ORTHO® T. cruzi ELISA Test System
Summary of Basis for Approval

Product Trade Name: ORTHO *T. cruzi* ELISA Test System
Proper Name: *Trypanosoma cruzi (T. cruzi)* Whole Cell Lysate Antigen
Applicant: Ortho-Clinical Diagnostics, Inc., 1001 US Highway 202, Raritan, NJ 08860-0606
Submission Tracking Number: BL 125161/0

Date Approved: December 13,2006

I. Intended Use

ORTHO *T. cruzi* ELISA Test System is an enzyme-linked immunosorbent assay for the qualitative detection of antibodies to Trypanosoma cruzi (*T. cruzi*) in human serum and plasma specimens.

This product is intended for use as a donor screening test to detect antibodies to *T. cruzi* in plasma and serum samples from individual human donors, including donors of whole blood, blood components or source plasma, and other living donors. It is also intended for use to screen organ and tissue donors when specimens are obtained while the donor's heart is still beating. This test is not intended for use ori specimens from cadaveric (non-heart-beating) donors. This test is not intended for use on samples of cord blood.

The ORTHO *T. cruzi* ELISA Test System is intended for use in a fully manual mode, in semi-automated mode using the Ortho Summit™ Sample Handling System (Summit) or in automated mode with the Ortho Summit™ System (OSS).

This assay is not intended for use as an aid in diagnosis.

II. Brief Description of Device and Principles

A. Summary and Explanation of the Test

Trypanosoma cruzi is a flagellated, protozoan parasite, which is endemic to regions of Latin America. It is the causative agent of Chagas' Disease. Infection is chronic, usually asymptomatic, treatment options are limited, and later complications of the disease are potentially fatal. Methods of transmission are vectorial (Reduviid bug),congenital, organ transplant, and blood transfusion. Organ transplant and blood transfusion cases in the USA have been demonstrated.

The ORTHO *T. cruzi* ELISA Test System is an enzyme-linked immunosorbent assay (ELISA). ELISA technology utilizes the principle that antigens or antibodies bound to the solid phase can be detected by complementary antibodies or antigens labeled with an enzyme capable of acting on. a

chromogenic substrate. When substrate is applied, the presence of antigens or antibodies can be detected by development of a colored end product.

This screening assay was developed to detect human antibodies to *T. cruzi* for blood screening. The assay utilizes microwells coated with a whole-cell lysate containing *T. cruzi* antigens as the solid phase. Any specimen that reacts in an initial test (is initially reactive) with the ORTHO *T. cruzi* ELISA Test System must be retested in duplicate. The ORTHO *T. cruzi* ELISA Test System detects antibodies to *T. cruzi* in blood and thus is useful in screening blood and plasma donated for transfusion and further manufacture in establishing prior infection with *T. cruzi*. It is recommended that repeatedly reactive specimens be investigated by additional testing for antibodies to *T. cruzi* before a specimen is considered positive, indicating *T. cruzi* infection. Additional testing for Leishmania, Malaria, Syphilis, and Paracoccidioides brasiliensis (P. brasiliensis) should be considered. Follow appropriate FDA recommendations and regulations for specimens found repeatedly reactive.

B. Description of Kit and Components

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. cruzi* 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 a-phenylenediamine (OPO) and hydrogen peroxide is added to the test well. If bound Conjugate is present, the OPO 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 at the wavelength appropriate for the colored end product.

Operational Modes

Manual testing is performed with handheld pipette sample handling, AutoReader IV, AutoWash 96, Model 120 Incubator or equivalent microwell incubator capable of maintaining 37°C, and Ortho Assay Software (OAS).

Automated testing is performed with the Ortho Summit System (OSS), defined as the Ortho Summit Sample Handling System (Summit), Ortho Summit Processor (OSP), and Ortho Assay Software (OAS).

Semi-automated testing is performed with the Ortho Summit Sample Handling System (Summit), AutoReader IV, AutoWash 96, Model 120 Incubator or equivalent microwell incubator capable of maintaining 37°C, and Ortho Assay Software (OAS).

Under circumstances of limited sample volume or limited number of samples, handheld pipette sample handling, may be combined with the Ortho Summit Processor (OSP), and Ortho Assay Software (OAS).

An Ortho Assay Protocol Disk (OAPD) for ORTHO *T. cruzi* ELISA Test System is also used in the testing of the samples by all processing methods.

The protocol to run this test on the automated Ortho Summit System (OSS) is contained on the ORTHO *T. cruzi* ELISA Test System Ortho Assay Protocol Disk (OAPD) for the Ortho Assay Software (OAS) and the instructions in the OSS User's Guide.

Label Abbreviations	480 Test Kit Product Code 6901968	2400 Test Kit Product Code 6901969	Component Description
T. cruzi	5 plates	25 plates	*T. cruzi* Lysate-Coated Microwell Plates (96 wells each)
CON	1 bottle (125 mL)	5 bottles (125 mL each)	Conjugate Reagent: Antibody to Human IgG (Murine Monoclonal) – anti-human IgG heavy chain (murine monoclonal) conjugated to horseradish peroxidase with bovine protein stabilizers Preservative: 1% ProClin™ 300
SD	1 bottle (190 mL)	4 bottles (190 mL each)	Specimen Diluent – phosphate-buffered saline with bovine protein stabilizers Preservative: 1% ProClin™ 300
PCal	1 vial (3 mL)	5 vials (3 mL each)	Positive Calibrator (Human) Source: Human plasma containing antibodies to *T. cruzi* antigens and non-reactive for HBsAg and antibodies to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), and hepatitis C virus (HCV). Preservative: 1% ProClin™ 300
NC	1 vial (2 mL)	5 vials (2 mL each)	Negative Control (Human) Source: Human plasma nonreactive for HBsAg and antibodies to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), *T. cruzi*, and hepatitis C virus (HCV). Preservative: 1% ProClin™ 300

OPD	1 vial (30 tablets)	5 vials (30 tablets each)	OPD Tablets-contains *o*-phenylenediamine • 2HCl
SB	1 bottle (190 mL)	4 bottles (190 mL each)	Substrate Buffer-G – citrate-phosphate buffer with 0.02% hydrogen peroxide Preservative: 0.1% 2-chloroacetamide
	21	84	Plate Sealers, disposable

STORAGE REQUIREMENT
Store unopened and opened components at 2 to 8° C

ANCILLARY KIT COMPONENTS
In addition to the ELISA kit components, 20X Wash Buffer Concentrate and 4N Sulfuric Acid are necessary for performing an ELISA. These ancillary reagents are supplied under separate part numbers for all Ortho donor screening assays. They are the same reagents for all Ortho licensed donor screening assays.

III. Manufacturing and Controls

A. Description of Manufacturing Facilities

All manufacturing processes for ORTHO *T. cruzi* ELISA Test System are performed by:

Ortho-Clinical Diagnostics
1001 US HWY 202
Raritan, NJ 08869-0606

US License Number 1236
Establishment Registration Number 2250051

The Ortho-Clinical Diagnostics facility (OCD Raritan) located on U.S. Highway 202 in Raritan, New Jersey is a multi-use biologics manufacturing facility. ----------
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B. Stability Program

Components of the ORTHO *T. cruzi* ELISA Test System were entered into the stability program to define the recommended storage conditions and to establish the expiration period for each component. The three different kit lots of each component were manufactured, tested, assembled into kits, and evaluated during storage through ------ months. These kit lots were also used in the clinical studies for the assay and to validate the manufacturing processes for the assay kit components. The expiration date of the complete test kit lot is the same as that of the shortest dated kit component. The -------- kit components have ------ months expiration dating, however the ---------- -- only has 12 months expiration dating.

The results of the stability studies completed to date support the *T. cruzi* specific kit components expiration dates in the following table.

ORTHO To cruzi ELISA Test System
Final Product Stability
Kit Expiration Date:
The expiration date of the complete test kit lot is the same as that of the shortest dated kit component.

	Component Description	**Proposed Expiration Date (Months)**	**Storage Temperature**	**Stability Test**
Plate	*T. cruzi* Lysate-Coated Microwell Plates (96 wells each)	----- months from the date of manufacture	2-8°C	---------- ---------- ----
1 bottle (125 mL)	Conjugate Reagent: Antibody to Human IgG (Murine Monoclonal) -anti-human IgG heavy chain (murine monoclonal) conjugated to horseradish peroxidase with bovine protein stabilizers Preservative: 1% ProClin™ 300	------- months from the date of filling	2-8°C	------- ---------- ---------- ---------- ----------
I bottle (190 mL)	Specimen Diluent -phosphate-buffered saline with bovine protein stabilizers. Preservative: 1% ProClin™ 300	------- months from the date of filling	2-8°C	------- ---------- ---------- ----------
1 vial (2mL)	Negative Control (Human) Source: Human plasma nonreactive for HBsAg and antibodies to human immunodeficiency virus type 1 (HIY-l) and type 2 (HIY-2), *T. cruzi*, and hepatitis C virus (HCY). Preservatives: 1% ProClin™ 300	------- months from the date of filling	2-8°C	------- ---------- ---------- - ---------- ----------

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1 vial (30 tablets)	OPD Tablets -contains *o*-phenylenediamine' 2HCI	------- months from the date of manufacture	2-8°C	---------- ---------- ----
I bottle (190 mL)	Substrate Buffer-G -citrate-phosphate buffer with 0.02% hydrogen peroxide. Preservative: 0.1 % 2-chloroacetamide	------- months from the date of filling	2 -8°C	---------- ---------- ----

C. Methods of Validation

All test kit components are monitored by in-process testing. ----------. Product performance is assessed through quality release evaluations of the final test kit against in-house panel containing negative control specimens and specimens that are known to positive for antibodies to *T. cruzi*. The test kit lot must meet all OCD performance requirements prior to submission to FDA for evaluation, testing, and approval for kit lot release to distribution.

D. Labeling

The product labeling, including immediate container labels, carton labels and package insert have been reviewed for compliance with 21 CFR 610.60,610.61; 610.62, 801 and 809.10 and were found acceptable. The package insert clearly states the intended use of the ORTHO *T. cruzi* ELISA Test System as a qualitative in vitro test for the detection of antibodies to Trypanosoma cruzi (*T. cruzi*) in human serum and plasma specimens.

E. Establishment Inspection

A Pre License Inspection (PLI) of the manufacturing facilities where ORTHO *T. cruzi* ELISA Test System is manufactured, packaged, and tested was conducted from September 11 through September 15, 2006. Facilities and procedures for this product were found to be in substantial conformity with the Quality System Regulation and current good manufacturing procedures.

F. Environmental Impact Analysis, Claims for Categorical Exclusion

Ortho-Clinical Diagnostics claimed a Categorical Exclusion from the submission of an Environmental Impact Statement with the ORTHO *T. cruzi* ELISA Test System Biologic License Application. This claim for a Categorical Exclusion was made pursuant to 21 CFR 25.31(a). The manufacture of the ORTHO *T. cruzi* ELISA Test System is performed under controlled conditions and in compliance with the appropriate federal, state, and local environmental regulations. The disposal of waste from the use of this product is performed in compliance with appropriate federal, state and local environmental regulations. Based on the materials, concentrations, volumes used in this product, the method of product disposal, it is unlikely that the release of any of the substances of this product at the expected level of exposure will be handful to the environment or toxic to organisms in the environment.

IV. Performance Characteristics

A. Nonclinical Studies Summary

1. Potentially Interfering Serologies -Interference with Bilirubin, Hemoglobin, Triglycerides and Total Protein

Bilirubin, Hemoglobin, Triglycerides: The effect of bilirubin, hemoglobin and triglycerides on the performance of the assay were evaluated in 30 reactive and 30 nonreactive matched samples. Bilirubin was evaluated at concentrations between 5 and 30 mg/dL. Hemoglobin was evaluated at concentrations between 150 and 800 mg/dL. Triglycerides were evaluated at concentrations between 750 and 3000 mg/dL. No clinically significant differences were observed. Therefore, bilirubin, hemoglobin and triglycerides were found to have no effect on the performance of the assay.

Elevated Total Protein: The effect of elevated (>9.0 g/dL) total protein on the performance of the assay was evaluated in 41 reactive and nonreactive matched samples. No clinically significant differences were observed. Therefore, elevated total protein samples were found to have no effect on the performance of the assay.

2. Potentially Interfering Serologies -Human Anti-Mouse Antibody (HAMA) Samples and Heterophilic Antibody Sample

Human Anti-Mouse Antibody (HAMA) Samples: A total of 15 human antimouse antibody (HAMA) samples presumed negative for T cruzi antibody were tested and found to be nonreactive. Therefore, no interference with known human anti-mouse antibodies was observed.

Heterophilic Antibody Samples: A total of 15 heterophilic antibody samples presumed negative for T cruzi antibody were tested and found to be nonreactive. Therefore, no interference with known human anti-mouse antibodies was observed.

3. Sample Stability

The effect of storing samples collected in plain glass serum tubes, plastic serum· tubes, EDTA glass tubes, EDTA plastic tubes, lithium heparin plastic tubes, ACD (Solution B) glass tubes, and citrate glass tubes was evaluated in 30 reactive and 30 nonreactive matched samples at 24 hours at 25°C, up to 11 days at 2-8°C, up to 4 weeks at -20°C undergoing up to 5 freeze/thaw cycles, and up to 6 months at -20°C undergoing 1 freeze/thaw cycle. No clinically significant differences were observed for any test condition as compared to baseline. Therefore, samples may be stored for up to 24 hours at 25°C, up to 10 days at 2-8°C, up to 4 weeks at -20°C undergoing up to 5 freeze/thaw cycles, or tip to 6 months at -20°C undergoing 1 freeze/thaw cycle.

4. Sample Handling:

Sample Storage on the Clot/Cells: The effect of storing matched serum and EDTA plasma samples on the clot/cells after centrifugation was evaluated in 30 reactive and 30 nonreactive samples at Day 0 (baseline), 24 hours at 25°C, 10 and 11 days at 2-8°C. No clinically significant differences were observed for any test condition as compared to baseline. Therefore, serum and EDTA plasma samples may be stored after centrifugation on the clot/cells up to 24 hours at 25°C or up to 10 days at 2-8°C.

Delayed Centrifugation Study: The effect of storing matched serum and EDTA plasma samples prior to centrifugation was evaluated in 30 reactive and 30 nonreactive samples at Day 0 (baseline), 24 hours at 25°C, and up to 11 days at 2-8°C. No clinically significant differences were

observed for any test condition as compared to baseline. Therefore, serum and EDTA plasma samples may be stored prior to centrifugation up to 24 hours at 25°C or up to 10 days at 2-8°C.

Anticoagulant/Matched Serum Study: The effect of using CPD, CPDA-I, and CP2D anticoagulant types versus serum was evaluated in 50 reactive and nonreactive matched samples. No clinically significant differences were observed for any anticoagulant type as compared to serum. Therefore, CPD, CPDA-I and CP2D anticoagulant types are acceptable for blood collection.

Specimen Collection Device Study: Whole blood from each of 50 volunteer study participants was collected into plain glass serum with no additives, serum plastic, EDTA plastic, EDTA glass, Lithium heparin plastic, ACD glass and citrate glass. The samples were split and half was spiked with anti-*T. cruzi* antibodies and the other half tested unspiked. No clinically significant differences were observed for any anticoagulant type as compared to serum. Therefore, EDTA plastic, EDTA glass, Lithium heparin plastic, ACD glass and citrate glass anticoagulant types and container types are acceptable for blood collection.

SST Collection Device Comparison and Sample Stability: The effect of using SST blood collection devices was evaluated in 10 reactive and 10 nonreactive unmatched samples. A plain glass serum tube was used as the control for the collection device comparison. Additionally, SST serum sample stability was evaluated in the same 10 reactive and 10 nonreactive samples. The SST samples were tested at the following conditions/time points:

- Day 0 (Centrifuged and processed following collection)
- Centrifuged and stored at 25°C for 24-30 hours with serum in contact with gel
- Centrifuged and stored at 25°C for 24-30 hours with the tube on its side so that the serum was in contact with both the gel and stopper
- Stored at 25°C for 24-30 hours prior to centrifugation and processing
- Stored at 2-8°C for up to 12 days with serum on gel
- Stored for 4 weeks at -20°C undergoing 5 freeze/thaw cycles
- Stored for 4 weeks at -20°C undergoing 1 freeze/thaw cycle. These samples were refrozen after testing and at 4 weeks and up to 6 months at -20°C with 4 freeze/thaw cycles.

No clinically significant differences were observed for any test condition as compared to serum/baseline. Therefore, SST samples stored at any of the conditions listed above are acceptable for use.

C. Clinical Trials Summary

1. Assay Cutoff Constant Verification:
For the Clinical Study, the cutoff values in the range of ----- times cutoff to ------ times cutoff (cutoff = mean Positive Calibrator times cutoff constant of were evaluated in order to establish the optimal cutoff. At the completion of the study, the cutoff constant was decreased by ------ to -------- to optimize sensitivity without affecting specificity.

2. Clinical Specificity:
In addition to the following studies, data from analytical testing and clinical trials demonstrated equivalent results for all modes of operation of the ORTHO *T. cruzi* ELISA Test System.

The specificity of the ORTHO *T. cruzi* ELISA Test System is based on a population of presumably healthy volunteer blood donors from four geographically distinct sites in the United States.

A total of 40,665 human serum and plasma samples were tested by the automated processing method. Among the 40,665 volunteer blood donor samples tested, 40,663 (99.995%) were nonreactive, 2 (0.005%) Were initially reactive, and 1 (0.002%) was repeatedly reactive. The only repeatedly reactive sample was negative by *T. cruzi* Radioimmune Precipitation Assay (RIPA), which was used as a confirmatory test. Rates of reactivity for the four sites are shown in Tables 1 and 2. The observed specificity of the ORTHO *T. cruzi* ELISA Test System in the volunteer blood donor population in this study was 99.998% (40,664/40,665) with a 95% exact confidence interval of 99.986% to 100.000%.

Table 1. Frequency of the ORTHO *T. cruzi* ELISA Test System Reactivity in Volunteer Blood Donors: Ortho Summit System [Ortho Summit Sample Handling System (Summit), Ortho Summit Processor (OSP) and Ortho Assay Software (OAS)]

Test Site	Number of Samples	Sample Matrix	Nonreactive(%)	Repeatedly Reactive (%)	Confirmed Positive with RIPA
1	4523	Serum	4523 (100.000)	0(0.000)	NA
2	9219	Serum	9219 (100.000)	0(0.000)	NA
3	12118	Plasma	12117.(99.992)	1 (0.008)	0
4	14805	Plasma	14805 (100.000)	0(0.000)	NA
Total N = 40665			40664 (99.998)	1(0.002)	0

The ORTHO *T. cruzi* ELISA Test System was used to test 2,121 additional donor samples by both automated and semi-automated processing methods at three sites. Semi-automated processing consists of the Ortho Summit Sample Handling System (Summit) with the AutoWash 96, Model 120 Incubator, AutoReader IV, and Ortho Assay Software (OAS). Automated processing consists of the Ortho Summit System (OSS) defined as the Summit, Ortho Summit Processor (OSP), and OAS. There was 100% agreement between the T cruzi ELISA results of automated and semi-automated processing methods.

Table 2. Frequency of the ORTHO T. cruzi ELISA Test System Reactivity in Volunteer Blood Donors by Processing Method.

Test Site	Number of Samples	Sample Matrix	Ortho Summit System	Semi-Automated

			[Summit, OSP and OAS] Nonreactive (%)	Processing (Summit, AutoWash 96, AutoReader IV, and OAS) Nonreactive (%)
1	713	Serum	713 (100.00)	713 (100.00)
2	738	Serum	738 (100.00)	738 (100.00)
3	670	Plasma	670 (100.00)	670 (100.00)
Total N = 2121			2121 (100.00)	2121 (100.00)

An additional study was conducted using volunteer blood donor samples from three geographic locations in the United States, including one site where previous cases of *T. cruzi* have been reported.[20] A total of 30,095 human serum and plasma samples were tested by the automated processing method. Among the 30,095 volunteer blood donor samples tested, 30,084 (99.963%) were nonreactive, 11 (0.037%) were initially reactive, and 10 (0.033% were repeatedly reactive, nine of which were confirmed positive and one negative by the *T. cruzi* Radioimmune Precipation Assay (RIPA) used as a confirmatory test. Ten samples in total were confirmed positive by the RIPA, including one ELISA nonreactive samples [initial S/C 0.964, repeat S/C 1.204 1.084], which was repeat tested according to the study protocol, representing a false negative in this study. Rates of reactivity for the three sites are shown in Table 3. The observed specificity of the ORTHO ELISA Test System in random, presumably health, linked, volunteer blood donors in these specific geographic locations was 99.997% (30,084/30,085) with a 95% exact confidence interval of 99.982% to 100.00%.

Table 3. Frequency of the ORTHO T. cruzi ELISA Test System Reactivity in Volunteer Blood Donors from a High Prevalence Area[a]

Site	Number of Samples	Nonreactive (%)	Repeatedly Reactive (%)	Confirmed Positive with RIPA
A	19381	19372 (99.954)	9 (0.046)	8
B	6228	6228 (100.000)	0(0.000)	0
C	4486	4485 (99.978)	1 (0.022)	1
Total N=30095		30085 (99.967)	10 (0.033)	9

[a]Testing was performed With the Ortho Summit System

3. Clinical Sensitivity:

The sensitivity of the ORTHO T. cruzi ELISA Test System in a positive population was evaluated by testing a total of 106 samples from subjects included as parasite positive by historial identification of T. cruzi parasites by one of the following methods: blood smear (i.e., Giemsa), hemoculture, or xenodiagnosis. The samples were obtained from the endemic countries of Bolivia, Chile, Colombia, and Nicaragua. Testing was performed at one site by the automated and semi-automated processing methods. All specimens initially reactive with the ORTHO T. cruzi ELISA Test System were retested in duplicate. Table 4 shows the overall results of the testing of the 106 positive samples by the automated processing method. Equivalent results were obtained with the semi-automated processing methods.

Table 4. Frequency of ORTHO T.cruzi ELISA Test System Reactivity in Positive Samples*

Number of Samples	Repeatedly Reactive (%)	Nonreactive (%)
106	106 (100.0)	0 (0.0)

*Testing was performed on the automated and semi-automated systems with the same outcomes

The overall sensitivity of the ORTHO *T. cruzi* ELISA Test System in this study was observed to be 100.0% (106/106) for parasite positive samples with a 95% exact confidence interval of 96.6% to 100.0%.

Sensitivity and Specificity in a High Risk Population

A total of 574 samples from study subjects from countries endemic for *T. cruzi* infection were tested with the ORTHO *T. cruzi* ELISA Test System and IFA to determine sensitivity and specificity in a population at risk. The samples were obtained from the endemic countries of Bolivia, Colombia, Guatemala, Mexico, and Nicaragua. Testing was performed at two sites by the semi-automated processing method. Table 5 compares the ORTHO *T. cruzi* ELISA Test System results with the most probable *T. cruzi* antibody status for the High Risk population.

Table 5. ORTHO *T. cruzi* ELISA Test System Results and Most Probable *T. cruzi* Antibody Status for High Risk Samples

Observed Results[a]	Most Probable *T. cruzi* Antibody Status -- Positive	Most Probable *T. cruzi* Antibody Status -- Negative	Most Probable *T. cruzi* Antibody Status -- Indeterminate	TOTAL
Repeatedly Reactive	92[b]	3[b]	0	95
Nonreactive	1[b]	478[c]	0	479
TOTAL	**93**	**481**	**0**	**574**

[a]Testing was performed by the semi-automated processing method
[b]Based on RIPA results
[c]Based on negative *T. cruzi* IFA

The observed sensitivity of the ORTHO *T. cruzi* ELISA Test System in the High Risk population in this study was 98.9% (92/93) with a 95% exact confidence interval of 94.2% to 100.0%.

The observed specificity of the ORTHO *T. cruzi* ELISA Test System in the High Risk population in this study was 99.4% (478/481 with a 95% exact confidence interval of 98.2% to 99.9%.

Additional Positive Performance Data

In additional to the samples from parasite positive individuals, another group of samples that were serological presumed positive were tested. A total of 810 samples were included in this *T. cruzi* serological positive population. The samples were obtained from the endemic countries of Bolicia, Brazil, Chile, Guatemala, Mexico, and Nicaragua. Serological presumed positive samples were included based upon two positive serological test for *T. cruzi* antibodies (i.e., ELISA, IFA, RIPA, hemagglutination, or complement fixation). Testing was performed at two sites by the semi-automated processing method. All specimens initially reactive with the ORTHO *T. cruzi* ELISA Test System were retested in duplicate. Six hundred sixty-four (664) samples gave repeatedly reactive results with the ORTHO *T. cruzi* ELISA Test System. Two of the 664 repeatedly reactive samples had S/C results <1.500 and both were tested with RIPA. Both samples were RIPA negative. The agreement between the ORTHO *T. cruzi* ELISA Test System and most probably *T. cruzi* antibody status was 100% (662/662) for samples with a *T. cruzi* antibody status of positive. All 146 samples that were ORTHO *T. cruzi* ELISA nonreactive were negative by RIPA.

Table 6 shows the ORTHO *T. cruzi* ELISA Test System results for the serological presumed positive population compared to the most probably *T. cruzi* antibody status.

Table 6. ORTHO *T. cruzi* ELISA Test System Results and Most Probable *T. cruzi* Antibody Status for Serological Presumed Positive Samples

Observed Results[a]	**Most Probable *T. cruzi* Antibody Status -- Positive**	**Most Probable *T. cruzi* Antibody Status -- Negative**	**Most Probable *T. cruzi* Antibody Status -- Indeterminate**	**TOTAL**
Repeatedly Reactive	662[b]	2[b]	0	664
Nonreactive	0	146[b]	0	146
TOTAL	**662**	**148**	**0**	**810**

[a] Testing was performed by the semi-automated processing method, except for 20 samples with limited volume that were pipetted manually

[b] Most probable *T. cruzi* antibody status was determined by RIPA for samples that were nonreactive or had S/C results <1.500 in the *T. cruzi* ELISA

Analytical Sensitivity (Dilutional Panel Precision Study)

Analytical sensitivity was determined by testing a 20-member dilutional panel and comparing results across multiple sites and multiple kit lots. Three replicates of each panel member were tested on a single occasion per day on three different days by one technologist at three sites, for a total of 540 observations. The dilutional panel was prepared from five unique *T. cruzi* antibody positive plasmas/serums, each diluted to provide 4 samples (dilutionallevels) with signal to cutoff (S/C) values targeted in descending order around the cutoff of 1.000. Analytical sensitivity testing was performed by the automated processing method. The reactive panel members were reactive across all sites with all kit lots and the

nonreactive panel members were nonreactive across all sites with all kit lots. The mean S/C, standard deviation (SD) and coefficient of variation (CV%) results are shown in Table 7 for each dilutional level.

Table 7. Dilutional Panel Member Precision by Dilutional Level[a]

Dilutional Level	Mean ORTHO *T cruzi* ELISA S/C	Between Site SD	Between Site CV(%)	Between Lot SD[†]	Between Lot CV(%)	Total SD[‡]	Total CV(%)[‡]	Number of Observations
DLl	4.992	0.000	0.0	0.134	2.7	0.486	9.7	135
DL2	2.417	0.000	0.0	0.000	0.0	0.222	9.2	135
DL3	1.787	0.059	3.3	0.000	0.0	0.253	14.2	135
DL4	0.271	0.027	N/A[+]	0.000	N/A[+]	0.100	N/A[+]	

[a]Testing was performed by the automated processing method
[*]Between Sites: Variability of the assay performance from site to site.
[†] Between Lot: Variability of the assay performance from lot to lot.
[‡]Total: Variability of the assay incorporating factors of site and lot
[+] % CVs are not meaningful when SIC is very small

Analytical Specificity -Potentially Cross Reacting Samples
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. This testing was performed by the semi-automated processing method. Samples from the following conditions or disease states were included in the testing: Leishmania; Malaria; Schistosomiasis; Syphilis; Influenza Vaccine; Paraproteins, Autoantibodies and Alloantibodies; Virally Infected and other Disease States. Table 8 shows the numbers and types of samples tested:

Table 8. Reactivity of the ORTHO T. cruzi ELISA Test System with Samples from Subjects With Potentially Cross Reacting Conditions or Disease States[a]

Potentially Cross Reacting Condition or Disease State	Number of Samples	Nonreactive (%)	Repeatedly Reactive (%)	Positive with RIPA (%)
Leishmania	100	26 (26.0)	74 (74.0)	21 (21.0)[*]
Malaria	96	95 (99.0)	1 (1.0)	0(0)
Schistosomiasis	30	30 (100.0)	0(0)	0(0)
Syphilis	30	29 (96.7)	1 (3.3)	0(0)

Influenza Vaccine [A]	70	70 (100.0)	0(0)	0(0)
Paraproteins, Autoantibodies, and Alloantibodies [B]	120	120 (100.0)	0(0)	0(0)
Virally Infected and Other Disease States [C]	170	168 (98.8)	2 (1.2)	2 (1.2)[**]
Total	**616**	**538 (87.3)**	**78 (12.7)**	**23 (3.7)[*]**

[a]Testing was performed by the semi-automated processing method
[*]Leishmania specimens cannot reliably be confirmed as *T. cruzi* antibody positive by RIPA. Leishmania samples were collected in India where *T. cruzi* is not .endemic and these samples are presumed to be *T. cruzi* antibody negative.
[**]These two RIPA positive samples were *P. brasiliensis* specimens that were obtained from Argentina, where *T. cruzi* infection is endemic.
[A.] Unlinked Paired Pre-and Post-Vaccination Samples from 35 Persons Receiving the Influenza Vaccine
[B.] Unlinked Samples from Individuals with Paraproteins, Autoantibodies, and Alloantibodies: Lupus Erythematosus (N=30, ANA titer> 1:640), Rheumatoid Arthritis (N= 30, RF > 30 IU or titer > 1:320), Polyclonal Gammopathies (N=15), Monoclonal Gammopathies (N=15), Multiple Leukocyte Alloantibodies (N=15), Multiple Red Cell Alloantibodies (N=15). .
[C.] Unlinked Samples from Individuals with Antibodies: Cytomegalovirus (N=20), Epstein - Barr virus (N=20), Herpes Simplex Virus Type 1 (N=20), Rubella (N=20), Hepatitis C (N=20), Hepatitis B (N=20), Human Immunodeficiency Virus (N=20), Human T-Cell Lymphotropic Virus (N=20), *Toxoplasma gondii* (N=5), *Paracoccidioides brasiliensis* (N=5)

Among the 100 subjects with Leishmania infection, 24 (24.0%) were nonreactive, 76 (76.0%) were initially reactive, and 74 (74.0%) were repeatedly reactive. Although 21 (21.0%) of the samples were positive by RIPA, the samples were obtained in India where *T. cruzi* is not endemic and, therefore, the most probable *T. cruzi* antibody status of the 100 Leishmania samples is negative. The ORTHO *T. cruzi* ELISA Test System may yield falsely reactive results among test subjects with Leishmania infection.

Of the 516 non-Leishmania samples, 511 (99.0%) were nonreactive, five (1.0%) were initially reactive, and four (0.8%) were repeatedly reactive. Two of the four repeatedly reactive samples (one syphilis and one malaria, *P. falciparum*) were RIPA negative. Two of the four repeatedly reactive samples were obtained from among the five test subjects with *P. brasiliensis* infection. These two samples 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.

Reproducibility
The intra-assay (within plate) and inter-assay (between plate) reproducibility of the ORTHO *T. cruzi* ELISA Test System was evaluated using an eight-member reproducibility panel. The reproducibility 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 and both the automated and semi-automated processing methods. 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 on both processing methods) per processing method. Mean signal to cutoff (S/C), standard deviation (SD), and coefficient of variation (CV %) results are presented in Table 9 and Table 10 for the two processing methods.

Table 9. Reproducibility Panel Testing: Ortho Summit Sample Handling System Summit), AutoWash 96, AutoReader IV, and Ortho Assay Software (OAS)

Panel Member	Number Tested	Mean ORTHO *T. cruzi* ELISA S/C	Inter-assay SD*	Inter-assay CV(%)*	Intra-assay SD†	Intra-assay CV(%)†	Total SD‡	Total CV(%)‡
RI	540	5.501	0.239	4.3	0.299	5.4	0.455	8.3
R2	540	5.935	0.283	4.8	0.299	5.0	0.463	7.8
R3	540	6.141	0.3 12	5.1	0.3 19	5.2	0.512	8.3
R4	540	1.798	0.082	4.6	0.132	7.3	0.175	9.7
R5	540	1.763	0.090	5.1	0.118	6.7	0.166	9.4
R6	540	2.008	0.104	5.2	0.123	6.1	0.191	9.5
R7	539	0.077	0.010	N/A+	0.023	N/A+	0.028	N/A+
R8	540	0.093	0.012	N/A+	0.027	N/A+	0.032	N/A+

*Between Plate (Between Run (Lot x Site x Technologist)): Variability of the assay performance from plate to plate.
†Within Plate (Between Replicate): Variability of the assay performance from replicate to replicate.
‡Total: Inter-assay and Intra-assay variability
+ % CVs are not meaningful when S/C approaches zero

Table 10. Reproducibility Panel Testing: Ortho Summit System (OSS) [Summit, Ortho Summit Processor (OSP), and OA

Panel Member	Number Tested	Mean ORTHO *T. cruzi* ELISA S/C	Inter-assay SD*	Inter-assay CV(%)*	Intra-assay SD†	Intra-assay CV(%)†	Total SD‡	Total CV(%)‡
RI	540	5.501	0.239	4.3	0.299	5.4	0.455	8.3
R2	540	5.935	0.283	4.8	0.299	5.0	0.463	7.8
R3	540	6.141	0.3 12	5.1	0.3 19	5.2	0.512	8.3
R4	540	1.798	0.082	4.6	0.132	7.3	0.175	9.7
R5	540	1.763	0.090	5.1	0.118	6.7	0.166	9.4
R6	540	2.008	0.104	5.2	0.123	6.1	0.191	9.5
R7	539	0.077	0.010	N/A+	0.023	N/A+	0.028	N/A+

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