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

A. 510(k) Number:

K161947

B. Purpose for Submission:

To obtain a substantial equivalence determination for a new device

C. Measurand:

IgG antibodies from *Trypanosoma cruzi*

D. Type of Test:

Qualitative immunochromatographic assay

E. Applicant:

InBios International, Inc

F. Proprietary and Established Names:

Chagas Detect™ *Plus* Rapid Test

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3870 *Trypanosoma spp.* serological reagent

2. Classification:

   Class I

3. Product code:

   MIU

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   The Chagas Detect™ *Plus* (CDP) Rapid Test is a rapid immunochromatographic strip assay for the qualitative detection of human lgG antibodies to *Trypanosoma cruzi* (*T. cruzi*) in human serum and whole blood matrices (venous and capillary (finger prick) whole blood). CDP is a non-invasive diagnostic test for use in a primary care setting by personnel trained to obtain whole blood or serum samples. Reactive test results will be presumptive evidence of infection with *T. cruzi*. The CDP when used in conjunction with other serological and clinical information is useful for the diagnosis of individuals with Chagas disease. Definitive diagnosis of an acute phase infection (including acute congenital infection) must be made by alternative methods, e.g., hemoculture, blood smear. This test is not intended for use on cord blood or for screening blood or plasma donors.

2. Indication(s) for use:

   Same as Intended Use.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Not applicable

I. Device Description:

The CDP Rapid Test is a qualitative, membrane-based immunoassay for the detection of antibodies to *T. cruzi* in human serum. The rapid test membrane is pre-coated with a recombinant antigen on the test line region and utilizes a separate control to assure assay flow and performance. During testing, the test sample is added to the sample pad and a proprietary blend of a stable liquid conjugate labeled with protein A is added to the sample pad. The conjugate and serum mixture migrates upward on the membrane (via capillary action) to react with recombinant *T. cruzi* antigen on the membrane. If antibodies to the *T. cruzi* antigen are present, a red line will appear at the test line. The red line at the control region should always appear if the assay is performed correctly. The entire procedure takes approximately 20 minutes.

Kit Components

1. Fifty (50) rapid tests in plastic cassette housing, individually pouched. Store at room temperature.
2. One (1) vial of Gold Solution, 3ml. Store at room temperature.
3. One (1) vial of Chase Buffer Type A, 6ml. Store at room temperature.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ORTHO *T. cruzi* ELISA Test System

2. Predicate 510(k) number(s):

   K072732

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for use	The Chagas Detect *Plus* (CDP) Rapid Test is a rapid immunochromatographic strip assay for the qualitative detection of human lgG antibodies to *Trypanosoma cruzi* (*T. cruzi*) in human serum and whole blood matrices (venous and capillary (finger prick) whole blood). CDP is a non-invasive diagnostic test for use in a primary care setting by personnel trained to obtain whole blood or serum samples. Reactive test results will be presumptive evidence of infection with *T. cruzi*. The CDP when used in conjunction with other serological and clinical information is useful for the diagnosis of individuals with Chagas disease. Definitive diagnosis of an acute phase infection (including acute congenital infection) must be made by alternative methods, e.g., hemoculture, blood smear. This test is not intended for use on cord blood or for screening blood or plasma donors.	Enzyme-linked immunosorbent assay for the qualitative detection of antibodies to *Trypanosoma cruzi* (*T. cruzi*) in human serum, plasma, and cadaveric 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 volunteer donors of whole blood, blood components, source plasma, and other living donors. It is also intended for use to screen organ donors when specimens are obtained while the donor's heart is still beating and in testing blood specimens to screen cadaveric (non-heartbeating) 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 semiautomated 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
Analyte	IgG	same
Interpretation	Quantitative	same
Specimen type	Serum, whole blood	Serum, plasma, whole blood

Differences		
Item	Device	Predicate
Sample volume	5 µl	20 µl
Antigen	Recombinant antigen	*T. cruzi* (Tulahuen)
Reading method	Visual, manual	Spectrometer
Technology	Immunochromatographic assay	EIA

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

Not applicable

L. Test Principle:

Lateral flow immunochromatographic assay.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility study was conducted at three study sites. Two test operators from each site participated in this study, for a total of six test operators over the period of 5 days. All tests were performed in singlet according to the product insert. Each site tested two panels per day (not simultaneously). Panels consisted of 9 samples, a weak positive, near LOD, and a true negative samples. A total of 180 data points were collected, (90 per site) for each of the three panel members. The results were recorded as negative or positive. Reproducibility was 99.4% for the one negative panel samples and 93.3% and 99.4 % for the near LoD and Low Positive panel members respectively.

b. Linearity/assay reportable range:

Not Applicable

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

Controls:
Each test cassette contains a run control line. Positive control serum is not supplied with the kit. If no control line appears, regardless whether a test line is seen, the test result should be considered invalid. It is recommended to retest using a new CDP Rapid Test and fresh serum or whole blood sample.

Sample stability:

Finger prick whole blood and serum:

Testing has been validated for use with various samples and testing should be performed as soon as possible after sample collection. Samples should not be left at room temperature for prolonged periods. Capillary blood samples collected with a capillary tube should be tested immediately after collection. Capillary blood should be expelled from the tube. 5µl of finger prick blood is then pipetted onto the test cassette. Testing has not been validated for capillary blood samples that have been refrigerated or frozen for extended periods of time.

Blood obtained by venipuncture should be allowed to clot at room temperature (20-25°C) for 30 to 60 minutes and then centrifuged to obtain serum for testing with the CDP Rapid test cassette. If assays are not completed at time of serum collection, serum should be frozen at or below -60°C immediately. Testing has not been validated for serum that has been refrigerated for extended periods of time.

d. Detection limit:

Not applicable

e. Analytical specificity:

Cross reactivity:
Eighty-six disease-positive specimens were tested for cross-reactivity with the Chagas Detect™ *Plus* Rapid Test. Ten confirmed positive serum samples from patients infected with each of the following were tested: Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Herpes Simplex Virus 1 (HSV-1), and Systemic Lupus Erythematosus (SLE). Five confirmed positive serum samples from patients with each of the following conditions were tested: Malaria, Schistosomiasis, Toxoplasmosis, Syphilis, Cytomegalovirus (CMV), Epstein-Barr Virus Nuclear Antigen (EBV), Human Immunodeficiency Virus 1 and 2 (HIV 1/2), Rubella, and Rheumatoid Factor (RF). One Visceral Leishmaniasis (VL) sample was also tested. Information regarding sample matrix, confirmatory test used, and confirmatory test results of each disease specimen is shown below in Table 1.

Table 1. Results of testing disease-positive human samples with CDP Rapid

Disease	Total Specimens	Positive	Negative	Positive/Total Ratio	% Cross-Reactivity
HBV	10	0	10	0/10	0%
HCV	10	1	9	1/10	10%
HSV-1	10	0	10	0/10	0%
SLE	10	0	10	0/10	0%
Malaria	5	0	5	0/5	0%
Schistosomiasis	5	0	5	0/5	0%
Toxoplasmosis	5	1	4	1/5	20%
Syphilis	5	2	3	2/5	40%
CMV	5	0	5	0/5	0%
EBV	5	0	5	0/5	0%
HIV 1/2	5	0	5	0/5	0%
Rubella	5	0	5	0/5	0%
RF	5	0	5	0/5	0%
VL	1	0	1	0/1	0%
Total	**86**	**4**	**82**	**4/86**	**4.65%**

The following specimens were not available, and were not included in this study, Cutaneous Leishmaniasis, *Paracoccidioides brasiliensis*, Giardiasis, Polyclonal Gammopathies, pre- and post- influenza vaccine, *T. rangeli* or other species of trypanosome, and as a result cross reactivity was not assessed. The results of the cross reactivity testing were acceptable with the following limitations in the labeling:

- Chagas Detect™ *Plus* may give false positive results in patients infected with hepatitis C, toxoplasmosis, or syphilis.
- Cross-reactivity with antibodies against Cutaneous Leishmaniasis, *Paracoccidioides brasiliensis*, Giardiasis, Polyclonal Gammopathies, pre- and post-influenza vaccine, *T. rangeli* or other species of trypanosoma have not been assessed.

Interference:
Potentially interfering substances tested in this study are listed below in, Table 2. Normal concentrations found in human whole blood (and serum), along with the concentrations tested in this study are included in the table below. Each interfering substance and its solvent control were added to serum prior to running the rapid test. Testing was performed per product insert, except that each sample was tested in duplicate. If no interference was demonstrated at high concentration, then testing with low concentration was bypassed A panel of simulated clinical specimens was tested. Chagas-positive serum was diluted in normal human serum (NHS) to generate one

negative sample, and three positive samples (one medium positive and two borderline positives). The stock serum was confirmed Chagas-positive by immunofluorescence assay (IFA).

Table 2. Interference testing

Interfering Substance	Normal concentration	Concentrations tested	Result
Bilirubin	0.002 – 0.01 mg/mL >0.025 mg/mL jaundiced	0.2 mg/mL	No interference
Triglycerides	<1.30-2.00 mg/mL	15 mg/mL	No interference
Hemoglobin	<0.01-0.05 mg/mL for serum, 110-180 mg/mL for whole blood	160 mg/mL	No interference
Cholesterol	1.70-1.90 mg/mL normal, 2.80-3.20 mg/mL elevated	5 mg/mL	No interference
Protein	60-83 mg/mL	150 mg/mL (albumin)	No interference
HAMA	0-188 ng/mL	7-46 ng/mL	No interference
Sodium citrate	0.1 mg/mL or 11mM in blood collection tubes	1.0 mg/mL	No interference
Heparin	10-50 IU/mL or 0.1-0.2 mg/mL in blood collection tubes	2.0 mg/mL	No interference
EDTA	0.5-2 mg/mL	20 mg/mL	No interference

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

A matrix equivalency study was conducted comparing a matched set of serum and venous whole blood with various anticoagulants (citrate, EDTA, heparin) from a single donor. Chagas negative serum and venous blood were purchased from a commercial vendor. A pool of serum was obtained from ten Chagas positive donors. The positive serum pool was diluted down to and confirmed positive (at a 1:32 dilution) by immunofluorescence assay (IFA). At dilutions below 1:32 the serum was negative by IFA. The positive serum pool was then diluted into each of the Chagas

negative matrices for testing on CDP. The matrix equivalency study tested two titer dilutions that are positive (1:16 and 1:32) and two titer dilutions (1:64 and 1:128) that are considered negative by IFA, along with true negative (matrix only). For each matrix, a larger volume of each titer dilution and blank were prepared and coded for blinded testing by two operators. Each operator tested the five coded samples concentrations in 30 replicates, and recorded the number of replicates that tested positive vs. negative. LOD and β-value (false negative rate) was then estimated as the dilution at which ~95% rapid tests demonstrated reactivity, and only ~5% of low concentration samples will erroneously show negative reactivity are shown below, Table3. For serum, LOD was demonstrated at 1:16 dilution, while for venous blood with any of the anticoagulants, LOD was at 1:32 dilution.

Table 3. Matrix equivalency results

Matrix	Dilution of reference stock	Sample code #	%positive for operator #2	%positive for operator #3	%positive average of 2 operators	Beta value (% false negative)
Normal Human Serum	1:16	17	100	100	100	0
	1:32	20	23.3	96.7	60.0	40.0
	1:64	13	0	0	0	0
	1:128	2	0	0	0	0
	0	7	0	0	0	N/A
Venous blood with citrate	1:16	10	100	100	100	0
	1:32	6	96.7	96.7	96.7	3.3
	1:64	19	63.3	90.0	76.7	23.3
	1:128	14	13.3	3.3	8.3	91.7
	0	16	0	3.3	1.7	N/A
Venous blood with EDTA	1:16	12	100	100	100	0
	1:32	1	100	86.7	93.3	6.7
	1:64	18	80.0	70.0	75.0	25.0
	1:128	8	6.7	0	3.3	96.7
	0	4	0	0	0	N/A
Venous blood with heparin	1:16	3	100	100	100	0
	1:32	15	100	100	100	0
	1:64	5	76.7	0	38.3	61.7
	1:128	9	0	0	0	0
	0	11	0	0	0	N/A

The limit of detection varies for each matrix, with each of the venous blood matrices demonstrating similar or slightly better sensitivity than serum. All matrices demonstrated an acceptable range and are similar to the limit of detection established by IFA, which tested positive for the reference stock down to a dilution of 1:32.

3. Clinical studies:

a. Clinical Sensitivity:

Clinical performance - Non-endemic population:

Venous serum and capillary blood samples were prospectively (n=200) collected from an area not endemic for *T. cruzi*. Subjects were recruited at the Johns Hopkins School of Public Health and the Center for Immunization Research. Both are located in Baltimore, MD, USA. All study samples were de-identified. Subjects who already knew they had positive serology for Chagas disease were excluded. As Chagas infection can be asymptomatic, study inclusion criteria did not specify minimum symptoms. Prospective samples were collected from adults (age 18 and older) with informed consent, Table 4a and 4b below.

CDP was run immediately on finger prick capillary blood. Venous serum was isolated and stored frozen until tested in batches. Validated IFAs were performed in a laboratory at Universidad Peruana Cayetano Heredia in Lima, Peru. Because this study was performed in a non-endemic area, samples were expected to be negative and reference testing was based on IFA alone. The reference test method was considered negative when either IFA or Indirect hemagglutination assay (IHA) reference test provided a negative test result. The result, 100% specificity was demonstrated in both venous serum and whole capillary blood. 95% confidence intervals were calculated based on the Wilson score method. CDP shows very high specificity in this non-endemic area.

Table 4a. US, prospective, serum

Serum		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	0	0	0
	Negative	0	200	200
	Total	0	200	200
Specificity: 200/200 = 100.0% [95% CI: 98.1-100.0%]				

Table 4b. US, prospective, finger prick

Capillary Whole Blood		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	0	0	0
	Negative	0	200	200
	Total	0	200	200
Specificity: 200/200 = 100.0% [95% CI: 98.1-100.0%]				

Clinical performance

A. Low prevalence endemic population:

Evaluation of performance of the CDP was done by testing prospective and retrospective venous serum and capillary blood samples at a site in Chile, a low endemic area for Chagas disease. Serum CDP samples were collected from patients who presented at the Parasitology Health Clinic in the Gustavo Fricke Hospital in Santiago, Chile. Capillary blood samples were collected with EDTA anticoagulant. Fresh confirmed positive retrospective serum and capillary blood samples were from patients who had previously been diagnosed as positive for Chagas disease and who had reported to the clinic for annual serological and clinical monitoring. All the bioethical standards required for the participation and acceptance of the patients were followed according to the bioethical committee of the Faculty of Medicine at the University of Chile. The collected samples were refrigerated and sent overnight to be analyzed at the Campus Research Laboratory in Santiago, Chile (Sanalab SA) by IFA and IHA.

The prospective (n=542) and confirmed positive retrospective (n=473) sample subsets of the Chilean study group were analyzed separately. Tables 5a and 5b show descriptive statistics and specificity determinations for prospective serum and finger prick samples. The incidence of new cases of Chagas disease in recent years has been very low in Chile. Therefore, there are no positive cases to allow sensitivity calculations and only specificity was calculated. CDP showed high specificity in this low risk endemic area.

Table 5a. Chile, prospective, serum

Serum		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	0	0	0
	Negative	0	542	542
	Total	0	542	542
Specificity: 542/542 = 100% [95% CI: 99.3-100%]				

Table 5b. Chile, prospective, finger prick

Capillary Whole Blood		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	0	0	0
	Negative	0	542	542
	Total	0	542	542
Specificity: 542/542 = 100% [95% CI: 99.3-100%]				

Fresh serum and finger prick blood samples from patients previously diagnosed positive for *T. cruzi* were tested to evaluate sensitivity. Almost all the symptomatic

patients in the Chilean sample pool were from the confirmed positive retrospective samples, Table 6a and 6b shows the sensitivity of the CDP.

Table 6a. Chile, retrospective, serum

Serum		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	452	7	459
	Negative	14	0	14
	Total	466	7	473
Sensitivity: 450/466 = 96.6% [95% CI: 94.5-97.9%]				

Table 6b. Chile, retrospective finger prick

Capillary Whole Blood		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	450	7	457
	Negative	16	0	16
	Total	466	7	473
Sensitivity: 452/466 = 97.0% [95% CI: 95.0-98.2%]				

B. High prevalence endemic population:

Evaluation of performance of the CDP was done in Bolivia, a high endemic area for Chagas disease. Specimens tested by CDP were de-identified samples from two prospective studies and one set of archived serums samples. Specimens were prospectively collected from male and female adults (N=108) at San Juan de Dios Hospital in the city of Santa Cruz. Table 7a and 7b show the results for serum and capillary blood samples, respectively.

An additional prospective study was conducted from pregnant women (n=243) at Camiri Municipal Hospital in the Bolivian Chaco, an area in the extreme south of Bolivia. Table 8a and 8b show the results for serum and finger prick samples, respectively. In this same study, CDP was run immediately on finger prick capillary blood. Venous blood serum specimens were divided into several aliquots and transported to a laboratory at the Hospital Japones for CDP and IHA testing. IFAs were performed in a laboratory at Universidad Peruana Cayetano Heredia in Lima, Peru.

Table 7a Bolivia, prospective, serum

Serum		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	77	4	81
	Negative	0	27	27
	Total	77	31	108
Sensitivity: 77/77 = 100.0% [95% CI: 95.2-100.0%] Specificity: 27/31 = 87.1% [95% CI: 71.1-94.9%]				

Table 7b Bolivia prospective, finger prick

Capillary Whole Blood		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	76	1	77
	Negative	1	30	31
	Total	77	31	108
Sensitivity: 76/77 = 98.7% [95% CI: 93.0-99.8%] Specificity: 30/31 = 96.8% [95% CI: 83.8-99.4%]				

Table 8a. Bolivia pregnant prospective, serum

Serum		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	122	4	126
	Negative	1	116	117
	Total	123	120	243
Sensitivity: 122/123 = 99.2% [95% CI: 95.5-99.9%] Specificity: 116/120 = 96.7% [95% CI: 91.7-98.7%]				

Table 8b. Bolivia pregnant prospective, finger prick

Capillary Whole Blood		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	117	2	119
	Negative	6	118	124
	Total	123	120	243
Sensitivity: 117/123 = 95.1% [95% CI: 89.8-97.7%] Specificity: 118/120 = 98.3% [95% CI: 94.1-99.5%]				

Archived serum specimens from pediatric subjects (n=200) in Cordillera province, were tested by CDP, IFA, and IHA at the laboratory at Universidad Peruana Cayetano Heredia in Lima, Peru. Table 9 shows the results for serum samples from this population

Table 9. Bolivia pediatric retrospective, serum.

Serum		Reference testing		
		Positive	Negative	Total
Chagas Detect *Plus*	Positive	78	2	80
	Negative	0	120	120
	Total	78	122	200
Sensitivity: 78/78 = 100% [95% CI: 95.3-100%] Specificity: 120/122 = 98.4% [95% CI: 94.2-99.5%]				

b. Clinical specificity:

See section M3a

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

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

In a non-endemic population in the United States, CDP Rapid Test demonstrated positive results in 0% (0/200) of human whole blood samples and in 0% (0/200) of human serum samples. The nonendemic study population was 47% female and 53% male with an age range of 20 to 54 years old.

In a low risk endemic population in Chile, CDP Rapid Test demonstrated positive results in 0% (0/542) of human serum. The low endemic study population was 47% male and 53% female with an age range of 18 to 87 years old.

In a highly endemic population in Bolivia, CDP Rapid Test demonstrated positive results in 55.8% (196/351) of human whole blood samples and in 59.0% (207/351) of human serum samples. The highly endemic study population was 84% female and 16% male with an age range of 18 to 83 years old.

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 equivalence decision.