Rapid Response™

Chagas IgG Test Cassette
(Whole Blood/Serum/Plasma)

REF CHA-13C40
CHA-13C25

Product Insert

A rapid test for the qualitative detection of IgG anti-Trypanosoma cruzi (T. cruzi) in human's whole blood, serum or plasma specimen.

For professional *in vitro* diagnostic use only.

Intended Use

The Rapid Response™ Chagas IgG Test Cassette is a lateral flow chromatographic immunoassay for the qualitative detection of IgG anti-Trypanosoma cruzi (T. cruzi) in human serum, plasma or whole blood. It is intended to be used as a screening test and as an aid in the diagnosis of infection with T. cruzi. Any reactive specimen with the Rapid Response™ Chagas IgG Test Cassette must be confirmed with alternative testing method(s) and clinical findings.

Introduction

Chagas disease is an insect-borne, zoonotic infection by the protozoan T. cruzi, which causes a systemic infection of humans with acute manifestations and long-term sequelae. It is estimated that 16- 18 million individuals are infected worldwide, and roughly 50,000 people die each year from chronic Chagas disease (World Health Organization)[1].

Buffy coat examination and xenodiagnosis used to be the most common methods[2,3] in the diagnosis of acute T. cruzi infection. However, both methods are either time consuming or lack sensitivity.

Recently, serological test became the mainstay in the diagnosis of Chagas's disease. In particular, recombinant antigen-based tests eliminate false-positive reactions which are commonly seen in the native antigen tests[4-5].

The Rapid Response™ Chagas IgG Test Cassette is an instant antibody test which detects IgG antibodies the T. cruzi within 15 minutes without any instrument requirements. By utilizing T. cruzi specific recombinant antigen, the test is highly sensitive and specific.

Principle

The Rapid Response™ Chagas IgG Test Cassette is a qualitative, membrane-based immunoassay for the detection of IgG anti-Trypanosoma cruzi (T. cruzi) in whole blood, serum or plasma. The membrane is pre-coated with recombinant mouse anti-human IgG on the test line region of the cassette. During testing, the whole blood, serum or plasma specimen reacts with recombinant Chagas antigen conjugated colloid gold. The mixture migrates upward on the membrane chromatographically by capillary action to react with mouse anti-human IgG on the membrane and generate a colored line. The presence of this colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

Reagents

The test cassette contains recombinant Chagas antigen conjugated colloid gold, mouse anti-human IgG coated on the membrane.

Precautions

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink, or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.

Materials

Materials provided

- Individually packed test cassettes
- Droppers
- Buffer solutions
- Product insert

Materials required but not provided

- Specimen collection container
- Heparinized capillary tubes and dispensing bulb
- Centrifuge
- Timer
- Lancets

Storage and Stability

The kit can be stored at room temperature or refrigerated (35.6-86°F; 2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

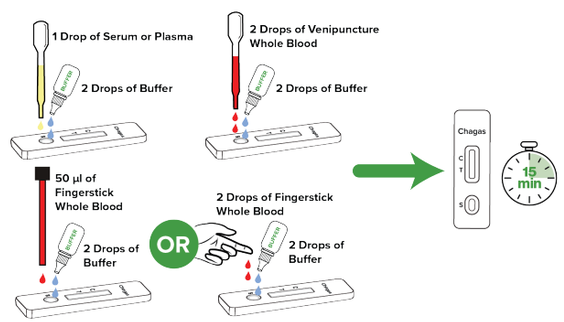
Collection and Storage of Specimens

- The Rapid Response™ Chagas IgG Test Cassette can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.
- To collect Fingerstick Whole Blood specimens:
  - Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
  - Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  - Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
  - Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
- Add the Fingerstick Whole Blood specimen to the test by using a capillary tube:
  - Touch the end of the capillary tube to the blood until filled to approximately 50 µL. Avoid air bubbles.
  - Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense the whole blood to the specimen area of the test cassette.
- Add the Fingerstick Whole Blood specimen to the test by using hanging drops:
  - Position the patient's finger so that the drop of blood is just above the specimen area of the test cassette.
  - Allow 2 hanging drops of fingerstick whole blood to fall into the center of the specimen area on the test cassette or move the patient's finger so that the hanging drop touches the center of the specimen area. Avoid touching the finger directly to the specimen area.
- Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 35.6-46.4°F (2-8°C) for up to 3 days. For long term storage, specimens should be kept below -4°F (20°C). Whole blood collected by venipuncture should be stored at 35.6-46.4°F (2-8°C) if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with federal regulations for transportation of etiologic agents.

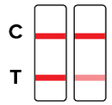
Test Procedure

Allow test cassette, specimen, and/or controls to equilibrate to room temperature (59-86°F; 15-30°C) prior to testing.

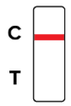
1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the cassette on a clean and level surface.
   For Serum or Plasma specimen: Hold the dropper vertically and transfer 1 drop of serum or plasma (approximately 25µL) to the specimen area, then add 2 drops of buffer (approximately 80 µL) and start the timer, see illustration below.
   For Venipuncture Whole Blood specimen: Hold the dropper vertically and transfer 2 drops of whole blood (approximately 50 µL) to the specimen area, then add 2 drops of buffer (approximately 80 µL) and start the timer. See illustration below.
   For Fingerstick Whole Blood specimen:
   - To use a capillary tube: Fill the capillary tube and transfer approximately 50 µL of fingerstick whole blood specimen to the specimen area of test cassette, then add 2 drops of buffer (approximately 80 µL) and start the timer. See illustration below.
   - To use hanging drops: Allow 2 hanging drops of fingerstick whole blood specimen (approximately 50 µL) to fall into the specimen area of test cassette, then add 2 drops of buffer (approximately 80 µL) and start the timer. See illustration below.
3. Wait for the colored line(s) to appear. The test result should be read at 15 minutes. Do not interpret the result after 20 minutes.



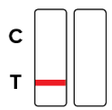
Results Interpretation



POSITIVE: Two colored lines appear. One colored line should be in the control region (C) and another colored line should be in the test region (T).
NOTE: The intensity of the color in the test line region (T) will vary depending on the concentration of Chagas antibodies present in the specimen. Therefore, any shade of color in the test region should be considered positive.



NEGATIVE: One colored line appears in the control region (C). No colored line appears in the test region (T).



INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Quality Control

Internal procedural controls are included in the test. A color line appearing in the control region (C) is an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Limitations

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of anti -T. cruzi antibody in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Rapid Response™ Chagas IgG Test Cassette is limited to the qualitative detection of anti-T. cruzi antibody in human serum, plasma or whole blood. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable anti-T. cruzi antibody. However, a negative test result does not preclude the possibility of exposure to or infection with T. cruzi.
4. A negative result can occur if the quantity of the anti-T. cruzi antibody present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

Performance Characteristics

Sensitivity and Specificity

A total of 264 samples from susceptible subjects were tested by the Chagas IgG Test Cassette and by a commercial Chagas ELISA kit. Comparison for all subjects is shown in the following table.

Method		TPPA		Total Result
Rapid Response™ Chagas IgG Test Cassette	Results	Positive	Negative	
	Positive	13	4	17
	Negative	1	246	247
Total Result		14	250	264

Relative Sensitivity: 92.9% (95% CI*: 66.1%-99.8%)
Relative Specificity: 98.4% (95%CI*: 96.0%-99.6%)
Accuracy: 98.1% (95%CI*: 95.6%-99.4%)
*Confidence Interval

Precision

Intra-Assay

Within-run precision has been determined by using 10 replicates of 4 specimens: a negative, a low positive, a medium positive and a high positive. The negative, low positive, medium positive and high positive values were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same 4 specimens: a negative, a low positive, a medium positive and a high positive. Three different lots of the Chagas IgG Test Cassette have been tested using negative, low positive medium positive and high positive specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

The Chagas IgG Test Cassette has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, Syphilis, HIV, H. Pylori, MONO, CMV, Rubella and TOXO positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to Chagas negative and positive specimens.

Acetaminophen: 20 mg/dL	Caffeine: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL	Gentisic Acid: 20 mg/dL
Ascorbic Acid: 2g/dL	Albumin: 2 g/dL
Creatin: 200 mg/dL	Hemoglobin 1000mg/dL
Bilirubin: 1g/dL	Oxalic Acid: 60mg/dL

None of the substances at the concentration tested interfered in the assay.

Bibliography

1. World Health Organization. Control of Chagas disease: report of a WHO expert committee. 1991
2. Frasch ACC, Reyes MB, Sanchez DO. Diagnosis of Chagas disease: present and future. In: Chagas' disease and the nervous system. Washington, DC: pan American Health Organization. 1994: 47-53
3. Frasch AC, Reyes MB. Diagnosis of Chagas disease using recombinant DNA technology. Parasitol Today. 1990,6(4):137-9
4. Lorca M, Gonzalez A, Reyes V, Veloso C, Vergara U, Frasch C. [The diagnosis of chronic Chagas disease using recombinant antigens of Trypanosoma cruzi] Rev Med Chil. 1993 121(4):363-8.
5. da Silveira JF, Umezawa ES, Luquetti AO. Chagas disease: recombinant Trypanosoma cruzi antigens for serological diagnosis. Trends Parasitol. 2001, 17(6):286-91.

Glossary of Symbols

Consult instructions for use
Test per Kit
EC REP Authorized Representative
35.6°F 2°C 86°F 30°C Store between 35.6°F to 86°F
Use by
Do Not Reuse
LOT Lot Number
IVD For *in vitro* diagnostic use only
REF Catalogue #

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