CHAGAS STAT-PAK® ASSAY
A Rapid Two step Test for the Detection of Antibodies to Trypanosoma cruzi in Serum, Plasma or Whole Blood

FOR EXPORT ONLY
FOR IN VITRO DIAGNOSTIC USE
FOR PROFESSIONAL USE ONLY
READ INSTRUCTIONS FOR USE CAREFULLY BEFORE PERFORMING TEST

INTENDED USE
The Chembio CHAGAS STAT-PAK assay is a single use immunochromatographic screening test for the detection of antibodies to Trypanosoma cruzi in human blood, serum or plasma. The test is used for the diagnosis of Chagas in conjunction with other criteria.

SUMMARY AND EXPLANATION
Chagas disease is caused by T. cruzi, a protozoan parasite of humans and a wide variety of insects and animals. It is a major public health concern in Latin America and of growing concern in the United States as the number of infected immigrants increases [1]. In Latin America, it is estimated that 120 million people, i.e. 25% of the population, inhabit endemic areas where there is risk of infection. Approximately 16 to 18 million inhabitants of rural and urban areas are thought to be infected with T. cruzi [2]. Prevalence of congenital infection is 10% for children born to chagasic mothers. An estimated 90% of infected individuals enter the chronic period of infection. Among them about 30% are characterized by cardiomyopathy and/or megasymphdromes involving the esophagus or colon. The leading manifestation of Chagas is chronic myocarditis resulting in high morbidity and mortality [3-5].

The infection occurs via the bite of the hematophagous triatome insect which releases the infective trypomastigotes in its excreta when feeding on mammalian host. In endemic countries where not all blood samples are controlled and detection methods are not available, infection can occur through blood transfusion. Alternatively, infection can occur through organ transplantation, ingestion of contaminated food and congenitally.

No vaccine or chemotherapy is available for the prevention of disease. However, drug therapy is available and effective when administered to young children prior to onset of the chronic stage of the disease. Recombinant antigens provide a convenient tool to improve current methods of serological diagnosis of Chagas disease. Several assays such as immunofluorescence, hemagglutination, complementation fixation, radioimmunoassay and ELISA are currently available for its diagnosis [6-8].

The Chembio CHAGAS STAT-PAK assay is an immunochromatographic screening test which employs a unique combination of highly specific recombinant antigens for the detection of antibodies to T. cruzi. It is rapid, simple and easy to use and can be stored at room temperature.

PRINCIPLE OF TEST
The Chembio CHAGAS STAT-PAK assay is a rapid immunochromatographic screening test for the detection of antibodies to T. cruzi. The method employs a unique combination of a specific antibody binding protein which is conjugated to dye particles and antigens which are bound to the membrane (solid phase). The assay shows a high degree of sensitivity and specificity.

The test sample is applied to the SAMPLE well. As the test sample flows laterally across the membrane, the specific antibody binding protein dye conjugate binds to the human immunoglobulins in the sample. If the sample contains antibodies to T. cruzi, the complex binds to the antigens on the solid phase in the TEST area producing a pink/purple line. In the absence of T. cruzi antibodies there is no line in the TEST area. The Chembio CHAGAS STAT-PAK test also provides an internal IgG antigen control. The sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL zone demonstrating that the reagents are functioning properly.

MATERIALS PROVIDED
Each kit contains the following items to perform 20 tests
- 20 CHAGAS STAT-PAK test devices
- 20 Microsafe® Tubes (10µL) for fingerstick whole blood
- 1 Sample Diluent (6mL)
- 1 Product Insert

Materials Required But Not Provided
- Timer
- Pipettor (for 5µL serum or plasma samples or 10µL venous whole blood only)
- Sterile single use lancets (for fingerstick whole blood samples only)
- Sterile alcohol swabs (for whole blood samples only)

STORAGE AND STABILITY
The CHAGAS STAT-PAK assay should be stored at 8-30°C in the original sealed pouch. The diluent vial should also be stored at 8-30°C. The kit is stable until the date imprinted on the box label and/or pouch.

**NOTE:** Do not use expired test kits.

**CAUTION:** DO NOT FREEZE TEST KITS

**PRECAUTIONS**

1. The test is FOR IN VITRO DIAGNOSTIC USE only. For PROFESSIONAL USE only. Use the test only in accordance with instructions supplied with the kit.
2. Handle all specimens as recommended for potentially infectious human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 4th ed., 1999.
3. Use suitable protective clothing (gloves, lab coat, safety glasses) when handling samples. Avoid any contact between hands, eyes, nose or mouth during specimen collection and testing.
4. Do not pipette any material by mouth. Do not smoke, eat or drink in areas where specimens or kit materials are kept.
5. After the completion of the assay, autoclave all materials for 1 hour at 125°C. Alternatively, materials can be treated with 10% solution of bleach. Dispose of materials carefully in biohazard bags.
6. Do not mix reagents from different kit lots.

**SPECIMEN COLLECTION**

The Chembio CHAGAS STAT-PAK test is performed on whole blood, serum or plasma.

**Whole Blood:** Collect whole blood into tubes containing heparin, EDTA or sodium citrate. For fingerstick blood, prick the finger and wipe away the first drop. Collect the second drop with a disposable 10μL Microsafe® Tube (provided). **Do not squeeze the finger too hard.** Follow test procedure instructions.

**Serum:** Serum is used from whole blood collected aseptically by venipuncture into a clean tube without anticoagulant. Allow the blood to clot at room temperature. Centrifuge the blood at 2000 rpm for 10 minutes at room temperature. Remove the serum from the clot as soon as possible to avoid hemolysis.

**Plasma:** Collect whole blood with anticoagulants (heparin, EDTA or sodium citrate), centrifuge at 2000 rpm for 10 minutes and isolate the plasma supernatant.

Patient samples perform best when tested immediately after collection. Specimens should be refrigerated immediately following collection at 2-8°C and can be used up to 3 days. If testing within 3 days is not possible, the specimens should be frozen (-20°C or colder).

**NOTE:** If specimens are to be shipped they should be packed in compliance with regulations covering the transportation of etiologic agents.

**CONFIRMATION OF TEST PERFORMANCE**

The Chembio CHAGAS STAT-PAK assay is the only rapid assay that provides visual confirmation of control line reagents BEFORE running the assay. When viewing the test device, you will note a YELLOW band in the CONTROL area of the reaction card. This colored complex indicates that the reagents necessary for the test to function properly ARE indeed present and active. After addition of the sample, this YELLOW band migrates along the membrane at the leading edge of the dye conjugate and is removed from the test device completely.

When the test is complete, you will observe the familiar pink/purple line in the CONTROL area of the test device for negative as well as positive samples (it serves as an internal IgG control and gives confirmation of proper test performance). A pink/purple line in both the TEST and CONTROL areas indicate a positive result.

**TEST PROCEDURE**

1. If the test sample is refrigerated, remove it from the refrigerator and allow it to come to room temperature prior to testing.
2. Remove the required number of CHAGAS STAT-PAK test devices from their pouches by tearing along the notched area and place the device on a flat surface area.
3. Label the test device with patient name or identification number.
4. For fingerstick whole blood, prick the finger and wipe away the first drop. Collect the second drop with a Microsafe® Tube holding it in a horizontal position as shown. Touch the tip of the tube to the blood sample. Capillary action will draw the sample to the black fill line and stop.

   **Never squeeze the bulb of the tube while sampling.**

5. Add the specimen to the center of the SAMPLE well.

   **To push the sample out of the Microsafe® Tube, line up the tip of the tube with the SAMPLE well and squeeze the bulb.**

   **ONLY IF THE SAMPLE DOES NOT COME OUT OF THE TUBE,** hold the tube vertically and slide a finger over the vent hole near the black mark. Then line up the tip with the SAMPLE well and squeeze the bulb.

   If testing a sample other than fingerstick blood, use an accurate pipette and add the required amount of sample to the sample well.

   a) Serum or plasma - 5μL

   b) Whole blood - 10μL

6. Invert the diluent bottle and hold it vertically (not at an angle) over the sample well. Add the diluent slowly dropwise, 6 drops (~240μL) into the SAMPLE well.
7. Read results within 15 minutes after the addition of diluent. Allow a full 15 minutes to confirm a negative result. **Do not read any results after 15 minutes.**

**NOTE:** The sample volume is critical; use an accurate pipette. Adding more than 5µL of serum or plasma or 10µL of blood decreases sensitivity.

**QUALITY CONTROL**
A pink/purple line should always appear in the CONTROL area if the test has been performed correctly and the device is working properly. It serves as an internal IgG procedural control. A clear background in the TEST area is an internal negative procedural control.

Good Laboratory Practice (GLP) recommends the use of control materials along with the test samples to ensure proper performance of the test kit. Positive and negative serum or plasma based commercial controls should be used for this purpose. Use controls as per the TEST PROCEDURE instructions of this insert.

**INTERPRETATION OF RESULTS**

**Negative Result**
One pink/purple line in the CONTROL area with no visible line in the TEST area indicates a negative result. A negative result at 15 minutes indicates that there are no detectable antibodies to Chagas in the sample. A negative result does not preclude the possibility of Chagas infection.

**Positive Result**
Two pink/purple lines - one in the TEST area and one in the CONTROL area indicate a positive result. The line in the TEST area may look different from the line in the CONTROL area.

**NOTE:** Intensities of the TEST and CONTROL lines may vary. If any visible line appears in the TEST area and in the CONTROL area, the result is positive.

**Invalid Results**
A pink/purple line should always appear in the CONTROL area regardless if the TEST line appears or not. If there is no distinct pink/purple line visible in the CONTROL area, the test is invalid and should be repeated using a new device.

An invalid result indicates a problem with running the test, either related to the specimen or to the device. If you are unable to obtain a valid result upon repeat testing, contact Chebio Diagnostic Systems at +1-631-924-1135 ext 114 or info@chebio.com.

**EXPECTED RESULTS**
This is a qualitative test for the detection of antibodies to *T. cruzi* in blood, serum or plasma. The presence of antibodies suggest *T. cruzi* infection and the expected result will be positive. In the absence of infection, a negative result will be observed. This is based on findings with CHAGAS STAT-PAK testing.

**LIMITATIONS OF THE PROCEDURE**
The CHAGAS STAT-PAK procedure and the interpretation of the results must be followed closely. The assay is designed for detecting antibodies to *T. cruzi* in human serum, plasma or whole blood. Any result from the testing of other body fluids or of pooled serum, or plasma should not be used.

For positive samples it is recommended that a more specific reference test be performed, along with a clinical evaluation of the patient’s situation before a final diagnosis is made. Rapid testing alone should not be used to diagnose *T. cruzi* infection even if *T. cruzi* antibodies are present. A negative result at any time does not preclude the possibility of infection with *T. cruzi.* Additional follow-up testing using other clinically available methods is required if the Chebio CHAGAS STAT-PAK test result is negative and clinical symptoms persist or do not fit other clinical data available.

**PERFORMANCE CHARACTERISTICS**
Highly specific antibody binding protein and antigens are used for the CHAGAS STAT-PAK assay. The sensitivity of the CHAGAS STAT-PAK test was compared to a leading commercial test for Chagas and the detection limit found to be substantially equivalent.

**Sensitivity and Specificity**
Clinical trial studies were performed to evaluate Chebio’s CHAGAS STAT-PAK assay. In one study (9) a panel of 393 coded serum samples from patients from endemic areas of Brazil were tested. 200 of these were from well-defined chagasic patients and an additional 150 samples were selected from healthy individuals. The status of these 350 samples was confirmed by conventional serology (indirect immunofluorescence assay (IIFA), indirect hemagglutination assay (IHA), and ELISA. The following results were obtained:
Within run precision was determined by using 10 replicates of Intra Precision 3400 sera, there was 100% agreement between ELISA and CHAGAS STAT test were 99.8% and 100% respectively. Overall sensitivity and specificity as compared to the conventional serology tests (IHA, IHA, ELISA). These results are detailed below.

In another study (9), comprising 352 serum samples from 4 different Latin American countries, the Chembio CHAGAS STAT-PAK assay exhibited an overall sensitivity of 100% and specificity of 98.6% as compared to conventional serology (IHA, IHA, ELISA). These results are detailed below.

A study was also performed using a total of 5998 sera from Honduras, El Salvador, and Nicaragua to compare the performance of the Chembio CHAGAS STAT-PAK assay with that of a commercial ELISA test (10).

Overall sensitivity and specificity as compared to the ELISA test were 99.8% and 100% respectively.

Of the 5998 sera, 3400 were blood donor sera and were found to have a positive rate of 4.6% with both the ELISA and CHAGAS STAT-PAK assays. For these 3400 sera, there was 100% agreement between ELISA and CHAGAS STAT-PAK assay.

### Precision

#### Intra-Assay

Within run precision was determined by using 10 replicates of three different specimens containing different concentrations of antibodies. The negative and positive values were correctly identified 100 percent of the time.

#### Inter-Assay

Between run precision was determined by using the same three specimens in 10 different replicates with three different lots of test devices over a six month period. Again positive and negative results were observed 100 percent of the time.

#### Interference and Cross Reactivity

Test performance was evaluated using various sera containing different antibody titers of Chagas and samples containing various interfering substances. No interference was observed from bilirubin, hemoglobin and triglycerides with Chembio CHAGAS STAT-PAK assay. Rheumatoid Factor levels up to 80 IU/mL do not interfere with the test, levels higher than this may result in false positive results. No cross reactivity was observed from Leishmaniasis infected sera with Chembio CHAGAS STAT-PAK assay.

### REFERENCES

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