INTENDED USE
Trypanosoma cruzi IgG ELISA Kit is an Enzyme-Immunoassay (EIA) for the detection of IgG antibodies against Trypanosoma cruzi in human serum. For professional use only.

GENERAL DESCRIPTION
Trypanosoma cruzi is a parasitic organism which, via the blood and lymphatic system, infects the tissue cells of the heart muscle, nerves, skeletal muscle and smooth muscle of the gastrointestinal tract. The resulting condition is known as Chagas Disease. Early detection of T. cruzi, before nerve and muscle fibre damage occurs, significantly reduces the risk of developing chronic Chagas Disease. Chagas Disease is endemic to Central and South America resulting in one million new cases and 45,000 deaths each year. Transmission of the parasite occurs following the bite of a bloodsucking triatomid insect. The infected faeces of the insect are then rubbed into the wound and enter the bloodstream as the individual scratches. T. cruzi is also transmitted via blood transfusions and less commonly by transplacental transmission. Positive status in blood donors often exceeds 20% making detection of T. cruzi specific antibodies in these individuals important in order to limit new cases of the disease. The infection, as a result of T. cruzi in the bloodstream, is often long lasting with both an acute and chronic phase. The acute phase is most commonly seen in children and is hallmarked by large numbers of parasites in the blood. Symptoms may pass unnoticed or may include fever, increased heart rate and enlargement of lymph glands, the liver and the spleen. The chronic phase, which develops in those individuals surviving an acute attack often 10-20 years later, is conversely characterised by very low numbers of parasites in the blood. At this point, the disease may develop with signs of cardiac muscle damage and for loss of muscular action necessary for the movement of food. Severe damage to heart muscle leads to heart failure and in some endemic areas is responsible for up to 10% of adult deaths.

PRINCIPLE OF THE TEST
Specific, recombinant, Trypanosoma cruzi antigens are prepared, purified and coated onto microtiter wells. Test sera diluted 1/25 are applied. Specific antibodies to T.cruzi bind to the antigen in the wells. Unbound material is washed away and anti-human IgG, conjugated to Horseradish Peroxidase (Conjugate) is applied. The Conjugate binds to the human IgG antibodies bound to the antigen. Unbound material is again washed away. On addition of the Substrate (TMB), a colour will develop only in those wells in which enzyme is present, indicating the presence of human anti- Trypanosoma antibody. The enzyme reaction is stopped by the addition of Stop Solution and the absorbance is then measured at 450nm. The concentration of specific IgG antibody is directly proportional to the colour intensity of the test sample.

REAGENTS PROVIDED
1. Microtitre Plate(12 x 8 wells x 1): Breakable wells coated with specific antigens contained in a resealable foil bag with a desiccant.
2. Serum Diluent(2 X 50 ml): Tris based buffer containing stabilising proteins. Ready to use. (Orange)
3. Control(2ml): Negative Control. Clear solution, of human serum negative for IgG antibodies to Trypanosoma cruzi. Ready to use. (Blue)
4. Control(2.5ml): Low Positive Control. Clear solution, of human serum containing a low level of IgG antibodies to Trypanosoma cruzi. Ready to use. (Green)
5. Control(2ml): High Positive Control. Clear solution, of human serum containing a high level of IgG antibodies to Trypanosoma cruzi. Ready to use. (Red)
6. Wash Buffer concentrate(50ml): Tris based buffer containing detergents. (Colourless)
8. Substrate Solution(15ml): 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)
9. Stop Solution(11ml): Sulphuric Acid diluted in purified water. Ready to use. (Colourless)

STORAGE
1. Reagents must be stored at temperatures between 2°C to 8°C.
2. Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.
3. Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.
4. DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.
**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Micropipettes: 100μl, 200μl, 1000μl and 5000μl
2. Disposable pipette tips
3. Tubes for Sample Dilution
4. Vortex Mixer
5. Incubator: Temperature of 37°C +/- 1°C.
6. Absorbent paper
7. Microplate reader fitted with a 450nm filter
8. Graph paper
9. Thoroughly clean laboratory glassware.

**SPECIMEN COLLECTION AND HANDLING**

1. Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.
2. Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversely affect the results.
3. Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at −20°C for up to 1 year. Thawed samples must be mixed prior to testing.
4. Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.
5. Do not repeatedly freeze-thaw the specimens as this will cause false results.
6. SERUM DILUTION 1/25. Each test uses 100μl of serum at a 1/25 of the patients serum. This can be achieved by adding 20μl of serum to 480μl of Serum Diluent.

**ASSAY PROCEDURE**

1. **Preparation of Reagents**
   1) All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.
   2) Wash Buffer: Dilute the concentrated Wash Buffer using 1 part Wash Buffer concentrate with 19 parts distilled water. For every 8-well breakable strip, prepare 25ml of diluted Wash Buffer by adding 1.25ml of concentrated Wash Buffer to 23.75ml of distilled water. Prepare fresh diluted Wash Buffer prior to every assay run. Extra Wash Buffer is supplied to enable priming of automatic washing machines.
   3) The washing procedure is critical to the outcome of this test. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

2. **Assay Steps**
   1) Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assy.
   2) One set of control serum should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the control serum and the test serum on the EIA Data Recording Sheet provided.
   3) Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

3. **Hand Washing**
   Fill the wells with a minimum of 300μl of wash buffer per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Wash the empty wells 3 times.

4. **Machine Washing**
   Ensure that 300μl of wash buffer is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 3 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.

5. **Incubation**
   Dispense 100μl of Anti-Human HRP Conjugate into each well. Gently mix for 5 seconds before returning the plate to the incubator, ensuring that the plate is positioned on top of the moist absorbent paper.

6. **Incubation**
   Incubate for 30 minutes at 37 °C.

7. **Washing**
   Wash plate as described above.

8. **Incubation**
   Incubate in the dark for 15 minutes at 37 °C.

9. **Stop Reaction**
   Stop the reaction by adding 100μl of Stop Solution to each well.

10. **Reading of Results**
    The plate reader should be set at a wavelength of 450nm and blanked on air. In determining the absorbances of each specimen and control, it is preferable not to use a reference filter as it will change the expected values of the controls.

11. **Calculation**
    1. For each test and Control serum, determine the optical density (OD) obtained in the wells.
    2. Cut off level = Average OD of Low Positive Control + 1.5
    3. Assay Validation: The average OD’s for the Negative Control should be less than 0.2, the Low Positive Control should be greater than 0.35 and the High Positive Control should be greater than 0.6 for the assay results to be valid.
4. Negative Result: A negative result should have an OD less than the cutoff level.
5. Suspected Positive: A low or suspected positive result should have an OD less than the OD of the Low Positive Control but greater than that of the cut-off level. This is considered to be the Equivocal zone.
6. Positive Result: A positive result should have an OD greater than the Equivocal Zone.
7. If levels of controls or users known samples do not give expected results, test results must be considered invalid.

ASSAY CHARACTERISTICS

1. In a clinical trial, CHAGAS tests reported a sensitivity of 98.3% and a specificity of 98.5% (sample size was 176 positive and 453 negative). Full clinical data is available upon request.
2. The co-efficient is less than or equal to 10%.

REFERENCES