Chagatest HAI

screening A-V

Indirect hemagglutination assay for the detection of antibodies to *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 of the disease. During the acute phase, the diagnosis is performed by detection of parasites in blood. During the chronic phase serologic methods are used as screening tests.

PRINCIPLE

Indirect hemagglutination (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 *Trypanosoma cruzi* cytoplasmic and membrane antigens. **Chagatest HAI screening A-V** uses red blood cells from chicken. They accelerate the reaction visualization, being larger than goat red blood cells.
Interfering antibodies, which may cause unspecific agglutination, are eliminated by the addition of a protein solution, which includes inhibiting substances.

PROVIDED REAGENTS

A-V IHA Antigen: chicken red blood cells suspension sensitized with *T. cruzi* cytoplasmic and membrane antigens in phosphates buffer with 8.5 g/l ClNa and < 0.1% preservative.
A-V IHA Buffer: 20 mmol/l phosphate buffered saline solution (8.5 g/l ClNa), pH 7.1 and < 0.1% preservative.
A-V Protein Solution: 5 g/dl protein solution and < 0.1% preservative.
Positive Control: inactivated serum containing antibodies against *Trypanosoma cruzi* and <0.1% preservative.
Negative Control: non-reactive serum protein dilution and < 0.1% preservative.

INSTRUCTIONS FOR USE

A-V IHA Antigen: every time it is used homogenize by shaking avoiding foam formation.
A-V IHA Serum Diluent: add 0.2 ml of A-V Protein Solution to 5 ml A-V IHA Buffer. Mix, label and cap.
Positive and Negative Controls: ready to use.

WARNINGS

- All samples should be handled as if capable of transmitting infection. Controls are inactivated. However, they should be treated as infective material.
- The control sera have been examined for Hepatitis B Surface Antigen (HBsAg), hepatitis C virus antibodies (anti- HCV) and Human Immunodeficiency Virus (HIV) and have been found non-reactive.
- All the materials used to perform the test must be destroyed to guarantee the inactivation of pathogenic agents. The recommended method is autoclaving for one hour at 121ºC. Liquid waste can be disinfected with sodium hypochlorite (5% final concentration) for at least 60 minutes.
- Do not exchange reagents from different kits and lots.
- Reagents are for "in vitro" diagnostic use.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box. Do not freeze.
Slight turbidity or opalescence in the Protein Solution does not affect the reactive capacity.
A-V IHA Serum Diluent: stable for 24 hours in refrigerator (2-10ºC) from preparation date.

INSTABILITY OR DETERIORATION OF REAGENTS

- When the Negative Control and all sera dilutions are reactive, it might be an indication of self-agglutination of the A-V IHA Antigen. Verify this by using a well of the microtitration plate only to mix the A-V IHA Antigen and A-V IHA Serum Diluent, without the Sample. If agglutination persists, the reagent is deteriorated. Discard.
- The absence of reactivity in all sera dilutions and Positive Control may indicate reagents' deterioration.

SAMPLE

Serum
a) Collection: patient should be fasting. Obtain serum as usual. Do not use plasma. Samples inactivated by heat may yield false positive results.
b) Additives: not required. Do not add preservatives.
c) Known interfering substances: hemolysis or hyperlipemia (with chylomicronemia) may cause erroneous results.
d) Stability and storage instructions: serum should be preferably fresh. If sample is not to be processed immediately, it might be stored in refrigerator (2-10ºC) up to 72-96 hours from collection. For longer storage periods, samples should be frozen (-20ºC) avoiding repetition of such procedure.

REQUIRED MATERIAL

1- Provided

- 96 wells microtitration plates with V-bottom.

2- Non-provided

- Micropipettes for measuring the stated volumes. See PROCEDURE.
- Hemolysis tubes and adequate volumetric material.

PROCEDURE

Choose an unused microtitration plate with V-bottom wells. Wipe the bottom with a wet cloth and place in a horizontal position. See PROCEDURE LIMITATIONS.

I- QUALITATIVE TEST

1- Place 400 ul IHA Serum Diluent in one hemolysis tube and add 10 serum or controls (1/40 dilution).
2- Place 50 ul of the 1/40 serum or controls dilution in a microtitration plate well and add 25 ul A-V IHA Antigen.
3- Mix by gently tapping the sides of the microtitration plate for at least 30 seconds.
4- Let stand free from vibrations for 60 minutes at room temperature.
5- Read from 60 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- QUANTITATIVE TEST

1- In a series of 6 wells place 50 ul A-V IHA Serum Diluent in wells 2, 3, 4, 5 and 6.
2- Pipette 50 ul of the 1/40 serum or controls dilution in wells 1 and 2.
3- Transfer 50 ul from well 2 to well 3 an so on, discarding 50 ul from the last well and avoiding bubble formation.
4- Homogenize the A-V IHA Antigen by inversion.
5- Add 25 ul A-V IHA Antigen to each dilution.
6- Mix by gently tapping the sides of the microtitration plate for at least 30 seconds.
7- Let stand free from vibrations for 60 minutes at room temperature.
8- Read from 60 minutes on.
The serum titer corresponds to the reactive serum highest dilution.

Corresponding Dilutions

Wells	1	2	3	4	5	6
Titers	1/40	1/80	1/160	1/320	1/640	1/1280

VALIDATION CRITERIA

The assay is considered valid if the following conditions are simultaneously met:
- The Negative Control should yield a visible button-shaped sedimentation on the bottom of the well.
- The Positive Control should yield a smooth film of cells on the bottom of the well.

Retest in case one or both conditions are not met.

INTERPRETATION OF RESULTS

Non-reactive: button-shaped sedimentation or little ring with regular borders.
Weakly Reactive: small film of cells covering the bottom of the wells with more or less defined button-shaped sedimentation at the center.
Strongly Reactive: film formation on the bottom of wells sometimes with irregular borders.

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 constitute an auxiliary information for diagnosis. Hence, reports should be considered in terms of probability. In this case, higher or lower probability of *T. cruzi* parasitosis. Individuals whose sera are reactive with titers higher or equal than 1/40 are presumed to be parasitized.
Any Reactive result should be confirmed by other technique. Mind the "Fatala 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) particle agglutination, validated by the Centro Nacional de Referencia.

PROCEDURE LIMITATIONS

See "Known interfering substances" under SAMPLE. Other causes producing erroneous results are:
- Lack of proper preparation of the microtitration plate. To eliminate electrostatic charge wipe the bottom of the microtitration plate with a wet cloth.
- Deteriorated microtitration plate due to successive use. It is not recommended to re-use wells.
- Lack of homogenization of reagents before use.
- Inadequate mixing.
- Accidental vibrations during time reaction.
- Non-fresh, repeatedly frozen and thawed sera.
- Sera inactivated by heat.
- Accidental contamination of the reagents or of the material used in the assay.
- Scratched microtitration plate due to recurrent use. It is not recommended to reuse wells.
- Not recently prepared Serum Diluent.
- Sample Dilution prepared for more than 24 hours.

Be advised that each component of **Chagatest HAI screening A-V** forms a complete kit that should be regarded as a unit. Hence, the components from different kits should not be exchanged.

PERFORMANCE

a) Sensitivity and specificity

In endemic populations, using **Chagatest HAI screening A-V**, 100% of titers higher than 1/40 and 99% lower than 1/40 were confirmed by reference methods.
In non-endemic populations, 100% of healthy individuals showed titers lower than 1/40 using **Chagatest HAI screening A-V**.

b) Population-based studies

In a study performed over 159 samples from a hospital population a 95.3% sensitivity and a 99.2% specificity were obtained. Data confirmed by indirect hemagglutination and ELISA.
In other population of 328 individuals from a blood bank of a non-endemic zone a 100% sensitivity and a 98.7% specificity were obtained comparing ELISA and indirect hemagglutination methods.

WIENER LAB PROVIDES

Kit for 480 tests containing:
2 vials x 6.3 ml A-V IHA Antigen
10 ml A-V Protein Solution
270 ml A-V IHA Buffer
0.5 ml Positive Control
0.5 ml Negative Control
5 microtitration plates
(Cat. Nr 1293204).

REFERENCES

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- Fontenla S., Moretti E. y González G. - 50°Triduo de la ABA, Huerta Grande (Córdoba), 1985.
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- Ministerio de Salud y Acción Social, Instituto Nacional de Parasitología "Doctor Mario Fatala Chabén" - Normas para el diagnóstico de la infección chagásica - Resolución ministerial 523/97, 1998.

SYMBOLS EXPLANATION

Antígeno | HAI A-V	Buffer | HAI A-V
A-V IHA Antigen	A-V IHA Buffer
Sol. | Proteica A-V	**Control | -**
A-V Protein Solution	Negative Control
Control | +	
Positive Control	

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

CE — 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

IVD — "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:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL + — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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