Chagas ELISA IgM ELISA

Tcruzi-M-96

FOR EXPORT ONLY

Intended Use

For the qualitative determination of serum or plasma antibodies in humans, primarily IgM, to *Trypanosoma cruzi* using the ELISA technique.

Summary

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.[1]

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.[2]

A variety of diagnostic methods have been used, but detection of antibodies to *T. cruzi* antigens remains the strongest method to diagnose infection.[3]

Principle of Procedure

During the first incubation, the antibodies in the patients' serum or plasma bind to the antigens in the test well. The next incubation allows the enzyme complex to bind to the antigen-antibody complex. After a few washings to remove unbound enzymes, a substrate is added that develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction, turning the blue assay color to yellow.

Reagents

Item	Description	Symbol
Test Strips	Microwells containing *Trypanosoma cruzi* antigens - 96 test wells in a test strip holder.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of anti-human IgM (mu chain) Peroxidase (HRP) in a stabilizing buffer with Thimerosal.	CONJ
Positive Control	One (1) vial containing 2 ml of a surrogate positive control.	CONTROL +
Negative Control	One (1) vial containing 2 ml of diluted negative human sera in buffer with Thimerosal.	CONTROL −
Chromogen	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).	SUBS TMB
Wash Concentrate (20X)	Two (2) bottles containing 25 ml of concentrated buffer and surfactant with Thimerosal.	WASH BUF
Dilution Buffer	Two (2) bottles containing 30 ml of buffered protein solution with Thimerosal.	SPECM DIL
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.	SOLN

Precautions

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.
Wash concentrate may show crystallization upon storage at 4 °C. Crystallization will disappear after diluting to working strength.
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.
Do not add azides to the samples or any of the reagents.
Controls and some reagents contain Thimerosal as a preservative.
Treat all sera as if capable of being infectious.
The controls have 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 Conditions

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

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 1:100 dilution of patients' sera using the dilution buffer.
(e.g. 5 ul serum plus 500 ul of dilution buffer).

Collection And Preparation Of Serum

Serum or plasma (collected with heparin, EDTA or citrate) should be stored at 2-8° C if it is to be analyzed within 5 days. Samples may be held for extended storage at -20° C or lower for 1 year.

Do not heat inactivate serum.
Avoid repeated freezing and thawing of samples.

Materials

Materials Provided
Trypanosoma cruzi Serology Microwell ELISA Kit

Materials Required But Not Provided
MicroPipettes
Squeeze bottle for washing strips
DI water
ELISA plate reader with a 450/620-650 nm filter (optionally, results can be read visually)
Tubes for serum dilutions
Timer

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 30 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 10 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 10 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.

If using automated washers; add 1 minute dwell time between washings and increase number of washes from three to five.

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

Test Limitations

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

Reading of Results

Visually: Look at each well against a white background (e.g. paper towel) and record as clear or +, ++ or +++ reaction.
ELISA Reader: Zero reader on air. Set for bichromatic readings at 450/650-620 nm.

Interpretation of Results

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 0.50 OD units and the negative control must be under 0.10 units. Should the values fall outside these ranges, the kit should not be used.

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.