CHAGAS RAPIDO FIRST RESPONSE

Rapid immunochromatographic assay for the detection of specific antibodies against Trypanosoma cruzi in serum, plasma or whole blood

For in vitro diagnostic use only.

PRESENTATIONS: Kits for 25, 50, 100 or 200 determinations

CLINICAL ASPECT AND INTENDED USE

Chagas Disease is caused by the flagellated protozoan Trypanosoma cruzi, affecting mainly Latin American countries. The routes of transmission of Trypanosoma cruzi can be vector through the infected feces of the insect vector of the genus Triatominae, congenital through the transplacental route, transfusion through contact with infected blood, by organ transplant
or by work accident. Three evolutionary periods of the disease are recognized. The acute period, generally seen in children, is usually asymptomatic. Most acute cases resolve within two to three months.
This is followed by the period without clinical complications where seropositivity is evidence of the existence of the disease. The chronic disease is characterized by disorders in cardiac function that can lead to sudden death due to ventricular fibrillation or death due to progressive heart failure. Rapid immunochromatographic assays have been developed for the detection of specific antibodies in serum, plasma and whole blood that, due to their simplicity and not requiring additional instruments, can be very useful for epidemiological tests and for routine clinical trials and early infer Trypanosoma infection. cruzi.

PRINCIPLE OF THE TEST

CHAGAS RAPIDO FIRST RESPONSE is a rapid immunochromatographic assay for the detection of specific antibodies against Trypanosoma cruzi in serum, plasma or whole blood.
The product contains plastic cassettes with an itrocellulose membrane sensitized with specific Recombinant Antigens of Trypanosoma cruzi of the epimastigote and trypomastigote stages in the area identified with the letter “T” and apatch impregnated with Anti-Human Immunoglobulin Antibodies conjugated with colloidal gold placed on the height of the receptacle intended for the sample.
Added samples and Buffer in the container mix with the Conjugate and flow laterally through the nitrocellulose membrane. The antibodies of the sample bind to the Conjugate and, if the sample has specific antibodies against Trypanosma cruzi, these subsequently bind to the Antigen of the “T” area, developing a colored line.
In the absence of specific antibodies this line does not develop. Additionally, the product has a Control line in the area identified with the letter “C”, which must always appear as the sample runs, to ensure the validity of the test.

CONTENT

Cassettes: Individual containers each containing cassettes with an itrocellulose membrane sensitized with specific Recombinant Antigens of Trypanosoma cruzi and Anti-Human Immunoglobulin Antibody Conjugate with Colloidal Gold, a volumetric Blood Dispenser and a desiccant.
Quantity: 25, 50, 100 or 200 cassettes depending on presentation.

Buffer: Dropper bottle containing Sodium Carbonate 0.296% W/V, Sodium Bicarbonate 0.159% W/V, Sodium Chloride 0.700% W/V, PEG 12,000 0.500% W/V, Sodium EDTA 0.500% W/V and Azide Sodium 0.020% W/V as a preservative in purified water. Ready to use.

Volume: 3.5, 7.0, 14.0 or 28.0 ml depending on presentation

Instruction Manual: This document.

NECESSARY MATERIALS NOT PROVIDED

1. Indelible marker.
2. Stopwatch.
3. Medical Alcohol 96° or Ethyl Alcohol 70° for disinfection of the fingertip.
4. Absorbent paper or cotton to clean the capillary puncture area.
5. Sterilized lancets for individual use capillary puncture.
6. Materials for sample collection if obtained by venipuncture and dispensing micropipettes for this case.
7. Disposer of potentially infectious waste.
8. Biosafety materials.

STORAGE AND STABILITY

1. The product must be stored between 2 and 30°C and used before the expiration date. expiration declared on the labels. Do not freeze.
2. The product should be brought to room temperature before use.
3. Once the container containing the Cassette is opened, it must be used immediately. which is sensitive to humidity.

PRECAUTIONS AND WARNINGS

1. For “in vitro” diagnostic use only.
2. Do not use the product after the expiration date stated on the labels. He Product must be stored between 2 and 30°C.
3. Procedures must be performed carefully to obtain reliable clinical results and interpretations.
4. Do not mix components from different lots.
5. Do not use the cassette if its individual packaging is not intact.
6. The Cassette and Volumetric Blood Dispenser are for single use only.
7. Potentially infectious samples and materials must be handled with care following current biosafety regulations.
8. All objects in direct contact with samples and test waste should be treated as potentially infectious. The most effective procedures for inactivation are treatment with an autoclave at 121°C for 30 minutes or with Sodium Hypochlorite at a final concentration of 2.5% for 24 hours.
9. Avoid any contact of liquids with the skin and mucous membranes. Always use gloves, glasses, etc. for your protection, according to biosafety regulations.
10. Sodium Azide used as a preservative is toxic if ingested.
In contact with acids it emits toxic fumes. Occasionally, it can produce explosions on contact with metal ions. Therefore, in case of disposal through a drainage system, plenty of water must flow.

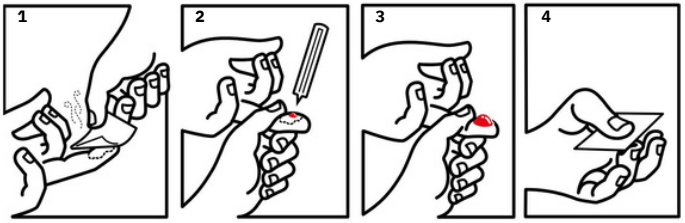
COLLECTION AND STORAGE OF SAMPLES

WHOLE BLOOD FROM CAPILAR PUNCTURE:

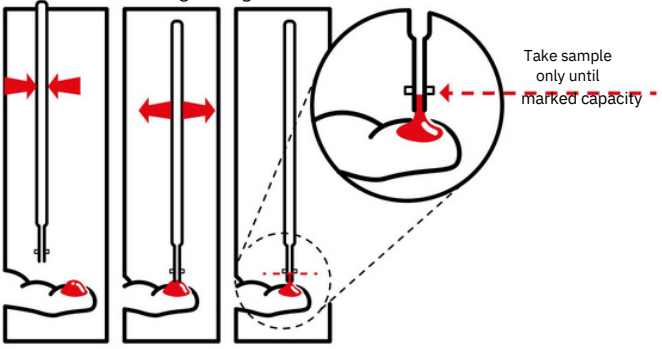
1. Use a sample obtained from the middle or ring finger. To facilitate blood flow

massage finger and/or apply gentle heat.
2. Using absorbent paper or cotton, disinfect the patient's fingertip with 96º Medical Alcohol or 70º Ethyl Alcohol. Wait for the moistened surface to dry.

3. Take a sterilized single-use hair puncture lancet from the back, avoiding touching its tip. Once the surface of the finger is dry and with the palm of the hand facing up, perform the puncture firmly on the tip of the finger.
4. Discard the lancet, and press the patient's finger to achieve bleeding.
5. Absorb the first drop of blood with absorbent paper or cotton to remove tissue fluid from the sample. See the following drawing:



6. Take the volumetric Blood Dispenser, gently press its bulb and place the open end in contact with the blood, allowing it to rise to the marked volume (10 µl), gently releasing the bulb. See the following drawing:



7. Perform the test immediately by depositing the blood in the cassette receptacle intended for this purpose.
8. Give the patient an absorbent paper. Instruct him to press for 1 to 2 minutes.

WHOLE BLOOD FROM VENIPUNCTURE:

Blood will be drawn from a patient following general guidelines. Collect Whole Blood obtained by venipuncture in tubes containing anticoagulants such as EDTA, Heparin or Sodium Citrate.

PLASMA:

Blood will be drawn from a patient following general guidelines. Collect blood obtained by venipuncture in tubes containing anticoagulants such as EDTA, Heparin or Sodium Citrate. Centrifuge the tube to separate the plasma obtained.

SERUM:

Blood will be drawn from a patient following general guidelines. Collect blood obtained by venipuncture in tubes without anticoagulants. Leave to rest for the clot to form for at least 30 minutes and centrifuge at 3000 rpm for 10-15 minutes to obtain the serum.

Venous blood samples should be used immediately or can be stored at 2 to 8°C for 3 days. Plasma or serum can be subjected to long-term storage by freezing at -20°C; They should be placed at room temperature before use. Avoid repeated freezing and thawing. Frozen samples should be homogenized and centrifuged before use. In the presence of turbidity, precipitates or clots, we recommend centrifuging at 2000 rpm for 20 minutes at room temperature or filtering with 0.22µ membranes. Do not use samples with bacterial contamination as they may produce false results. Do not use heat inactivated samples.

No interference has been observed due to hemolysis up to 10 mg/ml, triglycerides up to 15 mg/ml and bilirubin up to 0.30 mg/ml.

TEST PROCEDURE

1. Place the product components and samples required for testing at room temperature before performing the test procedure.
2. Immediately before use, remove the number of cassettes needed for the test from their packaging. Each cassette can be appropriately identified with an indelible marker.

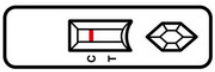
3. Place the selected cassettes on a clean, flat, dry surface and protect them from of vibrations.
4. Add to the receptacle intended for the samples: 10 µl of Whole Blood or 5 µl of Serum or Plasma. Use, as appropriate, the volumetric Blood Dispenser provided or micropipettes. Wait for it to be completely absorbed.
5. Next add a drop (approximately 40 µl) of Buffer to the same receptacle. Wait for it to be completely absorbed. Add another drop and start the stopwatch.

6. Observe the development of colored lines in the results window.
7. Interpret the results between 20 and 30 minutes. Do not interpret after 30 minutes as erroneous results may be obtained. Some positive samples react immediately, others do so within the interpretation time limit.
In some cases the background of the nitrocellulose membrane may remain faintly colored without disturbing the interpretation of the results. See the following drawing:

RESULTS ANALYSIS

Negative result:

A single reddish Control line appears in the area identified with the letter “C”.

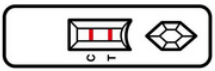


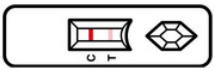
Positive result:

Two reddish lines appear. One in the area identified with the letter “T” and another Control line in the area identified with the letter “C”.

The color intensity of the “T” line may vary depending on the sample under study.

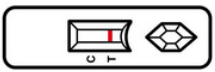
Any visible color, even if it is very faint, must be interpreted as positive. The intensities of the “T” and “C” lines may be different, however the result is positive.

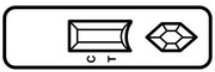




Invalid Result:

Absence of line Reddish control in the area identified with the letter “C” whether or not the line is in the area identified with the letter “T”. This may occur due to an error in the testing procedure, or a problem with the sample due to the presence of fibrin, or its high viscosity resulting in incomplete migration. In this case you must repeat the procedure using anew cassette with the sample centrifuged again.





VALIDATION OF RESULTS:

A reddish Control line in the area identified with the letter “C” must always appear as the sample runs, to ensure that the test was performed correctly and the product is working properly.

LIMITATIONS OF THE METHOD:

Serology only constitutes auxiliary data for the diagnosis. The immunochromatography technique, like any other serological technique, cannot be conceived as a definitive diagnostic method since it must be based on the correlation of the results of more than one test with clinical and epidemiological data. In that sense, every result must be interpreted as a greater or lesser probability of success in relation to the case studied, within the normal or parasitized population.
Any result obtained with this test must be confirmed by other alternative methods.

A reactive result must be verified by another technique such as indirect immunofluorescence, indirect hemagglutination and/or ELISA.
A non-reactive result does not exclude the possibility of exposure or infection with Trypanosoma cruzi. In very recent infections (less than 30/45 days of evolution) the technique may present non-reactive results.
False positive results could appear in autoimmune diseases, pregnancy, liver diseases, other parasites such as leishmaniasis or other infectious diseases.

PRODUCT PERFORMANCE CHARACTERISTICS

Sensitivity and specificity

653 samples from patients who were part of a double-blind study were analyzed.

carried out at the Reference Center for the Diagnosis of Chagas Disease in Argentina. Of the samples
analyzed, 308 samples were considered positive (47%) and 345 negative (53%), according to the results of
the National Reference Center and those obtained with commercial ELISA products at the Lemos SRL Laboratory. It was used as a reference for determine if the samples were positive, the reactive result of two
of the three diagnostic tests routinely performed by the Reference Center (HAI, IFI and ELISA), and negative, the non-reactive result of the three diagnostic tests routinely performed by the Reference Center. Reference
in combination with the results of four commercial ELISA type products, two of them based on the use of
total antigen and two based on the use of recombinant antigen.
The results were also compared with other commercial immunochromatography.
CHAGAS RAPIDO FIRST RESPONSE gave 96.4% sensitivity using capillary blood, 95.1 sensitivity using venous whole blood, and 92.2% sensitivity with serum samples. The sensitivity of alternative commercial immunochromatography with venous whole blood (95.4%) was similar to that of CHAGAS RAPIDO FIRST RESPONSE (95.1%).

		Positive (n =308)			
		Positive results	Negative results	Failed	Sensitivity (%)
CHAGAS FAST FIRST RESPONSE	Capillary Whole Blood	295	11	2	96.4
	Venous Whole Blood	291	15	2	95.1
	Serum	284	24	0	92.2
Other Inmuno chromatography	Venous Whole Blood	293	14	1	95.4

CHAGAS RAPIDO FIRST RESPONSE gave 96.0% specificity using capillary blood, 98.3 specificity using venous whole blood, and 98.5% specificity with serum samples. The specificity of alternative commercial immunochromatography with venous whole blood (98.3%) was equal to that of CHAGAS RAPIDO FIRST RESPONSE.

		Negatives (n =345)			
		Negative results	Positive results	Failed	Specificity (%)
CHAGAS RAPIDO FIRST RESPONSE	Capillary Whole Blood	315	13	17	96.0
	Venous Whole Blood	339	6	0	98.3
	Serum	339	5	1	98.5
Other Inmuno chromatography	Venous Whole Blood	339	6	0	98.3

In a study carried out with 270 serum samples from hemodiators provided by a Hemotherapy Service from individuals considered suitable as donors, the specificity obtained was 98.2%.

96 samples of Serum and Venous Whole Blood were evaluated in parallel. With Venous Whole Blood, an agreement of 98.0% was obtained for samples considered reactive and an agreement of 96.0% for samples considered non-reactive. With Serum, an agreement of 94.1% was obtained for samples considered reactive and an agreement of 98.0% for samples considered non-reactive.

In another study, 10 serum samples were evaluated in parallel compared to plasma samples from the same patient with different anticoagulants, and no differences were obtained between them.

The possible cross-reaction was studied with samples with reactive serology for other parasitosis such as Toxoplasmosis (56 samples), Hydatidosis (33 samples), Amebiasis (3 samples) and cysticercosis (7 samples), leishmaniasis (6 samples), samples from pregnant women. (30 samples), Rheumatoid Factor (12 samples) other infectious diseases (10 samples). In this population the total specificity was 98.7% over N =157.

Precision:

Intra-assay: 3 reactive samples with different reactivities and one non-reactive sample were used. Each one making 10 replicates. Positive and negative results were correctly identified in all cases.

Intertests: 3 reactive samples with different reactivities and one non-reactive sample were used. Each one doing 10 replicates for 10 different days reading two operators. Positive and negative results were correctly identified in all cases.

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