

UBI MAGIWEL™
Enzyme Immunoassay
Trypanosoma cruzi(Chagas)
Total IgG & IgM
Catalog No. PI-503

For Research Use Only

NAME AND INTENDED USE

The UBI MAGIWEL™ Trypanosoma cruzi (PI-503) qualitative is a solid phase enzyme-linked immunosorbent assay (ELISA). This test provides an easy method for detecting Chagas' antibodies (IgG & IgM) in human serum or plasma. *(For Professional Use Only)*

SUMMARY AND EXPLANATION OF TEST

Three types of Trypanosomiasis occur in humans. 1.) African trypanosomiasis caused by T. gambiense and T. rhodesiense 2.) Chagas' disease caused by T. cruzi, Central South America 3.) Benign American trypanosomiasis caused by T. rangelli. In all three types of trypanosomiasis, the flagellar trypanosome stage can be observed in the peripheral blood. The morphology of the blood stream stage is diagnostic for each of three types.

T. cruzi is the etiological agents of Chagas' disease. In 1909, Chagas discovered intermediate stage flagellates in the hindgut of the bug Triatoma megista in Brazil. The parasites as seen in the blood of early cases of Chagas' disease are of trypaniform type.

Chagas described the infection from a region having serious endemic goiter, and most of cases had marked thyroid pathology. The incubation period is one to two weeks.

Irregular fever and edema particularly of the eyelids, characterize the acute phase, and there is considerable enlargement of lymph nodes, spleen, and liver toward the end of the period.

The acute infection is rare except in small children, in whom occur almost the only deaths attributable to the acute infection. The chronic disease, which occurs in adults or following the acute stage in children, varies in

symptomatology with the localization of the parasites but in most often characterized by myocarditis. The transfusion of parasite containing blood is currently an important way of transmission of disease^{1,2,3}

Acquired immunity is lacking in the human disease, but experimental animal are immune to re-infection after recovery. Complement fixation test is widely used for laboratory diagnosis. Synthetic antigens and multiple epitope have been used recently for ELISA^{4,5,6}. Fluorescent antibody methods have also been shown to be of practical diagnostic value.

PRINCIPLE OF THE ASSAY

UBI MAGIWEL™ Trypanosomiasis (chagas) IgG & IgM is a solid phase enzyme-linked immunosorbent system employing plastic wells coated with recombinant T. cruzi antigens. Incubation of diluted serum samples in the coated wells results in the binding of anti- T. cruzi antibodies to the immobilized antigens. Subsequent addition of the enzyme conjugate (peroxidase) is in direct proportion to the amount of T. Cruzi antibody present in the serum sample. Unbound enzyme conjugate is washed from the wells and a substrate and chromogen solution is added. The intensity of the color formed as a result of enzyme activity is a direct measure of the anti-T. cruzi antibody present in the serum samples and may be quantified by use of a photometric well reader at 450 nm wavelength.

WARNING AND PRECAUTION

1. UBI MAGIWEL™ Trypanosomiasis (Chagas) IgG & IgM qualitative is designed for in vitro use only.
2. The components in this kit are intended for use as an integral unit. The components from different lots should not be mixed and used.
3. References contains human serum should be treated as potentially infectious. The references were found negative for Hepatitis B, C and HIV antibodies. However, because no test methods offer complete

assurance of the absence of the HIV I/II, Hepatitis B, Hepatitis C virus or other infectious agents, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Center for Disease Control/National Institutes of Health Manual" Biosafety in Microbiological and Biomedical Laboratories", 1984. Never pipet by mouth. Avoid contact with skin.

MATERIALS PROVIDED

1. Microwell Strips (96 wells): Highly purified recombinant T. cruzi Antigens coated wells. 8 x 12 strips.
2. Washing Buffer Concentrate (20X) (50 mL): Prepare working solution by adding purified water to 1 liter.
3. Enzyme Conjugate (11 mL): Human IgG & IgM antibodies conjugated to horseradish peroxidase.
4. Reference Control Set (1.0 mL each vial): Negative, Positive Control. Ready to Use, *DO NOT DILUTE*
5. Specimen diluent (11 mL): Buffered and stabilized protein solution.
6. Substrate Solution (11 mL): Buffer solution containing peroxide.
7. Chromogen Solution (11 mL): Tetramethylbenzidine solution.
8. Stop Solution: 2 N HCl.
9. Well holder with wells.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-well reader with wavelength at 450 nm.
2. Pipetor with tips for measuring 50 and 100uL.
3. Clean plastic washing bottle of 1000mL capacity for use in washing micro-wells with working washing buffer during testing procedure.

REAGENT PREPARATION

Prepare the working washing buffer by adding the entire contents of the Wash Buffer

Concentrate to 1000 mL distilled water in a clean plastic wash bottle. Mix gently to dissolve. Store at room temperature.

STORAGE AND STABILITY

1. Store the kits at 2-8°C and keep micro-wells in a dry bag with desiccants.
2. Unopened reagents are stable until expiration of the kit. Solution A and Solution B should be colorless; if the solution turns blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.

PRECAUTIONS

This kit is designed for in vitro diagnostic use only. The components of this kit are carefully matched and intended for use as an integral unit. Components of different lots should not be used interchangeably. Although all human materials used in the manufacture of this kit have been found negative for Hepatitis B antigen and for antibodies to HIV and HCV by required test methods, no test can offer complete assurance that infectious agents are not present, and therefore all calibrators, controls and samples should be handled as potentially infectious agents.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture and allow clotting. Separate the serum by centrifugation at room temperature. Do not heat and inactivate serum. If sera cannot be immediately assayed, they may be stored at – 20° C for at least six months. Avoid repeated freezing and thawing of samples. Specimens obviously contaminated with bacteria should not be use. Specimens turbid with high lipid concentrations should be clarified prior to assay.

PREPARATION FOR ASSAY

1. Bring all reagents and samples to room temperature (20-25°C) and mix gently before beginning the test.

- Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruptions to get the most reliable and consistent results.
- Use new disposable tips for each specimen.

ASSAY PROCEDURE (30/30/15)

- Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.
- Well #1 is the blank well. Dispense 100 uL of Negative Control to Wells #2 and #3. Add 100uL of Positive control to Wells # 4 and # 5. (*DO NOT DILUTE NEGATIVE AND POSITIVE CONTROL*). Add 100uL of sample diluent to the remaining wells (Example: Wells #6, #7 for Patient 1, #8, #9 for Patient 2, etc.). Add 25uL of Patient sample 1 to Wells #6, #7. Add 25ul of Patient Sample 2 to Wells #8, #9, etc.
- Incubate 30 minutes at room temperature
- Wash five times with the washing buffer.
- Dispense 100uL Enzyme Conjugate into each well except blank well.
- Incubate for 30 minutes at room temperature.
- Wash Five times with the Washing buffer.
- Dispense 100uL of Solution A and 100uL of Solution B.
- Incubate for 15 minutes at room temperature.
- Stop reaction by adding 50uL of Stop Solution in each well.
- Zero a microreader on the blank and measures absorbance of each well at 450 nm.

** Dilution of Patients samples 1:5 out side of glass tube, then transfer 100uL of diluted samples into each appropriate wells will produce better O.D. readings.

INTERPRETATION OF RESULTS

- Negative control: O.D should be less than 0.2. Calculate the mean absorbance of the replicates of the Negative Control.
Absorbance Well #2 = 0.10
Well #3 = 0.13
Mean Negative Control= 0.12
- Cut-Off Value:** Calculated by adding the mean of the Negative Control to the Factor of 0.2. For example: Cut-Off Value = 0.2 +0.12= 0.32
- Negative:** Samples that developed no color or less intensity than the Cut –Off Value are considered Negative
- Positive:** Samples that developed the color equal to or stronger than the Cut-Off Value are considered positive.

Specimens yielding absorbance reading within 10% of Cut-Off Value (gray zone) a new sample after one week should be retested with the old sample. If O. D. is less than Cut Off Value, the sample is considered Negative antibodies against T. cruzi parasites.

VALIDATION OF TEST

- Negative Control: mean absorbance value should be <0.2 units.
- Positive Control mean absorbance value should be greater than O.D. 0.5
- A test may be validated if the above criteria are met.

LIMITATIONS OF THE PROCEDURE

The results obtained by means of this kit should be used as an aid for diagnosis and should not be interrupted as diagnostic by itself. Should negative results be obtained and other clinical findings suggest infection by T. cruzi parasites, a second serum should be obtained one week after the first and testing repeated. Initial testing may have occurred prior to significant antibody production in response to infection. False-positive results may occur after other Leishmania antibody.

QUALITY CONTROL

Each laboratory should utilize internal controls several levels to monitor assay performance. The controls should be treated as unknown. Results obtained should be in agreement with the assigned values of the control.

PERFORMANCE CHARACTERISTICS

Assay reproducibility was determined by assaying three specimens (S1, S2 and S5) in duplicate in 5 separate assays. The **Intra-assay** coefficient of variations (CV's) were 3.46%, 2.92% and 9.25% for the S1, S2 and S5 respectively. The **Inter assay** CV's were 4.59%, 7.11% and 7.47% for the S1, S2 and S5 respectively.

The UBI MAGIWEL™ Trypanosoma ELISA is tested for the established reactivity.

Sensitivity of UBI MAGIWEL™ is 100%: For assay of non-chagasic 307 samples, the reactivities of 307 negative samples out of 307 were correctly identified, 29 of chagasic positive samples out of 34 were identified as positive.

Specificity of UBI MAGIWEL™ is 100%. No cross reactivities found with Leishmania patients in nine out of nine.

SPECIFICITY

UBI MAGIWEL™ Trypanomomiasis (Chagas) (PI-503) IgG & IgM is specific. No cross reactivities found with the following tests: Toxoplasma IgG, IgM; CMV IgG,IgM; Rubella IgG, IgM, HSV I,II IgG, IgM, HCV, HBsAg, HIV, Cysticercosis, Chlamydia Trachomatis, Rubeola RF factor, ANA, ENA, H.pyloi Mono, DNA etc. except it might cross react with Leishmania antibody and other strains of trypanomomiasis.

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