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Protocol for detect *Trypanosoma cruzi* by indirect methods as IIF, ELISA and IHA from serological samples

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We use this protocol and it's working

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Abstract

This is the protocol to be used in the diagnosis of Chagas disease in Colombia, according to the algorithm suggested by Health and Social Protection Ministery with the National Health Institute.

Guidelines

Follow the manufacter instructions for ELISA test.

Safety warnings

Use primary containment elements such as gown, glasses and gloves.

Before start

Condition the work surface by cleaning with 0.5% sodium hypochlorite solution. Avoid inappropriate procedures that result in punctures or accidents with samples.

Indirect Immunofluorescent (IIF) staining

1 **Indirect Immunofluorescent (IIF) staining**

1. Antigen-Antibody formation: The IIF staining was performed using antigen glass slides impregnated with trypomastigote forms of Chagas obtained from culture *in house.* A volume of 12 µL of the serum patients and controls (previously diluted with buffer in serial dilutions in base 2 from 1: 8 to 1: 256), were deposited in the wells with antigen, followed by incubation at 37 °C and finally they were washed.

2. Detection of Antigen-Antibody: An anti-human IgG labeled with fluorescein and Evans Blue was used as a conjugate, it was incubated and washed again.

3. Reading: After another washing step, allowed to dry at room temperature and it was observed under fluorescence microscopy. Samples with fluorescence greater than or equal to 1:32 were considered positive.

Enzyme-Linked ImmunoSorbent Assay (ELISA) Commercial kits forTrypanosoma cruzi

2 **Enzyme-Linked ImmunoSorbent Assay (ELISA) Commercial kits for *Trypanosoma cruzi***

Basis is the same, only they show variation in the nature of the antigens and in the interpretation of the results.

These are the main characteristics of polystyrene plates:
• E**LISA CHAGAS III GRUPOBIOS SA TEST.** Few impregnated with antigens activated with total extracts of *T. cruzi* strains (Tulahuén and MN), including highly immunogenic membrane antigens.

• **ELISA CHAGAS *T. cruzi* Ab DIAPRO TEST.** glasses coated with recombinant Chagas antigens.

1. Antigen complex antibody formation: Diluted patient serum at 37 °C and the corresponding kit controls were incubated on the antigen-coated plates, which, if the respective antibodies were present, formed a stable complex that was not removed by subsequent washing.

2. Complex reaction: 100 µL of conjugate was added, which consists of a peroxidase-labeled human anti IgG, which one bound to the complex after the respective incubation.

3. Detection: After washing, 100 µL of conjugate reacting with the peroxidase was added, revealing color, proportional to the amount of complex formed in each of the wells, by incubation time at room temperature. The reaction was stopped by adding sulfuric acid.

4. Reading: The color intensity was measured in a colorimetric reader for ELISA plates with a 450 nm filter.

5. Interpretation:

• ELISA CHAGAS III GRUPOBIOS SA TEST
Cut off calculation: (average of positive controls + average of negative controls) * 0.35
POSITIVE: When the absorbance of the sample is greater than cut off + 10%
NEGATIVE: When the absorbance of the sample is less than cut off + 10%
DOUBTFUL: When the absorbance of the sample is in a range of cut off +/- 10%

• ELISA CHAGAS *T.cruzi* Ab DIAPRO TEST
Cut-off value: average absorbance of negative controls + 0.35
Patient results: patient sample absorbance / cut-off value
POSITIVE: 1.1
NEGATIVE: <0.9
DOUBTFUL: 0.9 - 1.1

Indirect Hemaglutination (IHA) test

3 **Indirect Hemaglutination (IHA) test**
A commercial kit from Winner was used for the detection of antibodies against *Trypanosoma cruzi*.

1. Elimination of heterolipic antibodies: In order to eliminate nonspecific interfering antibodies that may interfere with the agglutination of red blood cells, 25 µL of patients and controls serum were incubated with 25 µL of 2% mercaptoethanol.

2. Dilutions: Serial dilutions were made on the basis of ½ to 1/64 from the first well diluted with mercaptoethanol.

3. Heterophilia control: 25 µL of non-sensitized red blood cells were added in the dilutions ½ and ¼.

4. Reactions: 25 µL of IHA antigen was added to the other dilutions, corresponding to ram red blood cells sensitized with antigens with cytoplasmic antigens of *T. cruzi.*

5. Reading: After waiting for 90 minutes at room temperature, the visualization was

performed. Formation of a mantle of at least 50% on the surface of the well was interpreted as positive and a small agglutination of irregular edges as negative result.