Chagatest

ELISA recombinante v.4.0

Enzyme-linked Immunosorbent assay (ELISA) for the detection of antibodies to *Trypanosoma cruzi*

SUMMARY

Chagas' disease is a parasitic infection caused by *Trypanosoma cruzi*. The laboratory diagnosis depends on the stage of the disease. During the acute phase, the diagnosis is done by identification of parasites in the blood or through immunological methods that detect IgM. During the chronic phase, immunological methods may be used, such as hemagglutination, immunofluorescence and enzyme immunoassay or western blot.

PRINCIPLE

Chagatest ELISA recombinante v.4.0 is an "in vitro" enzyme immunoassay for the qualitative detection of antibodies anti-*T. cruzi* in human serum or plasma samples. The sample is diluted in the well in which six recombinant antigens are immobilized (SAPA, 1, 2, 13, 30 & 36). These antigens are from specific proteins of epimastigote and trypomastigote stages of the *T. cruzi*, corresponding to highly conserved zones among different strains. If the sample contains specific antibodies, they will bind to the antigens and remain bound to the support. Unbound materials are eliminated by washing. Then, the conjugate is added (monoclonal human anti-IgG antibodies conjugated to peroxidase), which reacts specifically with immunocaptured anti-*T. cruzi* antibodies. The unbound conjugate is removed by washing. The presence of peroxidase bound to the complex is recognized by the addition of a chromogenic substrate, tetramethylbenzidine. The reactive samples develop a light blue color. The enzymatic reaction is stopped by the addition of sulfuric acid, turning the light blue into yellow color. The optical density is measured bichromatically at 450/620-650 nm or at 450 nm.

PROVIDED REAGENTS

Coated microtitration plate: microtitration plate with cutout strips and 96 wells coated with *Trypanosoma cruzi* recombinant antigens.
Sample Diluent: saline buffer with surfactant. Violet color.
Concentrated Conjugate: human anti-IgG monoclonal antibody conjugated to peroxidase (10x). Red color.
Conjugate Diluent: saline buffer with proteins.
Substrate: tetramethylbenzidine and hydrogen peroxide solution.
Stopper: 2 N sulfuric acid.
Concentrated Wash Buffer: saline buffer with surfactant (25x). Green color.
Positive Control: inactivated human serum containing antibodies to *Trypanosoma cruzi*. Orange color.
Negative Control: inactivated non-reactive human serum. Yellow color.

NON-PROVIDED REAGENTS

Distilled or deionized water

REQUIRED MATERIAL (non-provided)

- Micropipettes for measuring stated volumes
- Disposable tips
- Volumetric material to prepare stated dilutions
- 37°C incubator
- Absorbent paper
- Disposable gloves
- Timer or stopwatch
- Sodium hypochlorite
- Microtitration plate wash system (manual or automatic)
- Spectrophotometer for microtitration plate reading

WARNINGS

- The reagents are for "in vitro" diagnostic use.
- All patient samples should be handled as capable of transmitting infection.
- The control sera have been tested for Hepatitis B Surface Antigen (HBsAg) and antibodies to Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) and found to be non-reactive. However, because no test method can offer complete assurance that infectious agents are absent, they should be handled as potentially infectious material.
- All materials used to perform the test must be destroyed to ensure the inactivation of pathogenic agents. The recommended method is autoclaving for one 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 lots.
- Do not use reagents from other origin.
- Avoid touching the walls of the wells with the tips.
- Avoid using metal objects that may be in contact with the reagents.
- The microtitration plates should be incubated in incubator. Do not use water bath. Do not open the incubator during this process.
- Avoid contact of reagents with hypochlorite fumes from biohazards disposal containers or other sources, since hypochlorite affects the reaction.
- Avoid contact of the sulfuric acid (Stopper) with the skin and eyes. H315+H320: Causes skin and eye irritation. H314: Causes severe skin burns and eye damage. P262: Do not get in eyes, on skin, or on clothing. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P280: Wear protective gloves/ protective clothing/eye protection/face protection.

- Avoid the spill of liquids and the formation of sprays.
- Do not pipette by mouth. Use disposable gloves and eye protection during handling of samples and reagents.
- All reagents and samples should be discarded according to the regulations in force.

REAGENTS PREPARATION

All the material used for reagent preparation should be clean and free from detergent and hypochlorite.

Wash Buffer: constituents of the concentrated reagent may precipitate at low temperature. In such case, bring the solution to 37ºC until complete dissolution. To obtain a ready-to-use wash buffer, dilute 1 part Concentrated Wash Buffer (25x) with 24 parts distilled or deionized water. Example: 20 ml with 480 ml for one microtitration plate.

Conjugate: dilute 1 part Concentrated Conjugate (10x) with 9 parts Conjugate Diluent. Example: see table with concentrated Conjugate and Conjugate Diluent required volumes.

Nº of wells	Concentrated Conjugate	Conjugate Diluent
8	100 ul	0.9 ml
16	200 ul	1.8 ml
24	300 ul	2.7 ml
32	400 ul	3.6 ml
96	1200 ul	10.8 ml

Coated microtitration plate, Sample Diluent, Conjugate Diluent, Substrate, Stopper, Negative and Positive Controls: ready to use.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents are stable at 2-10ºC until expiration date stated on the box. Do not freeze.

Concentrated Wash Buffer and Stopper: they may be stored at room temperature (2-25ºC).

Wash Buffer: once diluted, it is stable for 3 months at room temperature (2-25ºC).

Conjugate: once diluted, it is stable for 6 hours at room temperature (2-25ºC).

Coated microtitration plate: do not open the pouch until performing the test and until it has reached room temperature. Otherwise, the well strip surface could get moistened. Unused well strips should be kept in the pouch with the desiccant, sealed and stored at 2-10ºC. Test strips stored in this manner are stable for 4 months if it does not exceed the date printed on the pouch label.

SAMPLE

Serum or plasma

a) Sample collection: obtain in the usual way.

b) Additives: not required for serum. Employ plasma collected using EDTA, heparin or sodium citrate as anticoagulants.

c) Known interfering substances: no interference has been observed with bilirubin up to 42 mg/dl, ascorbic acid up to 50 mg/dl, triglycerides up to 1,600 mg/dl, hemoglobin up to 290 mg/dl. Samples containing particles should be clarified by centrifugation.

d) Stability and storage instructions: sample should be stored at 2-10ºC. In case of do not perform the test within 72 hours, samples should be frozen at -20ºC. Samples should not be repeatedly frozen and thawed. This may lead to erroneous results. In case of using frozen samples, they should be homogenized and centrifuged before use. Heat inactivation may affect the result. Do not use samples with microbial contamination. If samples are to be transported, they should be packaged according to local regulations for biohazard material shipment.

TEST PROCEDURE

1- Bring the reagents and samples to room temperature before opening.

2- Prepare the necessary volume of diluted wash buffer.

3- Place the required wells in the strip holder for the number of determinations to be used, including 2 wells for Positive Control (PC) and 3 for Negative Control (NC).

4- Dispense the Sample Diluent, then the sample (S) and the controls according to the following scheme:

	S	PC	NC
Sample Diluent	100 ul	100 ul	100 ul
Positive Control	-	20 ul	-
Negative Control	-	-	20 ul
Sample	20 ul	-	-

Homogenize by loading and unloading the micropipette. When adding the sample, the Sample Diluent will change color, according to the following:

Sample type	Without sample	Serum or plasma	Positive Control	Negative Control
Color	Violet	Light blue	Dark orange	Green

Warning: turbid or hemolyzed samples may change the final color without affecting the results. Color change may depend on the added sample volume and its composition. A less intense color change may be due to a lower dispensed sample volume, the sample not being in the appropriate conditions, or a low protein level.

5- To avoid evaporation, cover the plate with the provided adhesive tape and incubate for 60 ± 2 minutes at 37 ± 1ºC. At the same time, prepare the diluted conjugate (see table in REAGENTS PREPARATION).

6- The liquid from each well must be thoroughly removed after incubation. Wash 5 times according to washing instructions (see WASHING PROCEDURE).

7- Add the Conjugate:

Diluted Conjugate	100 ul	100 ul	100 ul

To avoid evaporation cover the microplate with adhesive tape.

8- Incubate for 30 ± 2 minutes at 37 ± 1ºC.

9- Wash 5 times according to the washing instructions.
10- Dispense the Substrate. Transfer to a clean recipient only the required Substrate volume. Do not transfer the remaining Substrate back to the original bottle. Avoid reagent contact with oxidizing agents.

Substrate	100 ul	100 ul	100 ul

11- Incubate for 30 ± 2 minutes at room temperature (18-25ºC), protecting from light
12- Add the Stopper:

Stopper	100 ul	100 ul	100 ul

13- Read absorbance in spectrophotometer bichromatically at 450/620-650 nm or at 450 nm.
Note: bichromatic reading is recommended. In case the reading is monochromatic, perform a reagent blank that will have to be subtracted from all sample values.

STABILITY OF THE FINAL REACTION
Reaction color is stable for 10 minutes. Thus, results should be read within this period.

WASHING PROCEDURE
Remove the liquid from the wells by aspiration or inversion. The wells are washed with 300 ul diluted wash buffer. When filling the wells make sure not to spill. The wash solution should be in contact with the wells for 30 to 60 seconds. Make sure no residual liquid remains after the final washing step. Perform double aspiration to remove excess buffer. If after such procedure, it still persists, invert the plate onto absorbent paper and tap it several times. Otherwise, erroneous results may be obtained.
Note: the washing procedure is crucial for the test result. If excess wash buffer remains in the wells or if the wells are not completely filled, erroneous results may be obtained. Do not let the wells air dry during the procedure. Automatic washers should be rinsed with distilled or deionized water at the end of the day to avoid obstructions due to the presence of salt in the wash buffer.

SUMMARY OF THE PROCEDURE

STAGE	PROCEDURE	WARNINGS/OBSERVATIONS
Dilution	Prepare Wash solution	Dissolve salt crystals
Sample Diluent	Add 100 ul Sample Diluent in each well	
Samples	Add 20 ul S, PC and NC	Color change is observed when adding the sample and controls
Incubation	Cover the wells and incubate for 60 ± 2 minutes at 37 ± 1ºC	In incubator
Washing step	Wash each well with 300 ul diluted Wash Buffer (5 times)	Time of contact of the Wash solution from 30 to 60 seconds. Completely remove the residual liquid from the wells
Dilution	Conjugate	During incubation with the sample, dilute the Concentrated Conjugate (10x)
Conjugate	Add 100 ul diluted Conjugate	
Incubation	Cover the wells and incubate for 30 ± 2 minutes at 37 ± 1ºC	In incubator
Washing step	The same as above	
Substrate	Add 100 ul Substrate	Transfer the required Substrate volume to be used. Do not pipette from the original bottle. Discard the remaining reagent. Avoid contact with oxidizing agents.
Incubation	30 ± 2 minutos at 18-25ºC	Mantein the wells protected from light
Stop	Add 100 ul Stopper	
Reading	Read in spectrophotometer	Read within 10 minutes

ASSAY VALIDATION CRITERIA

The assay is considered valid if the following conditions are simultaneously met:

1- The mean optical density (O.D.) of the Negative Controls should be less than or equal to 0.100.
Example:
Reading 1 = 0.034; Reading 2 = 0.028; Reading 3 = 0.029
Mean = (0.034 + 0.028 + 0.029) / 3 = 0.030

2- Remove any Negative Control with O.D. greater than 0.100.

3- If any Negative Control has been removed, recalculate the Negative Control mean. An assay is valid when at least two of the Negative Controls are accepted.

4- The O.D. mean of the Positive Controls should be greater or equal to 1.300.
Example:
Reading 1 = 1.697; Reading 2 = 1.774
Mean = (1.697+1.774) / 2 = 1.736

5- The O.D. mean difference of the Positive and Negative Controls should be greater or equal to 1.200.

In case one of the above conditions is not met, repeat the assay.
Remember that the obtained readings will depend on the sensitivity of the instrument used.

INTERPRETATION OF RESULTS

a) With optical instruments

The presence or absence of antibodies anti-*T. cruzi* is determined associating the sample absorbance with the Cut-off value.

Cut-off = NC + 0.200

NC: O.D mean of the Negative Control

Example: 0.030 + 0.200 = 0.230

Non-reactive samples: samples with absorbances lower than the Cut-off value.
Reactive samples: samples with absorbances greater or equal to the Cut-off value.

b) Visual interpretation

If this type of interpretation is selected, every sample not presenting more color than the Negative Controls should be considered non-reactive. Otherwise, an evidently yellow sample is considered Reactive.

All samples initially reactive should be repeated by duplicate. If one or both repetitions are reactive, it should be considered reactive.
A sample initially reactive may be non-reactive in both repetitions. This may be due to:
- Cross contamination of a non-reactive well with a reactive sample.
- Sample contamination during dispensation, lack of precision in sample, conjugate and/or Substrate dispensation into the well.
- Tip re-utilization.
- Well contamination with hypochlorite or other oxidizing agents.

In certain cases a non-reactive sample may produce a falsely reactive reaction, both in the initial analysis as in its repetitions. Some probable causes of this effect may be:
- Sample contamination during collection, processing or storage.
- Presence of interfering substances, such as autoantibodies, drugs, etc.
- Ineffective dispensation and/or aspiration of the wash solution (obstructed system).

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
Do not use pooled samples.
Do not use other body fluids such as saliva, cerebrospinal fluid or urine.

SPECIFIC PERFORMANCE FEATURES

a) Sensitivity

- Clinical Sensitivity in Performance Panels: in a study performed on different international commercial panels, the following results were obtained:
PMT 201 (Anti-*T. cruzi* Performance Panel, BBI, USA): 14 out of 14 reactive samples were detected.
PP 0404 (Performance Panel for Chagas, Q Panel, Brazil): 16 out of 16 reactive samples were detected.
PP 0405 (Performance Panel anti-*T. cruzi*, Q Panel, Brazil): 16 out of 16 reactive samples were detected.

- Clinical Sensitivity in Panels of reactive anti-*T. cruzi* samples: in a study performed on 100 samples from children from endemic regions with *T. cruzi* infection, confirmed by different methods, all of the samples were found reactive with the Chagatest ELISA recombinante v.4.0 kit.
In a study of 116 reactive samples from a hospital institution, 115 samples were detected.

b) Specificity

In a study performed on 1192 sera and plasma samples from blood banks, the obtained specificity was 99.66%.
In a further study on 477 sera and plasma samples from two different health institutions, a specificity of 99.57% was obtained.
Over a panel of 474 plasmas from a high prevalence population, the obtained specificity was 98.30%.
A possible cross-reactivity was evaluated, assaying samples from 491 individuals with different clinical conditions that may be the cause of unspecific reactions for the Chagatest ELISA recombinante v.4.0 test. These conditions include pregnant women, hemodialyzed patients, patients with autoimmune diseases or infectious diseases other than Chagas (HIV, HTLV, Hepatitis C, Hepatitis B, Syphilis, others). For this population the specificity was 98.37%.

c) Precision

The test precision was evaluated following EP5-A protocol recommended by the NCCLS. The assays were performed

with samples having different reactivity levels and with controls. Two daily assays were performed testing each sample by duplicate during 20 days.

	Mean (O.D.)	Intra-assay		Total	
		S.D.	C.V.	S.D.	C.V.
Sample 1	0.360	0.029	8.07%	0.043	11.90%
Sample 2	0.550	0.036	6.55%	0.055	10.01%
Sample 3	0.870	0.063	7.23%	0.093	10.69%
(+)Control	1.750	0.085	4.83%	0.138	7.89%
(-)Control	0.028	0.003	10.35%	0.004	14.24%

It is recommended that any Reactive result be investigated by further diagnostic testing. The “Fatala Chabén” Institute recommends criteria by which the immunodiagnostic of the infection should be performed with a minimum of 2 of the following methods, validated by the Reference National Center: indirect immunofluorescence, indirect hemagglutination and EIA.

WIENER LAB. PROVIDES

- 96 tests (Cat. Nº 1293257).
- 192 tests (Cat. Nº 1293258).

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SYMBOLS EXPLANATION

Policubeta | Sensib.
Coated microtitration plate

Diluyente | Muestra
Sample Diluent

Conjugado | Conc.
Concentrated Conjugate

Conjugado | Diluy.
Conjugate Diluent

Revelador
Substrate

Buf. Lavado | Conc.
Concentrated Wash Buffer

Control | +
Positive Control

Control | -
Negative Control

Stopper
Stopper

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