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CE

Trypanosoma

REF

Catalog No.E6-517

For the Detection of Antibodies in *T.cruzi* Infected Human Serum or Whole Blood

Intended Use

The Immunospec *Trypanosoma* Rapid Test for the diagnosis of *T.cruzi* infection in humans is a rapid immunochromatographic strip assay based on proprietary multi-epitope recombinant antigen. The rapid tests are used for the qualitative detection of serum antibodies to *T.cruzi* derived antigens.
For research use only. Not for use in diagnostic procedures

Summary and Explanation

Chagas' disease is caused by the flagellated protozoa *Trypanosoma cruzi* and is an endemic infection in Central and South America that affects 16 to 18 million individuals (1). Over the last years, intensive eradication campaigns directed against the triatomine, vector transmission of *T. cruzi* diminished drastically, especially in rural areas, and does not exist today in many regions where the infection used to be endemic. However, the transfusion of parasite-containing blood continues to be an important way of transmission (2-4).
Migration and immigration of people led to a spread of the disease beyond the geographical borders of Latin America, and it has been detected in Europe, Asia, and the United States. Because Chagas' disease has become predominantly transfusion-related, a systematic screening of blood donors is useful not only in Latin America but also in developed countries that receive immigrants from areas of endemicity (1, 5).
Several strategies exist for the diagnosis of Chagas' disease. Direct detection of the parasite in the blood by microscopy, hemoculture, xenodiagnosis, or PCR is highly specific and confirms the existence of an infection (4, 5). However, these procedures are technically and operationally demanding. Other tests currently used include measurement of antibodies against crude lysate, complement fixation, indirect hemagglutination, and fluorescent antibody (IFA). All are lacking specificity and/or sensitivity (6-9).
Serologic tests that detect antibodies specific for antigens expressed by the different developmental stages of the parasite are well suited for a fast and easy diagnosis of the disease (10-14). In an attempt to improve the serological diagnosis of Chagas' disease, we have identified and used a Multi-epitope recombinant antigen derived from different *T. cruzi* antigens (15, 18). The antigen is composed of a total of nine different epitopes (15, 16).

Principle

This immunochromatographic test has been designed for the qualitative determination of serum antibodies against the *T.cruzi* antigen. This test detects antibodies in *T.cruzi* infected individuals.
The test is based on a proprietary gold mix containing target Gold Conjugate and its ability to bind to antibodies present in serum and whole blood. Once bound, the gold antibody complex will move laterally to form a complex with immobilized *T. cruzi* derived proteins present on the membrane forming a test line. The unbound gold will continue to move upward to bind forming a control line. The test is positive when a test line is observed (refer to Figure 1).

Precautions

- Do not use after expiration date.
- Handle all sera, whole blood and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of sera/whole blood and used kits.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly when finished.
- Avoid all contact between hands and eyes or mucous membranes during testing.
- Do not eat, drink or smoke in the area where the sera and kits are handled.
- Chase Buffer contains a preservative; avoid all possible contact with skin, mouth and mucous membranes.

Storage

The sealed pouch or vial containing the test strip(s) are designed to be stored at room temperature (20˚C-28˚C). In a sealed pouch or vial the test strips are designed to retain reactivity for the duration of its shelf life. The bottle containing the Chase Buffer is designed to be stored at room temperature for the duration of its shelf life. Exposure to temperatures over 30˚C can impact the performance of the test and should be minimized. The strips should not be frozen. The test should be used quickly (as quickly as possibly preferably within 2-5 minutes) after removal from the pouch or vial to prevent exposure to humidity. Do not store tests exposed to sunlight (store in shade or dark places to prevent temperature increase inside the pouches).

Sample Collection

- Serum/whole blood should be tested with this test strip. For whole blood collection, K2, or K3EDTA, or heparinized blood samples should be used.
- Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
- Test should be performed as soon as possible after sera/whole blood collection. Do not leave samples at room temperature for prolonged periods. Sera can be refrigerated at 2-8˚C up to 3 days. Otherwise sera should be stored frozen.
- Bring sera/whole blood to room temperature prior to testing. Frozen sera must be completely thawed prior to testing. Sera should not be repeatedly frozen and thawed.
- If sera/whole blood are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

Kit Contents

1.Twenty-five (25) test strips individually pouched or 25 test strips in a vial with desiccant in the cap.
2.One (1) vial of Chase Buffer solution.

Test Procedure

1.Allow the sera/whole blood to reach room temperature prior to testing.
2.Remove the test strip from the foil pouch or vial.
3. Add 10µl of human serum or 20µl whole blood to the test strip in the absorbent area beneath the arrow.
4. Separately add three or four drops (150-200 µl) of the Chase Buffer solution, provided with this test kit, into test tube, or assay well (not provided).

5. Place the test strip loaded with sample into a test tube or an assay well so that the end of the strip is facing downward as indicated by the arrows on the strip.
6. Within 10-20 seconds gold migration will be visible on the membrane region of dipstick. If no migration is observed, gently tap on sample tape region of dipstick until gold migration is free flowing.
7. Read the results in 10 minutes. It is significant that the background is clear before reading the test. This is especially true when sera have low titer of anti-*T.cruzi* antibody. In this case, only a weak, but unequivocal band may appear in the test region. **Results interpreted after 15 minutes can be misleading**.

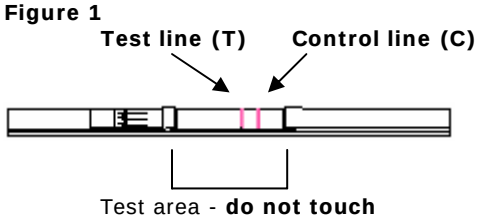
Note: Do not test this product with the Chase Buffer solution alone. 10µl of human serum or 20µl of whole blood must be added first.

Interpretation of Results

A Positive Result

The test is positive when a control line and test line appear in the test area as shown in Figure 1. A faint line is considered a positive result. As a guide for interpretation, the red color in the test region will vary depending on the concentration of anti-*T.cruzi* antibodies present. The test line for "weakly positive" samples may show a weak positive but distinct red line. ("Weakly positive" samples are those with low affinity antibodies.)

Figure 1

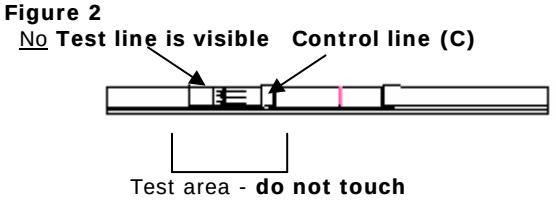


Note: The control line is faint blue before assay and turns red following gold migration.

A Negative Result

The test is negative when only the control line appears. No test line is present as in Figure 2.

Figure 2



An Invalid Result

No lines appear at either the control or test line areas. The test is also invalid if no control line appears, but a test line is seen. It is recommended to retest using a new test strip and fresh human serum.

Note: The red color in the test region will vary depending on the concentration of specific antibodies present in *T.cruzi* infected hosts. However, neither the quantitative value nor the rate of increase in antibodies can be determined by this qualitative test.

Performance Characteristics

Performance of Trypanosoma rapid test has been correlated with a number of archive sera in different countries. We have included one study performed in Chile.

Study 1: Chile Study

IFA/ELISA				
		+	-	Total
Trypanosoma Assay	+	51	0	51
	-	0	40	40
		51	40	91

Sensitivity: 100%; **Specificity:** 100%

All positive sera were confirmed positive by ELISA and IFA. The negative samples included 10 sera from patients with Toxoplasmosis.

Limitations

- For research use only.
- This test will only indicate the presence of antibodies to our recombinant antigen in human serum/whole blood and should not be used as the sole criterion for the diagnosis of *T.cruzi* infection. Note: As with all diagnostic tests, all results must be considered with other information available to the physicians.
- If the result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of *T.cruzi* infection.
- Do not use serum or whole blood samples containing any glycerol or other viscous materials. This will compromise the sensitivity of the assay dramatically.
- Do not use highly hemolyzed or aged samples. Highly hemolyzed samples will interfere with test performance.

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