INTENDED USE

The **Hemagen Chagas' Kit** (Catalog # 66101) is a presumptive, qualitative, enzyme-linked immunosorbent assay (ELISA) designed for the detection of circulating antibodies to *Trypanosoma cruzi*, the causative agent of Chagas' disease. When used according to instructions, the kit is useful in establishing prior exposure to *T. cruzi* and as an aid in the diagnosis of Chagas' disease. This product is not FDA-cleared for use in screening blood or plasma donors.

SUMMARY AND EXPLANATION OF THE TESTS

Chagas' disease, or American trypanosomiasis, is a parasitic disease caused by the hemoflagellate *T. cruzi*. It is transmitted to vertebrate hosts, including man, by hematophagous insects of the sub-family Triatominae.[1] The disease affects humans in the Americas from Mexico in the north to Argentina and Chile in the South, where its extent is related to the poor socioeconomic conditions of the population and to the domestic nature of the vector.[2]

There are two main phases of Chagas' disease, acute and chronic.[3] After one to two months in the acute phase, patients enter one of three forms of the chronic phase: indeterminate form, in which there are often no systemic complaints (in the indeterminate forms, seropositivity is frequently the only abnormal finding); cardiac form, which may be diagnosed by conventional electrocardiogram and/or chest X-ray; digestive form, characterized by megaesophagus or megacolon and diagnosed radiographically.

Seropositivity is frequently established in the clinical laboratory using such techniques as passive hemagglutination and ELISA.

TEST PRINCIPLE

Indirect enzyme-linked immunosorbent assays, or ELISAs, are commonly used for serum antibody evaluation.[4] In the **Hemagen Chagas' Kit** (EIA method), purified antigens extracted from cultured *T. cruzi* organisms have been attached to the inner surfaces of each microwell. During an initial incubation step, Chagas' antibodies in patient serum bind specifically to the immobilized antigen and remain in place after a washing step.

A second antibody, conjugated to the enzyme horseradish peroxidase, recognizes the "heavy + light" chain regions of human IgG and binds to the patient's Chagas' antibodies remaining from the first step. In those wells where the peroxidase conjugate remains bound, the enzyme then catalyzes a color change in the substrate 3,3',5,5'-tetramethylbenzidine (TMB). After the enzymatic reaction is stopped, the now-yellow color is read in an EIA Plate reader.

REAGENTS

The following components are included in the **Hemagen Chagas' Kit** (EIA method):

Chagas' Microplate

Plastic, flat-bottomed microplate, coated with purified *T. cruzi* antigens. Store at 2-8 °C in original pouch until ready to use. Allow package to reach room temperature before opening. Unused wells should be resealed in pouch with original desiccant and returned to refrigerated storage. May be stored at 2-8 ºC for six months in resealed pouch. 96 wells per kit.

HRP Conjugate

Affinity-purified goat anti-human IgG (H + L) linked to horseradish peroxidase. Ready to use. Store at 2-8°C. Allow HRP Conjugate to reach room temperature before using. 15 mL**.**

Negative Control

Processed human serum with sodium azide as a preservative. Ready to use. Does not contain antibodies to *T. cruzi.* Designed to give OD reading of less than 0.250 (after subtracting blank) at 450 nm when used according to instructions. Store at 2-8 °C. 1.5 mL

Chagas' Positive Control

Processed human serum with sodium azide as a preservative. Ready to use. Contains antibodies to *T. cruzi* and gives a positive OD reading of greater than 0.400 when used according to instructions. Store at 2-8 °C. 1.5 mL

Serum Diluent (45 mL)

Borate-buffered saline containing animal serum, eosin, stabilizers and 0.2% sodium azide. For dilution of patient samples and control sera. Store at 2-8 °C.

The following items are common components and are interchangeable throughout all of Hemagen's VIRGO EIA kits:

TMB (15 mL)

3,3',5,5'-tetramethylbenzidine for use as enzyme substrate. Ready to use. Store at 2 - 8 ºC.

Sulfuric Acid (10 mL)

1N sulfuric acid for stopping enzyme reaction. Ready to use. Store at room temperature or at 2 - 8 ºC.

Wash Solution Concentrate (50 mL)

Concentrated Tris Tween solution. Store at 2 - 8 ºC or at room temperature prior to use. Dilute contents of bottle to one liter with deionized or distilled water to prepare Wash Solution. Use as a rinsing and washing buffer between incubation steps as directed. Stable for six months after diluting.

Precautions

1. For *in vitro* diagnostic use.
2. Warning: This kit contains potentially biohazardous material. Each donor unit used in the preparation of control materials was tested by an FDA approved method for the presence of antibody to human immunodeficiency virus type I (HIV-I) as well as for hepatitis B surface antigen (HBsAg) and found to be negative. But because no test method can offer complete assurance that HIV, hepatitis virus, or other infectious agents are absent from biological materials, all reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control / National Institutes of Health manual, Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition, 1993.
3. The concentrations of anti-*T. cruzi* IgG / IgM in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
4. Some reagents contain sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. On disposal of reagents, flush with a large volume of water to help prevent azide build-up.
5. Sulfuric acid causes irritation and may cause burns. Avoid contact with eyes. In case of contact with skin, wash immediately with plenty of water.

Required but not supplied with the kit

The following items are needed in order to perform the assay but are not supplied with the kit:

- small test tubes or mini-tubes for initial serum dilutions
- micropipettors with tips
- multi-channel pipettors capable of delivering 50 µL and 100 µL per channel
- reagent boats
- squeeze bottle or plate washer
- EIA plate reader capable of reading at 450 nm

SPECIMEN COLLECTION AND PREPARATION

Serological specimens should be collected under aseptic conditions. Allow whole blood to clot; then centrifuge to remove particulate matter from the serum. Decant promptly. Samples may be held at 2-8 °C for several days or frozen at -20 °C for extended storage. Repeated freezing and thawing of serum is not recommended.

Caution: Human serum is a potential source of HIV, hepatitis virus, and other infectious agents. Therefore, all specimens should be handled as if capable of transmitting disease.

TEST PROCEDURE

A. Notes on Procedure

1. It is recommended that kit controls be run in duplicate. Patient samples may also be run in duplicate, if desired.
2. For best results, serum dilutions should be prepared in 1 mL mini-tubes. Aliquots may then be conveniently transferred to the test strips with an eight-channel pipettor.
3. Adequate washing of the test wells may be achieved using a squeeze bottle filled with Rinsing Solution, prepared as directed above. Alternatively, an automated plate washer, programmed to deliver 250-350 µL per well four times in succession, may be used.
4. Color-coded Chagas' microstrips are supplied in a break-apart format. We recommend that microwells be used in groups of two or more. **Important**: if an automated plate washer is used, be sure it can accommodate a partial strip of microwells before separating wells from each other.
5. **Important:** When adding reagents to the wells, be sure to extend the pipet tips all the way to the bottom of the test well. In particular, do not leave residual material from one step on the rim of a well where it could interfere with a reaction at a later step.

B. Quality Control

1. A blank (Serum Diluent), the Positive Control, and the Negative Control must be included in each run.
2. The Positive and Negative Controls have been pre-diluted and should not be treated in the same manner as patient samples.
3. In addition to testing the control sera provided by Hemagen, we recommend that each laboratory establish its own in-house serum standards and include them in its testing protocol on a routine basis.

C. Test Protocol

1. Bring reagents to room temperature (18-25 °C).
2. Dilute Wash Solution Concentrate to a volume of one liter with distilled water. Transfer to squeeze bottle or to automated plate washer.
3. Prepare serum dilutions. Dilute each patient sample, **do not dilute the Positive and Negative Controls**, 1:26 by adding 10 µL serum to 250 µL Serum Diluent in a labeled test tube or mini-tube.
4. Place microstrips securely into holder, and transfer 100 µL of each control (in duplicate) and each diluted sample to wells of 1 x 8 microstrips using an eight-channel pipettor. Be sure to include a well for the blank (Serum Diluent). Tap the plate gently to ensure a uniform distribution of sample.
5. Incubate plate 30 minutes at room temperature (18-25 °C).
6. Discard samples by flicking plate away from you and expelling contents into a suitable container for disposal, or insert the plate into an automated plate washer.
7. Wash plate 4 times with Wash Solution. Fill each well with a gentle stream, and then discard solution. If you are using the manual washing method, invert plate and tap gently against an absorbent material after the last wash.
8. Transfer 100 µL HRP Conjugate (second antibody) to each well using an eight-channel pipettor. Tap the plate gently as in step 4.
9. Incubate plate 30 minutes at room temperature (18-25 °C).
10. Discard HRP Conjugate by flicking plate away from you and expelling contents into a suitable container for disposal, or insert the plate into an automated plate washer.
11. Wash plate 4 times with Wash Solution as in step 7.
12. Transfer 100 µL TMB to each well. Tap the plate gently as before.
13. Incubate plate 30 minutes at room temperature (18-25 °C).
14. Transfer 50 µL 1 N H_2SO_4 to each well to stop the enzymatic reaction.
15. Tap the plate gently to disperse reagents and read the plate promptly in an EIA plate reader which has been set to 450 nm.

Blank the reader on Serum Diluent before proceeding to read samples.

INTERPRETATION OF RESULTS

Results are obtained by examining the plate in an EIA plate reader at 450 nm. The intensity of the final yellow color is related to the amount of Chagas' antibody present in the sample. The OD reading of the blank must be subtracted from each sample and control reading.

- Cut-Off. The cutoff value is determined by multiplying Positive Control OD value with the Cut-Off Coefficient provided in the kit. For example, if the mean OD of Positive Control is 0.725, the kit Cut-off Coefficient is 0.476, the Cut-Off is 0.345 (0.725 x 0.476).
- Negative reaction. Samples with OD's less than or equal to Cut-Off are considered negative for antibodies to *T. cruzi*. **NOTE:** In order to provide valid results, the Negative Control readings should both be less than or equal to 0.250 after blanking.
- Positive reaction. More intense color is present in positive wells. Specifically, samples whose OD's are more than 10% above the cutoff value described above are considered positive. In the example above, a sample whose OD is greater than 0.380 is reported as positive.
- Equivocal reaction. If the mean OD reading on a sample is between the cutoff value and 1.1 times the cutoff value, the result is considered equivocal, and the test should be repeated.

A positive result in this test indicates the presence of antibodies to *T. cruzi*, the causative agent of Chagas' disease. This information may be helpful in establishing prior exposure to the agent and as an aid in the diagnosis of Chagas' disease, especially the indeterminate form where clinical symptoms are lacking.

A negative result indicates that antibodies to *T. cruzi* have not been detected and that there is a high probability of non-infection. However, during the early phases of seroconversion, there may be low levels of antibody present which are undetectable by EIA methods. **Seronegativity should not overrule clinical or historical information which may be consistent with Chagas' disease**.

In a repeatedly equivocal sample, the presence or absence of antibody to *T. cruzi* cannot be established with this assay, and the sample should be tested using another method such as hemagglutination.

LIMITATIONS OF THE TEST

1. The tests described herein require an initial serum dilution of 1:26. Results obtained with less dilute sera (e.g., 1:5, 1:10) have no clinical significance.
2. The clinical significance of any test result depends upon its relationship to other medical patient data. Disease diagnosis and management should be based on an evaluation of all relevant patient information.
3. The second antibody used in this kit, because it is directed against "heavy + light" chains of human IgG, exhibits some reactivity to human IgM. Therefore, some IgM samples will give positive results in this assay. However, we recommend that the presence or absence of IgM antibodies to *T. cruzi* be established using a product or method designed specifically for that purpose.
4. Other species of *Trypanosoma* (in particular, *T. rangeli*) share common epitopes with *T. cruzi*; therefore, antibodies produced in response to other trypanosomal infections may cross-react with the *T. cruzi* antigen preparation used in this assay.

EXPECTED VALUES

Infected individuals usually begin producing antibodies to *T. cruzi* during the first month following exposure to the parasite. Antibody levels may fluctuate during the chronic phase of the disease and may become undetectable after several months. Uninfected individuals are not expected to have detectable levels of antibodies to *T. cruzi*.

Serum samples from hospitalized individuals were evaluated with the **Hemagen Chagas' Kit** at two different sites in the United States. At one clinical site, located in the Southwest, samples from 261 different patients were tested and found to be negative for antibodies to *T. cruzi*. At a second site, located in the Northeast, 251 out of 258 samples tested were negative, 3 samples were equivocal, and 4 samples were positive. The equivocal and positive samples were negative upon repeat testing with a commercially available EIA kit.

PERFORMANCE CHARACTERISTICS

A. Precision

Intra-run reproducibility for this assay was determined by testing two positive sera 16 times each in a single run. Inter-run reproducibility was checked in a separate study by testing two positive sera 5 times each day for 3 days (15 tests for each serum). The intra-run coefficients of variation (CV, based on O.D. readings) for the two sera were 3.5% and 2.7%; the inter-run CVs were 6.0% and 5.4%. (These values include the well-to-well variation inherent in the plastic strips, which ranges up to 5%, according to the plastics manufacturer.)

B. Accuracy

The **Hemagen Chagas' Kit** has been compared to a commercially available Chagas' kit which also uses the ELISA method. In this study, 394 serum samples were evaluated with both kits. These samples included 168 patients with immunofluorescence titers > 1:80 and clinical signs of Chagas' disease, as well as 226 normal blood donors. The results are summarized in the table below:

		Comparison Method		
		Positive	Negative	Equivocal
Hemagen Diagnostics	Positive	160	3	5[a]
	Negative	0	226	0

Product Comparison Table. Relative sensitivity, compared to the commercially available kit, is 160/160 = 100%. The 95% confidence interval is 97.7% to 100%. Relative specificity is 226/229 = 98.7%. The 95% confidence interval is 96.2% to 99.6%.

[a]These specimens were obtained from a serum library in which samples had an immunofluorescence titer ≥ 1:80.

C. Cross reactivity

In order to determine cross-reactivity between the antigen preparation used on the Hemagen plates and antibodies found in patients with other infections, twenty-eight specimens which were seropositive for leishmaniasis, toxoplasmosis, cysticercosis, toxocariasis, or amebiasis were tested on the **Hemagen Chagas' Kit**. All twenty-eight specimens were negative in this assay.

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OTHER SOURCES

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Hemagen® Chagas' Kit (EIA Method)

For *in vitro* diagnostic use.

Order No.: 66101

Description: 96 Test EIA Kit

Place label here with
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