T. cruzi* ELISA Test System among individuals at high or low risk for *T. cruzi* infection selected under well-defined inclusion and exclusion criteria but without regard to a known or previously determined *T. cruzi* antibody assay result. Statistical testing was performed to ensure that the distribution of ORTHO *T. cruzi* ELISA S/C values was homogeneous across the two testing sites participating in the study (Camp Hill, PA and Newark, NJ), and that the test results could be combined for analysis. Individuals presumed to be *T. cruzi* antibody positive by parasite detection methods or by serological methods were evaluated separately.

Specimens from subjects at high risk for *T. cruzi* infection (N=574) were collected in Bolivia (28.9%), Colombia (13.1%), Guatemala (23.3%), Mexico (8.7%) and Nicaragua (26.0%). The population was 44.9% female and 55.1% male, and ranged in age from 18 to 88 years.

Specimens from subjects at low risk for *T. cruzi* infection but with signs or symptoms similar to Chagas' disease (N=300) were collected in the U.S. from Black (7.7%) and Caucasian (92.3%) subjects. The population was 43.0% female and 57.0% male, and ranged in age from 21 to 93 years. Specimens from low risk pregnant women (N=200) were obtained in the U.S. from subjects in their first (21.5%), second (38.5%) or third (40.0%) trimester.

Comparator testing was performed with a validated *T. cruzi* IFA. Additional, more specific supplemental testing was performed with a validated *T. cruzi* radioimmunoprecipitation assay (RIPA).

ORTHO *T. cruzi* ELISA and *T. cruzi* IFA Results among High Risk and Low Risk Subjects

Specimens from 1074 subjects at high or low risk for *T. cruzi* infection were tested with a comparator *T. cruzi* IFA and with the ORTHO *T. cruzi* ELISA Test System. The results are presented in the following table.

ORTHO *T. cruzi* ELISA vs. *T. cruzi* IFA Results (N=1074)			
ORTHO *T. cruzi* ELISA Result	***T. cruzi* IFA Result**		**Total**
	Positive	**Negative**	
Repeatedly Reactive	82	16 [2]	98
Nonreactive	3 [1]	973	976
Total	85	989	1074

[1] These three specimens were also negative with the *T. cruzi* RIPA.
[2] Ten of these 16 specimens were also positive with the *T. cruzi* RIPA.

Percent Agreement

The table below summarizes the percent agreement between the ORTHO *T. cruzi* ELISA and the *T. cruzi* IFA. Data are listed by population and overall, with positive and negative percent agreement and 95% exact confidence intervals (CI).

Positive and Negative Percent Agreement of the ORTHO *T. cruzi* ELISA with the *T. cruzi* IFA by Study Population (N=1074)				
Population	**Positive Percent Agreement**	**95% Exact Confidence Interval**	**Negative Percent Agreement**	**95% Exact Confidence Interval**
High Risk	96.47% (82/85)	90.03% - 99.27%	96.93% (474/489)	94.99% - 98.27%
Low Risk			100% (300/300)	98.78% - 100%
Pregnancy Low Risk			99.50% (199/200)	97.25% - 99.99%
Total	96.47% (82/85)	90.03% - 99.27%	98.38% (973/989)	97.39% - 99.07%

ORTHO *T. cruzi* ELISA Results and Most Probable *T. cruzi* Antibody Status among High Risk and Low Risk Subjects

Because the *T. cruzi* IFA is a non-reference standard for detection of antibodies to *T. cruzi*, the most probable *T. cruzi* antibody status of the high and low risk study subjects was determined by ORTHO *T. cruzi* ELISA Test System, comparator *T. cruzi* IFA and supplemental *T. cruzi* RIPA testing according to a pre-specified testing algorithm. Specimens not tested with RIPA that were negative with both the ORTHO *T. cruzi* ELISA and the *T. cruzi* IFA were assigned a most probable *T. cruzi* antibody status of negative. Specimens tested with the RIPA were assigned a most probable *T. cruzi* antibody status of positive, negative or indeterminate based on the RIPA results.

A comparison of the ORTHO *T. cruzi* ELISA results to most probable *T. cruzi* antibody status is presented in the following table.

ORTHO *T. cruzi* ELISA Results and Most Probable *T. cruzi* Antibody Status in the High Risk and Low Risk Populations (N=1074)

ORTHO *T. cruzi* ELISA Results	**Most Probable *T. cruzi* Antibody Status**			
	Positive	**Negative**	**Indeterminate[1]**	**TOTAL**
Repeatedly Reactive	92	6	0	98
Nonreactive	1	975	0	976
TOTAL	93	981	0	1074

[1] There were no *T. cruzi* RIPA indeterminate results and therefore no specimens with a most probable *T. cruzi* antibody status of indeterminate among the high and low risk specimens.

Percent Agreement

The table below summarizes the percent agreement between the ORTHO *T. cruzi* ELISA and most probable *T. cruzi* antibody status. Data are listed by population and overall, with positive and negative percent agreement and 95% exact confidence intervals.

Positive and Negative Percent Agreement of the ORTHO *T. cruzi* ELISA with Most Probable *T. cruzi* Antibody Status by High Risk and Low Risk Study Population (N=1074)

Population	**Positive Percent Agreement**	**95% Exact Confidence Interval**	**Negative Percent Agreement**	**95% Exact Confidence Interval**
High Risk	98.92% (92/93)	94.15% - 99.97%	98.96% (476/481)	97.59% - 99.66%
Low Risk			100% (300/300)	98.78% - 100%
Pregnancy Low Risk			99.50% (199/200)	97.25% - 99.99%
Total	98.92% (92/93)	94.15% - 99.97%	99.39% (975/981)	98.67% - 99.78%

Performance with Presumed *T. cruzi* Antibody Positive Populations

Specimens Positive for *T. cruzi* by Parasite Detection Methods

The sensitivity of the ORTHO *T. cruzi* ELISA Test System was evaluated among subjects classified as parasite positive by historical identification of *T. cruzi* parasites (N=106). The samples were obtained from the endemic countries of Bolivia (27.3%), Chile (40.6%), Colombia (28.3%), and Nicaragua (3.8%), and were tested with the ORTHO *T. cruzi* ELISA at one testing site in St. Paul, MN. Specimens in this group were considered to have a most probable *T. cruzi* antibody status of positive. Assay sensitivity and 95% exact confidence interval are shown in the following table.

Sensitivity and 95% Exact Confidence Interval for the ORTHO *T. cruzi* ELISA in Parasite Detection Positive Specimens (N=106)		
Population	**Sensitivity (%)**	**95% Exact Confidence Interval**
Parasite Detection Positive	100% (106 / 106)	96.58% – 100%

Specimens Presumed Positive for Antibodies to *T. cruzi* by Serological Methods

ORTHO *T. cruzi* ELISA versus *T. cruzi* IFA

A total of 810 specimens were included in the *T. cruzi* serological presumed positive population based upon two positive serological tests for *T. cruzi* antibodies in use in the countries of origin (i.e., ELISA, IFA, hemagglutination, or complement fixation). The comparator *T. cruzi* IFA was not used to admit specimens to the study. The specimens were obtained from the endemic countries of Bolivia (17.8%), Brazil (24.7%), Chile (10.6%), Guatemala (2.2%), Mexico (32.5%) and Nicaragua (12.2%). ORTHO *T. cruzi* ELISA testing was performed at two testing sites in Camp Hill, PA and Newark, NJ. Direct comparison of the ORTHO *T. cruzi* ELISA with the *T. cruzi* IFA is presented in the following table.

ORTHO *T. cruzi* ELISA vs. *T. cruzi* IFA Results in Specimens Presumed Positive by Serologic Methods (N=810)			
ORTHO *T. cruzi* ELISA Result	***T. cruzi* IFA Result**		**Total**
	Positive	**Negative**	
Repeatedly Reactive	565	99 [2]	664
Nonreactive	5 [1]	141 [3]	146
Total	570	240	810

[1] These five specimens were also negative with the *T. cruzi* RIPA.
[2] Ninety-seven of these 99 specimens were also positive with the *T. cruzi* RIPA.
[3] All 141 specimens were negative with the *T. cruzi* RIPA.

Percent Agreement

Positive, negative and overall percent agreement of the ORTHO *T. cruzi* ELISA with the *T. cruzi* IFA and 95% exact confidence intervals are shown in the following table.

Positive, Negative and Overall Percent Agreement of the ORTHO *T. cruzi* ELISA with the *T. cruzi* IFA in the Serological Presumed Positive Population (N=810)

Population	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval	Overall Percent Agreement	95% Exact Confidence Interval
Serological Presumed Positive	99.13% (565/570)	97.96% - 99.71%	58.75% (141/240)	52.24% - 65.04%	87.16% (706/810)	84.66% - 89.39%

ORTHO *T. cruzi* ELISA versus Most Probable *T. cruzi* Antibody Status

Because the *T. cruzi* IFA is a non-reference standard for detection of antibodies to *T. cruzi*, the most probable *T. cruzi* antibody status of the study subjects presumed positive by serologic methods was determined by ORTHO *T. cruzi* ELISA Test System, comparator *T. cruzi* IFA and supplemental *T. cruzi* RIPA testing according to a pre-specified testing and interpretation algorithm. Specimens that were ORTHO *T. cruzi* ELISA repeatedly reactive and positive with the *T. cruzi* IFA were assigned a most probable *T. cruzi* antibody status of positive and were not tested with the *T. cruzi* RIPA. All specimens negative with both assays or with discordant results between the two assays were tested with the *T. cruzi* RIPA and assigned a most probable *T. cruzi* antibody status based upon the RIPA results. A comparison of ORTHO *T. cruzi* ELISA results and most probable *T. cruzi* antibody status is presented in the following table

ORTHO *T. cruzi* ELISA Results and Most Probable *T. cruzi* Antibody Status in the Serological Presumed Positive Population (N=810)

ORTHO *T. cruzi* ELISA Results	Most Probable *T. cruzi* Antibody Status			TOTAL
	Positive	Negative	Indeterminate[1]	
Repeatedly Reactive	662	2	0	**664**
Nonreactive	0	146	0	**146**
TOTAL	**662**	**148**	**0**	**810**

[1] There were no *T. cruzi* RIPA indeterminate results and therefore no specimens with a most probable *T. cruzi* antibody status of indeterminate among the serological presumed positive specimens tested with RIPA.

Percent Agreement

Positive, negative and overall percent agreement of the ORTHO *T. cruzi* ELISA with most probable *T. cruzi* antibody status and 95% exact confidence intervals are shown in the following table.

Positive, Negative and Overall Percent Agreement of the ORTHO *T. cruzi* ELISA with Most Probable *T. cruzi* Antibody Status for the Serological Presumed Positive Population (N=810)

Population	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval	Overall Percent Agreement	95% Exact Confidence Interval
Serological Presumed Positive	100% (662/662)	99.44% - 100%	98.65% (146/148)	95.20% - 99.84%	99.75% (808/810)	99.11% - 99.97%

4. Clinical cut-off**:**

   NA

5. Expected values/Reference range:

   NA

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalent decision.