Chagas Detect™ *Plus* Rapid Test

for the Detection of Antibodies to *T. cruzi* in Human Serum or Whole Blood

1 Intended 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.

Caution: **U.S. Federal Law restricts this device to sale by or on the order of a physician.**

2 Summary and Explanation

Chagas' disease is caused by the flagellated protozoa *Trypanosoma cruzi* and is an endemic infection in Central and South America that affects 16 to 18 million individuals[1]. Over the last several years with intensive eradication campaigns directed against the triatomine, vector transmission of *T. cruzi* diminished drastically, particularly in rural areas, and does not exist today in many regions where the infection used to be endemic. However, the transfusion of parasite-containing blood continues to be an important mode of transmission[2,3,4]. Several strategies exist for the diagnosis of Chagas' disease. Direct detection of the parasite in the blood by microscopy, hemoculture, xenodiagnosis, or PCR is highly specific and confirms the existence of an infection[4,5]. However, these procedures are technically and operationally demanding. Other tests currently used include measurement of antibodies against crude lysate, complement fixation, indirect hemagglutination, and fluorescent antibody (IFA). All are lacking specificity and/or sensitivity[5,6,7,8]. Serologic tests that detect antibodies specific for antigens expressed by the different developmental stages of the parasite are well suited for a fast and easy diagnosis of the disease[9,10,11,12,13]. In an attempt to improve the serological diagnosis of Chagas' disease, we have identified and used a multi-epitope recombinant antigen derived from different *T. cruzi* antigens.

3 Principle

The Chagas Detect™ *Plus* Rapid Test is a qualitative, membrane-based immunoassay for the detection of antibodies to *T. cruzi* in human serum and whole blood matrices (venous and capillary (finger prick) whole blood). 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 presence of this red line verifies that proper flow has occurred and catastrophic failure of the conjugate has not occurred.

The entire procedure takes approximately 20 minutes.

4 Materials Provided

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

Materials Required but not Provided:

1. MICROSAFE® capillary tubes (Safe-Tec Clinical Products LLC, catalog number 1005-25) or pipettor and tips capable of precisely dispensing 5µl.
2. Timer
3. Lancets for finger prick blood collection or venous blood collection supplies for serum collection
4. Alcohol wipes and/or gauze

5 Warning and Precautions

- A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
- Do not use the test after expiration date shown on kit box label.
- Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for the proper disposal of sera and used kits.

- Wear protective clothing, eye protection, and disposable gloves while performing the assay. Wash hands thoroughly when finished.
- Avoid all contact between hands and eyes or mucous membranes during the testing.
- Do not eat, drink, or smoke in the area where the sera and kits are handled.
- All solutions contain a preservative. Avoid all possible contact with the skin and mucous membranes.

6 Storage

The entire kit is designed to be stored at room temperature (20-30°C) for the duration of its shelf life. Exposure to temperatures over 30°C can impact the performance of the test and should be minimized. The kit should not be frozen. The cassette device should be used immediately after removal from the pouch to minimize exposure to humidity.

7 Specimen Collection and Preparation

- The usual precautions for capillary puncture or venipuncture should be observed. (CLSI Approved Guidelines – Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens).
- Use of lotions and hand creams should be avoided before testing.
- Hands should be washed in warm water with antibacterial soap, rinsed and dried thoroughly.
- Disinfect the entry site, and be sure that the alcohol dries completely before pricking the finger or vein.
- For capillary blood draw, use a sterile, disposable lancet to puncture the side of the fingertip. Wipe away the first drop of blood with a clean piece of gauze, then gently, without force, apply pressure to the fingertip to accumulate a drop of blood. Excessive squeezing of the finger may alter test results.
- If using a MICROSAFE® capillary tube (not provided):
  - Hold the capillary tube horizontal to the drop of blood. Do NOT squeeze the bulb as the tube will fill automatically by capillary action to the proper fill line.
  - Squeeze the bulb to expel the sample immediately (< 30 seconds).
- If using another capillary tube transfer pipet, ensure that it precisely dispenses 5µl, and follow the manufacturer's instructions for use.
- See the "Test Procedure" section for information on how to apply the blood to the test cassette.
- Testing should be performed as soon as possible after collection. Capillary blood should be tested immediately after collection. 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 on Chagas Detect™ *Plus* Rapid Test.
- Discard used materials properly. Caution: Handle and dispose of all materials coming in contact with blood according to universal precautions and guidelines.
- If assays are not completed at time of serum collection, serum should be frozen at or below -60°C.
- Avoid repeated freezing and thawing of serum samples since this can cause analyte deterioration. Frost-free freezers are not suitable for sample storage. Samples should not be frozen and thawed more than 5 times.
- Frozen serum samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin (5-10 seconds, 500g) before use.
- Do not use sera if any indication of microbial growth is observed.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

8 Test Procedure

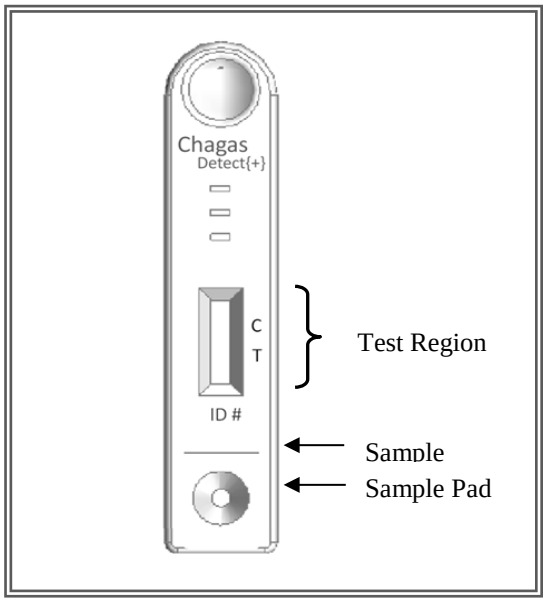
Before beginning, review all directions and limitations in this procedure.

Remove the Chagas Detect™ *Plus* Rapid Test cassette from the foil pouch and ensure that no physical damage (e.g., scratched membrane at the Test Region) is apparent on the cassette.

Bring all test samples to room temperature before beginning this procedure.

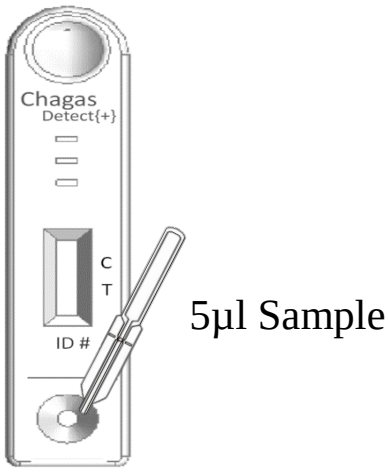
Observe the test format shown in Figure 1 below. Note the locations of the Test Region, Sample ID, and Sample Pad. The Test Region has two markings: (C) for the control line and (T) for the test line.

Figure 1 Test Format

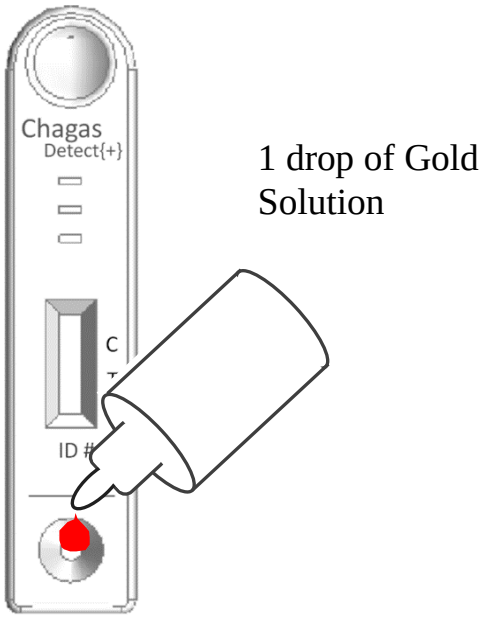


1 Lay the cassette onto a clean, flat surface. Write the Patient name or ID# using an indelible marker at the Sample ID line.

2 Transfer 5µl of serum sample or 5µl of whole blood directly to the Sample Pad. Use an appropriately sized pipetter and tip.

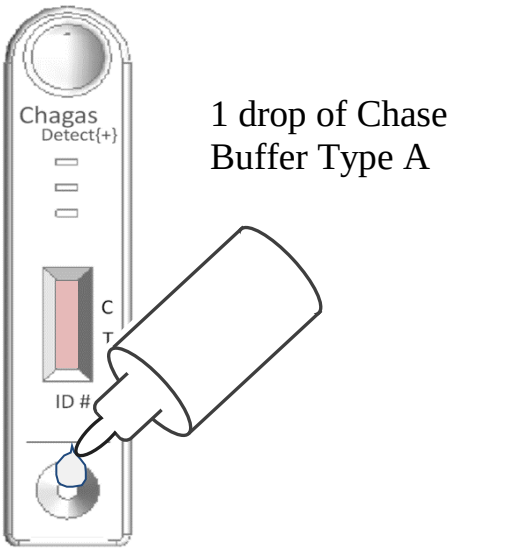


3 Slowly add one drop (approximately 40µl) of Gold Solution to the Sample Pad. It is important that only one full drop is added. Exceeding this amount could result in incomplete clearing of the test and interfere with result reading.

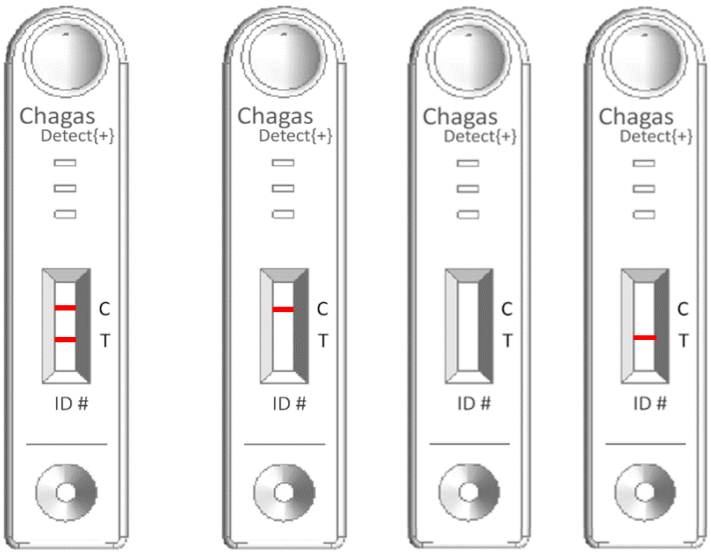


4 Wait 5 minutes (+/- 30 seconds).

5 Add one drop (approximately 40µl) of Chase Buffer Type A to the Sample Pad.



6 Read and interpret the rapid test after 15 additional minutes (20 minutes total test time). Do not interpret results at later time points.



Positive **Negative** **Invalid**

9 Interpretation of Results

A Positive Result

The test is positive for *T. cruzi* antibodies when the control line (C) and the test line (T) appear in the test area. A faint but clear test line is considered a positive result. As a guide for interpretation, the red color in the test region will vary depending on the concentration of the anti-*T. cruzi* antibodies present. The test line for 'weakly positive' sera samples may show a weak positive but distinctly red line. *The presence of a weak red test line should be considered a positive result*.

Note: The red color in the test region will vary depending on the concentration of antibodies present. However, neither the quantitative value nor the rate of increase in antibodies can be determined by this qualitative test.

A Negative Result

The test is negative when only the control line (C) appears. No test line is present.

An Invalid Result

The test is invalid if no control line appears, regardless whether a test line is seen. It is recommended to retest using a new Chagas Detect™ *Plus* Rapid Test and fresh serum or whole blood sample.

10 Troubleshooting

1. *The membrane did not clear up and remains pink and difficult to interpret*. Re-test the sample, making sure to follow instructions precisely. Optionally, remove the cap of the bottle of Gold Solution and measure 40µl using a pipetter for running the assay to ensure precise volumes.

2. *The test line is very strong but the control line remains weak*. It is possible for the test line to react so strongly that the flow of the solutions up the membrane is entirely blocked. This will cause the membrane beneath the test line to remain pink. In this situation, the sample should certainly be considered positive and as long as a minimally weak control line is present, the assay is considered valid.

11 Limitations

- For *in vitro* diagnostic use only.
- This test will only indicate the presence of antibodies to our recombinant antigen in human serum/whole blood.
- Performance with samples from congenital pediatric patients has not been assessed.
- Performance with venous blood samples has not been assessed in clinical settings.
- Chagas Detect™ *Plus* may give false positive results in patients infected with hepatitis C, toxoplasmosis, or syphilis.
- If the result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of Chagas disease.
- 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.
- Testing has not been validated for capillary blood samples that have been refrigerated or frozen for extended periods of time.

12 Expected Values

In a non-endemic population in the United States, Chagas Detect™ *Plus* Rapid Test demonstrated positive results in 0% (0/200) of human whole blood samples and in 0% (0/200) of human serum samples. The non-endemic 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, Chagas Detect™ *Plus* Rapid Test demonstrated positive results in 0% (0/542) of human serum samples and 0% (0/542) of human blood samples. The low endemic study population was 50% male and 50% female with an age range of 18 to 87 years old.

In a highly endemic population in Bolivia, Chagas Detect™ *Plus* 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.

13 Performance Characteristics

Clinical Performance: Non-endemic Population

Specificity of Chagas Detect™ *Plus* (CDP) Rapid Test was assessed at Johns Hopkins School of Public Health and the Center for Immunization Research in Baltimore, MD, a site non-endemic for Chagas disease. Reference testing consisted of immunofluorescent antibody (IFA) test. Two-hundred prospectively collected serum and capillary whole blood specimen pairs were collected for this study with informed consent. As Chagas infection can be asymptomatic, study inclusion criteria did not specify minimum symptoms. The results of this study are shown in Table 1 below for serum and capillary whole blood. Chagas Detect™ *Plus* Rapid Test demonstrated very high specificity.

Table 1: United States, a non-endemic area

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

Capillary Whole Blood		Reference testing		
		Positive	Negative	Total
Chagas Detect™ *Plus* Rapid Test	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: Endemic Populations

Sensitivity and specificity of Chagas Detect™ *Plus* Rapid Test was assessed at two sites endemic for Chagas disease. Reference testing included indirect hemagglutination assay (IHA) and immunofluorescent antibody test (IFA). Only samples that tested positive in both IHA and IFA were considered confirmed seropositive. The results are shown below for each site.

Site 1: Chile, a low risk endemic area

One thousand fifteen (1015) specimen pairs were collected for this low risk endemic site. Serum and capillary whole blood specimens tested by CDP in Chile were de-identified samples from one prospective study (Table 2) and one confirmed positive retrospective study (Table 3). Chagas Detect™ *Plus* Rapid Test demonstrated very high sensitivity and specificity in this population.

A prospective study was performed on serum and capillary blood specimens collected from 542 adults (49.6% male, 50.4% female, ages 19-82, average age 50 years old). Results are shown in Table 2 below.

Table 2: Prospective samples from adults in Chile

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

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

A retrospective study was performed on serum and capillary blood specimens from 473 adults (47.4% male, 52.6% female, ages 18-87, average age 51 years old) who were previously diagnosed as positive for Chagas disease. Results are shown in Table 3 below.

Table 3: Retrospective samples from adults in Chile

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

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

Site 2: Bolivia, a highly endemic site

Specimens from five hundred fifty-one (551) subjects were tested for this high risk endemic site. Serum and capillary whole blood specimens tested by CDP in Bolivia were de-identified samples from two prospective studies and one set of archive samples Chagas Detect™ *Plus* Rapid Test demonstrated very high sensitivity and specificity in this population.

One of the prospective studies was performed on serum and capillary blood specimens collected from 108 adults (49.1% male, 50.9% female, ages 22-83, average age 56 years old) in the city of Santa Cruz. Results are shown in Table 4 below.

Table 4. Prospective samples from adults at Santa Cruz, Bolivia

Serum		Reference testing		
		Positive	Negative	Total
Chagas Detect™ *Plus* Rapid Test	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%]				

Capillary Whole Blood		Reference testing		
		Positive	Negative	Total
Chagas Detect™ *Plus* Rapid Test	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%]				

A second prospective study was performed on serum and capillary blood specimens collected from 243 pregnant women (ages 18-42, average age 25 years old) in the Bolivian Chaco. Results are shown in Table 5 below.

Table 5. Prospective samples from pregnant women at Camiri, Bolivia

Serum		Reference testing		
		Positive	Negative	Total
Chagas Detect™ *Plus* Rapid Test	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%]				

Capillary Whole Blood		Reference testing		
		Positive	Negative	Total
Chagas Detect™ *Plus* Rapid Test	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%]				

The third study was performed on archived sera collected in Cordillera province of Bolivia, from 200 pediatric subjects (53% male, 47% female, ages 2-17, average age 9.6 years old). Results are shown in Table 6 below.

Table 6. Archived samples from pediatric subjects of Cordillera province, Bolivia

Serum		Reference testing		
		Positive	Negative	Total
Chagas Detect™ *Plus* Rapid Test	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%]				

15 Reproducibility Study

A reproducibility study of the Chagas Detect™ *Plus* Rapid Test kit was performed at three sites by two operators at each site for five days. Panels contained weak positive, near LOD, and negative samples. Each operator was given 10 randomized, blinded, coded panels of samples for a total of 90 tests per sample. Reproducibility was 99.4% for the weak positive and negative samples, and 93.3% for the near LOD sample. Chagas Detect™ *Plus* Rapid Test demonstrated high reproducibility among multiple operators at multiple test sites, even for samples near limit of detection.

16 Cross-reactivity Study

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.

Table 7. Results of testing disease-positive human samples with Chagas Detect™ *Plus* Rapid test.

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%**

Chagas Detect™ *Plus* Rapid Test did not cross-react with 11 out of the 14 disease states tested. Faint false positive results were observed for one HCV sample, one Toxoplasmosis sample, and two Syphilis samples.

17 Interference Study

Blood components and anticoagulants were evaluated to determine if they have a detrimental effect on Chagas Detect™ *Plus* Rapid Test. Potentially interfering substances tested in this study, normal concentrations found in human blood and serum, and concentrations tested in this study are listed in the table below. Each interfering substance (IS) and its solvent control was added to serum prior to running the rapid test. Concentrations in this study are expected to exceed those encountered in customer usage, demonstrating 'worst case' scenario of interference likelihood. 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). No interference was found with the substances listed in the table below with the Chagas Detect™ *Plus* Rapid Test.

Table 8. Interfering substance test results

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

18 Matrix (Venous Blood) Equivalence Study

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 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. For serum, LOD was demonstrated at 1:16 dilution, while for venous blood with any of the anticoagulants, LOD was at 1:32 dilution.

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IVD

EC REP **European Authorized Representative**

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