Chagas IgG
ELISA TEST SYSTEM

REACTIVA SEARCH

INTENDED USE
The Reactiva Search.'s Chagas IGg ELISA Test System is a semiquantitative Enzyme immunoassay for the detection of antibodies to Asca ris, in samples of human serum or plasma. This test is intended to be perfomed by trained medical technologists only.

SUMMARY AND EXPLANATION
Trypanosoma cruzi is a protozoan parasite, which is the causative agent of Chagas' disease. This disease ranges from southern United States to Northern Argentina and Chile.
The disease is transmitted to humans through the bite wound caused by reduviid bugs, blood transfusions, and in newborns, infection in utero.
In acute infections, there may be few or no symptoms of the disease. In chronic infections, there may be inflammatory cardiomyopathy, or severe dilation of the esophagus or colon known as megadisease. A variety of diagnostic methods have been used, but detection of antibodiesto T. cruzi antigens remains the strongest method to diagnose infection.

PRINCIPLE OF THE TEST
The micro wells are coated with Chagas recombinant antigen. During the first incubation with the diluted patients' sera, any antibodies which are reactive with the antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen (tetramethylbenzidine or TMB) is added. If the Enzyme Conjugate is presen t, th e p e ro xid a se will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color. The reaction may then be read visually or with an ELISA reader.

MATERIALS AND COMPONENTS PROVIDED
Microwells:
Containing Chagas antigen - 96 test wells in a test strip holder.
Enzyme Conjugate:
One (1) bottle containing 11 ml of Protein-A conjugated to peroxidase.
Positive Control:
One (1) vial containing 2 ml of diluted positive rabbit sera.
Negative Control:
One (1) vial containing 2 ml of diluted human sera .
Chromogen:
One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
Wash Concentrate (20X):
Two (2) bottles containing 25 ml of concentrated buffer and surfactant.
Dilution Buffer:
Two (2) bottles containing 30 ml of buffered protein solution.
Stop Solution:
One (1) bottle containing 11 ml of 1 Mphosphoric acid.

MATERIALS REQUIRED BUT NOT PROVIDED
- Micropipette
- Reagent grade (DI) water
- Graduated Cylinder
- Sample Dilution Tubes
- Timer

SUGGESTED MATERIALS
ELISA plate reader with a 450 nm and a 620 - 650 nm filter

PROPER TEMPERATURE
All incubations are at room temperature (15-25 °C)

STORAGE CONDITIONS
- Reagents, strips, and bottled components should be stored at 2-8 °C
- Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25 °C)

WARNINGS
- Do not deviate from the specified procedures when performing this assay. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect te sensitivity and specificity of the assay.
- For In Vitro Diagnostic Use Only.
- Do not interchange reagents between kits with different lot numbers.
- Do not use reag ents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents be yond the ir expiration dates may affect results.
- Unused microwells should be stored in the desiccated pouch to protect them from moisture .
- Do not use solutions if the y precipitate or be come cloudy. Exception : Wash concentrate may precip itate during refrigerated storage but will dissolve up on warming .
- Do not add azid es to the samp les or any of the reagents.
- Controls and some reagents contain Thime rosal as a preservative , which may be irrita ting to skin , eyes, and mucous membranes. In case of contact, flush eyes, or rinse skin with copious amounts of water.
- Do not use serum that may have supported micro b ia l growth or is cloudy due to high lipid content. Samples high in lip id s should be clarified before use.
- Tre at all reagents and samples as potentially in fectious materials. Negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV be required test methods. Use care to prevent aerosols and decontaminate any spills of samples.
- Stop solution is a 5% solution of phosphoric acid in wa ter. If spilled on the skin, wash with copious amounts of water. If acid gets in to the eyes, wash with copious amounts of water and seek medical attention.

SPECIMEN COLLECTION
Serum or plasma may be stored at 2-8 ºC for up to five days. Sample may be frozen below -20 ºC for extended periods. Freezing whole blood samples is not advised . Do not heat inactivate samples and avoid repeated freezing and thawing of samples.

REAGENT PREPARATION
- Before use, bring all reagents and samples to room temperature (1525 °C) and mix.
- (20X) Wash Concentrate may precipitate during refrigerated storage , but will go back into solution when brought to room temperature and mixed. Ensure that (20X) Wash Concentrate is completely in solution before diluting to wo rking concentration. To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one b ottle of Wash Concentrate to asqueeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

TEST PROCEDURE
Notes:
- Ensure all samples and reagents are at room temperature (1525°C)
- Negative and positive controls are supplied pre-diluted. DO NOT dilute further.

1. Break off number of wells needed (three fo r co ntrols plus number of samples) and place in strip holder.
2. Dilute patient sera 1:100 using the Dilution Buffer (e .g ., 2 µl sera and 200 µl dilution buffer).
3. Add 100 µl o f th e ne g a tive control to well #1 and well #2 , 100 µl of the positive control to well #3 and 100 µl of the diluted test samples to the remaining wells.
4. Incubate at room tempe rature for 30 minutes, then wash. *
5. Add 100 µl of Enzyme Conjugate to each well.
6. Incubate at room temperature for 10 minutes, then wash. *
7. Add 100 µl of the Chromogen to each well.
8. Incubate at room temperature for 10 minutes.
9. Ad d 100 µl o f th e Sto p Solutio n to each we ll. Mix contents by gently tapping the side of the strip holder.
10. Read within one hour of adding Stop Solution.

* Washings consist of 5 washings o f 300 µl per well for each step with a 30 second dwell time for each wash set. If possible , slap out excessive wash buffer from the wells against absorbent to weling before addition of the next reagent.
Proper and thorough washing is ke y to obtaining accurate and reproducible results.

READING RESULTS
ELISA Reader : Zero reader on air. Se t for bichromatic readings at 450/620-650 nm.

QUALITY CONTROL
The use of controls allows validation of kit stability. The kit should not be used if any of the controls are out of range. Expected values for the contro ls are :
Negative - 0.0 to 0.2 OD units
Po sitive - 0.5 OD units and above

CALCULATION OF RESULTS
1 – Calculate the average extinction value by taking the average OD value of the Negative Control.
2 – Add 0.200 to this average extinction value . This value is the cut-o ff value used in the Sample Index Calculation .
Example :
Negative Control 1 OD = 0.084
Negative Control 2 OD = 0.100
Average is 0.084 + 0.100 = 0.184 / 2 = 0.092 =
Average Extinction Value
Cut-o ff value is the Average Extinction Value + 0.20 0 (in th is example 0.092 + 0.200 = 0.292

PRESENTACIÓN:

CONT. CODIGO: RSET058-2

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3 – Determine the Sample Index by diving the patients OD value by the Cut-o ff value.
Exam p le :
Patient OD value of 0.982
Cut-off value of 0.292
0.982 / 0.292 = 3.36
4 – Evaluate the Sample Index.
Negative = less than 0.9 Samp le Index
Equivocal = 0.9 to 1.1
Positive = greater than 1.1

TEST LIMITATIONS

Diagnosis of Chagas infection should not be made solely based on results of the ELISA Chagas test alone, but in conjunction with other clinical signs and symptoms and other laboratory findings.
Epidemiologic factors, clinical findings, exposure to endemic regions, and other laboratory results should be considered when making a diagnosis.

PERFORMANCE CHARACTERISTICS

Study #1 – Versus Canadian Reference Center (NRC P) Samples
Negative Samples: 97/100 (97% Specificity)
Positive Samples: 24/25 (96% Sensitivity)

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.