Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Chagas ELISA Kit

Cat # 8100-35

IVD See external Label 2°C-8°C Σ 96 Tests

Test	Chagas ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Qualitative : Positive, Negative
Sample	5 µL serum
Total Time	~ 20 min.
Shelf Life	12 Months from the manufacturing date
Specificity	97%
Sensitivity	96%

INTENDED USE

The **Chagas ELISA Test** is an enzyme linked immunosorbent assay (ELISA) for qualitative identification of human serum antibodies to *Trypanosoma cruzi* antigen. The Chagas ELISA Test is a two-step incubation assay that predominantly detects IgG antibodies to *T. cruzi* antigens.

SUMMARY AND EXPLANATION

Of the various testing methods, the Chagas IgG ELISA Test is a diagnostic method to detect antibodies to T. cruzi antigens. The causative agents vary from a bite transmitted through a specific bug called reduviid bug, or the disease is transmitted through blood transfusion, or from mother to baby as in utero infection. Acute stage is marked by mild infection, or being symptom free. However, the chronic stage, if left untreated, can range from cardiac problems (i.e. cardiomyopathy) to an enlarged esophagus, or an enlarged colon.

TEST PRINCIPLE

The principle of the Chagas IgG ELISA test involves a two-step incubation procedure. The antibodies in the patient's serum bind to the antigens in the test well in the course of the first incubation stage. After this, the enzyme complex binds to the antigen-antibody complex, which occurs in the second incubation step. The unbound enzymes are then removed through repeated washings. In the presence of this enzyme complex and peroxide, a substrate is added, and as a result of this combination, the substrate color will turn blue. The reaction is ended with the stop solution, and what appears is a yellow color instead of the blue.

SPECIMEN COLLECTION AND PREPARATION

Coagulate blood and remove serum. Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8 °C if it is to be analyzed within a few days. Serum may be held for 3 to 6 months by storage at -20 °C or lower. Lipemic and strongly hemolytic serum should be avoided. Do not heat inactivate serum. Avoid repeated freezing and thawing of samples.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Plate:** Microwells containing *Trypanosoma cruzi* antigens – 96 test wells in a test strip holder.
2. **Enzyme Conjugate**: One (1) bottle containing 11 ml of anti-human Ig Peroxidase (HRP) in a stabilizing buffer with Thimerosal.
3. **Positive Control**: One (1) vial containing 1 ml of diluted Trypanosoma cruzi-positive sera in buffer with Thimerosal.
4. **Negative Control:** One (1) vial containing 1 ml of diluted Trypanosoma cruzi-negative sera in buffer with Thimerosal.
5. **TMB Substrate Solution:** One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
6. **Wash Concentrate 20X**: One (1) bottle containing 25 ml of concentrated buffer and surfactant with Thimerosal.
7. **Dilution Buffer:** Two (2) bottles containing 30 ml of buffered protein solution with Thimerosal.
8. **Stop Solution**: One (1) bottle containing 11 ml of 0.73 M phosphoric acid.

Materials required but not provided

1. Pipettes
2. Squeeze bottle for washing strips
3. DI water
4. ELISA Plate reader with a 450/620-650 nm filter (optionally, results can be read visually)
5. Tubes for serum dilutions

Preparation

Wash Buffer - Remove cap and add contents of bottle to 475 ml DI water. Place diluted wash buffer into a squeeze bottle.
Note: Washings consist of filling to the top of each well, shaking out the contents and refilling.
Avoid generating bubbles in the wells during the washing steps.

Test samples: Make a 1:64 dilution of patients' sera using the dilution buffer.

ASSAY PROCEDURE

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µl of negative control to well #1, 100 µl of positive control to well #2, and 100 µl of the diluted (1:64) test samples to the remaining wells.
   Note: Negative and positive controls are supplied as prediluted. Do not dilute.
3. Incubate at room temperature (15 °C to 25 °C) for 10 minutes.
4. Shake out contents and wash 3 times with diluted wash buffer.*
5. Add 100 ul of enzyme conjugate to each well.
6. Incubate at room temperature for 5 minutes.
7. Shake out contents and wash 3 times with wash buffer.
8. Add 100 ul of Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 100 ul of stop solution.
11. Zero ELISA reader on air, read wells at 450 nm with a reference filter at 620-650 nm or read results visually.

* Washings consist of using the diluted wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 times.

Avoid generating bubbles in the wells during the washing steps.
Controls must be included each time the kit is run.

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RESULTS

Troubleshooting

Problem: Negative control has substantial color development.
***Correction*:** Inadequate washings. Rerun test with more vigorous washings.

Interpretation of the Test

Spectrophotometer:
Zero ELISA reader on air. Read all wells using a bichromatic reading with filters at 450 nm and 620-650 nm.

Positive - Absorbance reading greater or equal to 0.2 OD units.
Negative - Absorbance reading less than 0.2 OD units.

Visual
A sample should be interpreted as positive if the degree of color development is obvious and significant**.**

QUALITY CONTROL

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.3 OD units and the negative control must be under 0.2 units. Should the values fall outside these ranges, the kit should not be used.

PERFORMANCE CHARACTERISTICS

Precision
Intra-run reproducibility for this assay was determined by testing two positive sera 16 times each in a single run. Inter-run reproducibility was checked in a separate study by testing two positive sera 5 times each day for 3 days (15 tests for each serum). The intra-run coefficients of variation (CV, based on O.D. readings) for the two sera were 3.5% and 2.7%; the inter-run CVs were 6.0% and 5.4%. (These values include the well-to-well variation inherent in the plastic strips, which ranges up to 5%, according to the plastics manufacturer.)

Accuracy
The Diagnostic Automation, Inc. Chagas Kit has been compared to a commercially available Chagas' kit which also uses the ELISA method. In this study, 391 serum samples were evaluated with both kits. These samples included 165 patients with immunofluorescence titers >1:80 and clinical signs of Chagas' disease, as well as 226 normal blood donors. The results are summarized in the table below:

	Comparison Method		
	Positive	**Negative**	**Equivocal**
Positive	154	6	5[a]
DAI			
Negative	6	223	0

Product Comparison Table. Relative sensitivity, compared to the commercially available kit, is 154/160 = 96.3%. The 95% confidence interval is 93.4% to 99.2%. Relative specificity is 223/229 = 97.3%. The 95% confidence interval is 95.2% to 99.3%.

These specimens were obtained from a serum library in which samples had an immunofluorescence titer ≥ 1:80.

Sensitivity: 154/160 = 96.3% Specificity: 223/229 = 97.3%

Cross reactivity
In order to determine cross-reactivity between the antigen preparation used on the Diagnostic Automation Inc. plates and antibodies found in patients with other infections, twenty-eight specimens which were seropositive for leishmaniasis, toxoplasmosis, cysticercosis, toxocariasis,or amebiasis were tested on the Diagnostic Automation, Inc. All twenty-eight specimens were negative in this assay.

LIMITATIONS OF PROCEDURE

Serological results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

PRECAUTIONS

1. Do not use solutions if they precipitate or become cloudy. Dilution buffer is a colloidal solution and will appear opaque. In addition, a gelatinous precipitate may form at the bottom of the bottle. Do not attempt to resuspend this precipitate.
2. Wash concentrate may show crystallization upon storage at 4 °C. Crystallization will disappear after diluting to working strength.
3. Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content. Samples high in lipids should be clarified before use.
4. Do not add azides to the samples or any of the reagents.
5. Controls and some reagents contain Thimerosal as a preservative.
6. Treat all sera as if capable of being infectious.
7. The negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. Since no test can offer complete assurance that infectious agents are not present, this product should be used under appropriate safety conditions that would be used for any potentially infectious agent.

STORAGE

Reagents, strips and bottled components:
Store between 2 – 8 °C.
Squeeze bottle containing diluted wash buffer may be stored at room temperature.

REFERENCES

1. Brener, Z. "Biology of *Trypanosoma cruzi*." Annu Rev Microbio. 1993; 27. pp 347-82.
2. Kirchhoff, L.V. "*Trypanosoma* Species (American Trypanosoniais, Chagas Disease): Biology of Trypanosomes. Principles and Practice of Infectious Diseases. 1990. pp. 2077-84.
3. Peralta, J.M. et al. "Serodiagnosis of Chagas' Disease by Enzyme-Linked Immunosorbent Assay Using Two Synthetic Peptides as Antigens." ASM 1994, 32. pp 971-4.

ISO 13485
ISO 9001

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EC REP	**CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands.** www.cepartner4u.eu

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