



# Chagatest

*HAI*

**Indirect hemagglutination assay (IHA) for the detection of antibodies against *Trypanosoma cruzi***

## SUMMARY

Chagas' disease is a parasitic infection produced by the *Trypanosoma cruzi*. In most of the cases, the disease evolves towards a chronic phase.

The laboratory diagnosis depends on the phase in which the disease is. During the acute phase, the diagnosis is done directly through the finding of parasites in blood. During the chronic phase, easy immunological methods can be used, which make their use possible as screening tests.

## PRINCIPLE

The IHA is based on the property of the antibodies (anti-*T. cruzi* in this case) of producing specific agglutination in presence of sensitized red blood cells with the corresponding antigens.

Since unspecific antibodies are present, not only in the infected patients but also in the non-infected ones, their presence must be carefully studied, specially for the heterophile antibodies capable of agglutinating red blood cells in different classes. Such investigation is done confronting the serum with non-sensitized red blood cells. The interfering antibodies are eliminated by treating them with 2-mercaptoethanol.

## PROVIDED REAGENTS

**IHA Diluent buffer:** saline solution buffered at pH 7.

**IHA Antigen:** lyophilized sheep red blood cells sensitized with *T. cruzi* cytoplasmic antigens.

**Non-sensitized red blood cells:** suspension of 1% goat erythrocytes, for the control of heterophilia.

**IHA Buffer:** phosphate buffered saline solution, pH 7.5, with inert dye.

**Protein Solution:** 10% bovine albumin solution.

**2-Mercaptoethanol:** ampoule with 2-Mercaptoethanol (2-ME).

**Positive Control:** inactivated serum containing antibodies against *Trypanosoma cruzi*.

**Negative Control:** inactivated non-reactive serum.

## NON-PROVIDED REAGENTS

Saline Solution.

## INSTRUCTIONS FOR USE

**IHA Antigen:** prepare it with 6.1 ml of IHA Diluent buffer. It can be used after an hour, mixing every 20 minutes to achieve a proper rehydration of the reagent. Every time it is used, homogenize by agitating, avoiding the formation of foaming.

**Non-sensitized red blood cells:** homogenize by agitating before use, avoiding the formation of foaming.

**IHA Serum Diluent:** add 0.2 ml of Protein Solution every 10 ml

of IHA Buffer. Mix, label and date.

**2-Mercaptoethanol:** once opened the ampoule, transfer the contents to the empty bottle provided which should be covered immediately after use.

**1% 2-Mercaptoethanol:** with the 2-ME provided, prepare a dilution of 1/100 with Saline Solution in a sufficient quantity according to the number of wells used. For example, for 96 wells: 25 ul of 2-ME in 2.5 ml of Saline Solution.

**Positive and Negative Controls:** ready to use.

## WARNINGS

- Reagents are for "in vitro" diagnostic use.

- All samples should be handled as capable of transmitting infection. Controls are inactivated, however, they should be handled as infectious material.

- Control sera have been tested and found non-reactive to hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV).

- Discard all materials used to perform the test in order to ensure the inactivation of pathogenic agents. The recommended method is autoclaving for 1 hour at 121°C. Liquid waste may be disinfected with sodium hypochlorite (5% final concentration) for at least 60 minutes.

- Do not exchange reagents from different kits and lots.

## STABILITY AND STORAGE INSTRUCTIONS

**Provided Reagents:** stable in refrigerator (2-10°C) until the expiration date shown on the box. Do not freeze.

**IHA Serum Diluent:** stable for 5 days in refrigerator (2-10°C) from the preparation date.

**1% 2-Mercaptoethanol:** use immediately after preparation.

**IHA Antigen:** once reconstituted, it is stable for 2 months stored in refrigerator (2-10°C). Do not freeze.

## INSTABILITY OR DETERIORATION OF REAGENTS

- When all the sera dilutions are reactive, it might be an indication of self-agglutination of the IHA Antigen. Verify this by using a well of the microtitration plate only to mix the IHA Antigen and IHA Serum Diluent, without the Sample. If agglutinations persists, the reagent is deteriorated. Discard.

- The absence of reactivity in all the sera dilutions, it might be an indication of deterioration of the reagents. Process the Sample with known positiveness.

## SAMPLE

Serum

**a) Collection:** patient should be fasting. Obtain serum as usual. Do not use plasma.

- b) Additives:** not required. Do not add preservatives.
- c) Known interfering substances:** hemolysis, hyperlipemia (chylomicronemia) may cause erroneous results.
- d) Stability and storage instructions:** serum must be fresh. If sample is not to be processed immediately, it might be stored in refrigerator (2-10°C) up to 72-96 hours from collection. For longer storage periods, samples should be frozen (-20°C), avoiding to repeat such procedure. Non-fresh sera are inclined to become gel when in contact with the 2-ME, causing false positive results.

## REQUIRED MATERIAL

### 1- Provided

- 1 empty bottle (to transfer the 2-ME of the ampoule)
- 5 microtitration plates with U-bottom 96 wells
- 60 disposable plastic droppers
- 2 rubber bulbs
- 4 calibrated loops

### 2- Non-provided

- microdilutors (25 ul)
- microdroppers (25 ul)
- assay tubes and proper volumetric material
- adhesive tape
- filter paper

## PREVIOUS RECOMMENDATIONS

### Microdilutors

- Previous conditioning: before using the microdilutors, put them in a distilled water recipient and onto a filter paper to be sure of a proper collection of sample.
- Washing: before collecting a new sample with the dilutors, transfer the residual volume onto filter paper. Then transfer the microdilutors to two recipients with distilled water and put them onto filter paper.

### Microtitration plate

To eliminate the electrostatic load use a wet piece of cloth for the bottom of the microtitration plate.

## PROCEDURE

Choose an unused microtitration plate with U-bottom wells.

### I- TITRATION WITHOUT 2-ME

- 1- Place a drop of IHA Serum Diluent with a 25 ul microdropper, in all the wells of the microtitration plate.
- 2- Take an aliquot of each serum to be processed with 25 ul microdilutors (one for each sample). Place each microdilutor in the first well and rotate it at least 10 times to be sure of a proper dilution of sample.
- 3- Make serial dilutions from the first well (1/2 dilution), transferring the microdilutors to the next well (1/4 dilution) and then consecutively up to the desired dilution (for example: 1/8, 1/16, 1/32), rotating the microdilutor in each step at least 10 times to be sure of a proper dilution of the sample.
- 4- In the wells with the 1/2 and 1/4 dilutions, place a drop (25 ul) of unsensitized red blood cells to control the heterophilia.
- 5- In the other wells, add a drop (25 ul) of IHA Antigen.

- 6- Mix by slightly tapping the sides of the microtitration plate.
- 7- Settle during 90 minutes, free from vibrations.
- 8- Read from 90 minutes on.  
To clear the result read on a mirror, putting the plate to light with a white paper between the microtitration plate and the source of light.

### II- TITRATION WITH 2-ME

- 1- Place a drop of serum in the first well using disposable droppers in a vertical position (one for each serum).
- 2- Dilute 1/2 adding a drop of 1% 2-Mercaptoethanol to the same wells (use only one disposable dropper).
- 3- Seal these wells with adhesive tape and mix by slightly tapping the sides of the microtitration plate.
- 4- Incubate at 37°C during 30-60 minutes or at room temperature during 90 minutes.
- 5- Take off the adhesive tape, use a wet piece of cloth for the bottom of the microtitration plate, and with a microdropper of 25 ul, place a drop of IHA Serum Diluent in the other wells to use, up to the desired dilution.
- 6- Perform steps 3 and 8 previously described in Titration I.

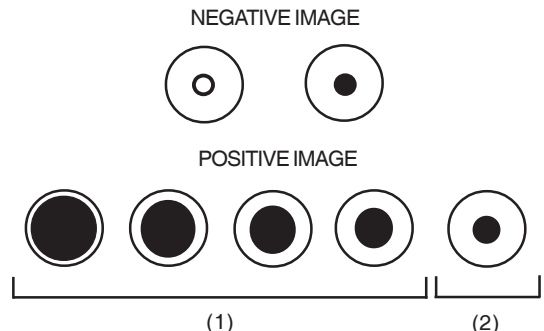
### III- ABSORPTION WITH UNSENSITIZED RED BLOOD CELLS

In those sera with heterophilia, the heterophile antibodies can be adsorbed onto unsensitized red blood cells as follows: in an hemolysis tube, place 50 ul of unsensitized red blood cells provided + 50 ul of serum in assay. Cover to prevent evaporation. Settle the suspension during 30 minutes at 37°C, agitating occasionally. Then centrifuge at 2000 rpm during 5 minutes.  
Take 50 ul from the Supernatant and it is used as 1/2 dilution, placing it in the first well. If it is used in titration with 2-ME, this well equals the 1/4 dilution.

## INTERPRETATION OF RESULTS

**Non-reactive:** button-shaped sedimentation or little ring with regular borders.

**Reactive:** smooth film of cells covering 50% or more of the bottom of wells. Warning: if microdilutors are not used, the smooth film could be smaller.



- (1) Film.  
(2) End Point (50%).

## 1 TITER SCREENING TECHNIQUE

### PROCEDURE

- 1- With 25 ul microdropper place 1 IHA serum diluent drop in every well of the microtitration plate to be used (one well for each sample).
- 2- Immerse 1 clean loop (provided) in sample.
- 3- Place loaded loop in the well containing the IHA serum diluent, rotating it to obtain a homogeneous blend. Distribute the drop over the entire bottom of the well. Withdraw the loop and dry with filter paper. Perform washing in two recipients with distilled water. Repeat drying with filter paper. Proceed in the same way for each new sample.
- 4- With 25 ul microdropper add 1 drop of reconstituted and homogenized IHA antigen to each well.
- 5- Stir the microtitration plate by tapping its slides with the fingers for at least 30 seconds, to obtain a homogeneous blend.
- 6- Settle for 2 hours free from vibrations.
- 7- Perform reading. If reading of results is performed after 2 hours, the microtitration plate should be sealed with a transparent adhesive tape to avoid evaporations.

### INTERPRETATION OF RESULTS

Non-reactive: button-shaped sedimentation.

Reactive: smooth film formation on the bottom of wells. Sample should be considered as indefinite and be re-tested using other method in the presence of a little ring with regular borders.

POSITIVE IMAGE



NEGATIVE IMAGE



INDEFINITE IMAGE



### REFERENCE VALUES

Among immunological techniques, IHA is considered a reliable method for the determination of specific antibodies. However, the results, as those of any other serological method, only constitutes an auxiliary information for the diagnosis. It is for this reason that reports should be considered in terms of probability; in this case, higher or lower probability of T. cruzi parasitosis.

Verify any Reactive result using an additional technique. Mind the "Fatale Chaben" Institute recommendations, according to which the immunodiagnosis of the infection should be performed with a minimum of two of the following methods: indirect immunofluorescence, indirect hemagglutination, ELISA, (latex) particles agglutination, validated by the Centro Nacional de Referencia.

### Titration procedure

Sera with titers higher or equal than 1/16 are considered reactive for anti-T cruzi antibodies.

When observing positive results (reactive sera) as well as the presence of a film in the wells designed for heterophilic control (1/2 and 1/4 dilutions), an additional titration should be performed with the corresponding sera, previously treated with 2-ME or adsorbed with non-sensitized Red Blood Cells. The purpose of such treatment is to eliminate the non-specific reaction. In the first case, the reducing agent (2-ME) eliminates the agglutinating capacity of the heterophilic antibodies, while the non-sensitized Red Blood Cells are eliminated by adsorption.

### 1 Titer screening technique

Under assay conditions, sera with titers higher or equal than 1/12 are considered reactive for anti-T cruzi antibodies. The image presented by a reactive serum is a membrane or film in the bottom of the well.

### QUALITY CONTROL METHOD

A Positive Control and a Negative Control may be processed as a reference point for the reaction. Employ them in the same way as the sample.

### PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.

Other causes producing erroneous results are:

Lack of proper preparing of the microtitration plate and microdilutors (See PREVIOUS RECOMMENDATIONS).

Deteriorated microtitration plate due to successive use. It is not recommended to re-use wells.

Lack of homogenization of reagents before use.

Deficiencies in mixing.

Casual vibrations during time reaction.

Non-fresh, repeatedly frozen and thawed sera.

Casual contaminations of the reagents or of the material used in the assay.

Excess or failure of IHA Serum Diluent in the wells of the microtitration plate.

IHA Serum Diluent with more than 5 days from elaboration.

Not to keep the indicated incubation time and temperature in the treatment with 1% 2-ME.

1% 2-ME not prepared at the time to be used.

### PERFORMANCE (titration with 2-ME)

Experimental experiences which were performed on endemic and non-endemic population-based samples and which were assayed by immunofluorescence and complement-fixation reaction (Machado & Guerreiro) showed that:

- 1) In endemic populations, using IHA method, 98% of those titers lower than 1/8 and 95% of those higher or equal than 1/8 were confirmed by reference methods.
- 2) In non-endemic populations, 100% of healthy individuals showed titers lower than 1/8 determined by IHA.
- 3) In 100% of individuals with positive serology confirmed by reference methods and parasitosis confirmed by xenodiagnosis and/or hemoculture, higher or equal titers than 1/32 were observed determined by Chagatest IHA.

## WIENER LAB PROVIDES

- 96 tests of 5 titers or 480 tests of 1 titer (Cat. 1293205).

## REFERENCES

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- Cerisola, J.A. - La Prensa Médica Argentina 49/34:1761 (1962).
- Fontenla S., Moretti, E. y González, G. - 50° Triduo de la ABA, Huerta Grande (Córdoba), 1985.
- Basso, A. y col. - 50° Triduo de la ABA. Huerta Grande (Córdoba), 1985.
- Lorenzo, L.; Capriotti, G.; Rojkin, F. - Rev. Arg. Transf. XVII/ 1: 51, 1991.

## SYMBOLS EXPLANATION

**Antígeno** | **HAI**

IHA Antigen

**Buffer** | **HAI**

IHA Buffer

**Reconstituy.** | **HAI**

IHA Diluent buffer

**GR** | **no sensibil.**

Non-sensitized red blood cells

**Sol.** | **Proteica**

Protein Solution

**2-ME**

2-Mercaptoethanol

**Control** | **+**

Positive Control

**Control** | **-**

Negative Control

The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

**EC** | **REP**

Authorized representative in the European Community

**MD**

"In vitro" diagnostic medical device



Contains sufficient for <n> tests



Use by



Temperature limitation (store at)



Do not freeze



Biological risks



Volume after reconstitution

**Cont.**

Contents

**LOT**

Batch code



Manufactured by:

**Xn**



Harmful



Corrosive / Caustic

**XI**



Irritant



Consult instructions for use

**Calibr.** | **+**

Calibrator

**CONTROL** | **+**

Control

**CONTROL** | **+**


Positive Control

**CONTROL** | **-**

Negative Control

**REF**

Catalog number

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