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bioelisa CHAGAS

3000-1236 96 tests
3000-1237 480 tests

ELISA test for detection of antibodies to *Trypanosoma cruzi* in human serum or plasma.

Summary
Chagas' disease is a chronic parasitic infection caused by a flagellate protozoan, *Trypanosoma cruzi*. This parasite is normally transmitted to humans or other mammals by triatomine bugs, of the family Reduviidae. *T. cruzi* can also be transmitted congenitally or by the transfusion of contaminated blood or the transplantation of contaminated organs. The life cycle of the parasite is long and complex, with several developmental stages both in the triatomine vector and in the vertebrate host. Three stages are recognized in Chagas disease: a short acute stage and a long-lasting chronic stage, separated by a long clinically asymtomatic phase, called the indeterminate stage. Different organs may be involved at any time in the first and third stages, and the disease can be fatal in either. It is estimated that up to 30% of persons with indeterminate form of the infection will suffer from cardiac, digestive or neurological dammage 10-20 years after having contracted the disease. The antibodies appear soon after infection and rise to high levels and may persist, along with infection, for many years. **bioelisa CHAGAS** allows a very sensitive and specific detection of antibodies to *T. cruzi* in the acute and chronic stages of the infection with the ELISA technique, thanks to the use of recombinant antigens. It is a low-cost method and can be completely automated to screen large number of sera in blood banks and clinical laboratories.

Principle
bioelisa CHAGAS is an immunoenzymatic method in which the wells of a microtiter plate are coated with recombinant antigens representing four immunodominant epitopes of *T. cruzi* licensed from Corixa Corporation (US Patented). Serum or plasma samples are added to these wells. If antibodies specific for *T. cruzi* are present in the sample, they will form stable complexes with the antigens on the well. After washing to remove the unbound material, a rabbit conjugate anti-human IgG and anti-human IgM labeled with horseradish peroxidase is added and, if the antigen/antibody complex is present, the conjugate will bind to the complex. After a second wash, an enzyme substrate solution containing a chromogen is added. This solution will develop a blue colour if the sample is positive. The blue colour changes to yellow after blocking the reaction with sulphuric acid. The intensity of colour is proportional to the concentration of anti-*T. cruzi* antibodies in the sample. Wells containing negative samples remain colourless.

Components

1. **MCPL** MICROPLATE:
   12 x 8 wells coated with *T. cruzi* antigens.

2. **CONJ 51x** CONCENTRATE CONJUGATE:
   Rabbit anti-human IgG and anti-human IgM antibodies conjugated with peroxidase. Contains red dye, 0.02% thimerosal and stabilisers protein. To be diluted 1/51 with the conjugate diluent before use.

3. **DIL CONJ** CONJUGATE DILUENT:
   Tris buffer containing yellow dye, additives and 0.02% thimerosal. To dilute the concentrate conjugate.

4. **DIL SAMP** SAMPLE DILUENT:
   Tris buffer with stabilisers protein, < 0.1% sodium azide, < 1.0% Triton X-100 as preservative and 8% Ethylene Glycol. Ready to use.

5. **WASH SOLN 10x** CONCENTRATE WASHING SOLUTION:
   Phosphate buffer concentrate (10x) containing 1% Tween 20 and 0.01% thimerosal. To be diluted 1/10 in distilled or deionised water before use.

6. **SUBS BUF** SUBSTRATE BUFFER:
   Citrate-acetate buffer containing hydrogen peroxide.

7. **SOLN TMB** CHROMOGEN:
   3,3', 5,5'-Tetramethylbenzidine (TMB) dissolved in dimethylsulphoxide (DMSO).

8. **CONTROL +** POSITIVE CONTROL:
   Diluted heat inactivated human serum containing antibodies to *T. cruzi*. Contains green dye and < 0.1% sodium azide as preservative. Ready to use.

9. **CONTROL –** NEGATIVE CONTROL:
   Diluted human serum negative for antibodies anti-*T. cruzi*. Contains yellow dye and < 0.1% sodium azide as preservative. Ready to use.

10. **H_2SO_4 1N** STOPPING SOLUTION (only in 1 plate kit):
    1N sulphuric acid. Ready to use.

11. **SEALS** ADHESIVE SEALS:
    To cover the microplate during incubation.

12. **BAG** RESEALABLE BAG:
    For storage of unused strips.

Precautions
bioelisa CHAGAS is intended for IN VITRO diagnostic use.
For professional use only.

The **Sample Diluent** contains < 0.1% Sodium azide and < 1.0% Triton X-100. The following are the appropriate risk (R) and safety (S) phrases:

R22 Harmful if swallowed.
S46 If swallowed, seek medical advice immediately and show this container or label.

WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL.
The positive control has been heat inactivated. All human source material used in the preparation of this product was found to be negative for the presence of HIV-1/HIV-2 and HCV antibodies, as well as for the hepatitis B surface antigen, using a commercial licensed method. Nevertheless, because no test method can offer complete assurance of the absence of infectious agents, this product should be handled with caution:

- Avoid contact of reagents with the eyes and skin. If that occurs, wash thoroughly with water.
- Wear gloves.
- Do not pipette by mouth.
- Do not smoke.
- Dispose all used materials in a suitable biohazardous waste container. Remains of samples, controls, aspirated reagents and pipette tips should be collected in a container for this purpose and autoclaved 1 hour at 121°C or treated with 10% sodium hypochlorite (final concentration) for 30 min before disposal. (Remains containing acid must be neutralised prior addition of sodium hypochlorite).
- Certain reagents in this kit contain sodium azide as preservative. Sodium azide may react with lead or copper pipes and plumbing creating highly explosive metal azides. Flush drains with water thoroughly after disposing of the remains of reagents.

Handling instructions:

- Adjust washer to the plate used (flat bottom) in order to wash properly.
- Do not mix reagents from different lots.
- Do not use reagents after expiration date.
- Do not use the reagent if you observed any change in appearance of components included in the kit.
- Extreme care should be taken to avoid microbial contamination and cross contamination of reagents.
- Use a new pipette tip for each specimen and each reagent.
- Soaps and/or oxidising agents remaining in containers used for preparation of substrate-TMB solution can interfere with the reaction. If glass containers are used to prepare the solution, they should be washed with 1N sulphuric or hydrochloric acid, rinsed well with distilled water and dried before use. We recommend using disposable plastic containers.
- It is very important to prepare the substrate-TMB solution just 5-10 minutes before use. Keep it in a well-sealed container and avoid light exposure.

Storage and stability
The components will remain stable through the expiration date shown on the label if stored between 2-8°C. The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells. Once opened the bag, microplate wells are stable for 3 months at 2-8°C in the plastic bag tightly sealed, with the silicagel. Once diluted, the washing solution is stable for two weeks if stored between 2-8°C. Once diluted, the conjugate must be used in the same day. Store the chromogen in the dark. As the substrate-TMB solution is not stable once prepared, instructions for its use should be closely followed.

Available packaging

- 1 plate kit (96 tests), **REF** 3000-1236.
  Contains: 1 plate, 1 x 0.35 ml concentrate conjugate, 1 x 15 ml conjugate diluent, 1 x 30 ml sample diluent, 2 x 50 ml concentrate washing solution, 1 x 14 ml substrate buffer, 1 x 1.5 ml chromogen, 1 x 2 ml positive control, 1 x 3 ml negative control, 1 x 12 ml stopping solution, 1 resealable bag and adhesive seals.

- 5 plates kit (5 x 96 tests), **REF** 3000-1237.
  Contains: 5 plates, 1 x 1.3 ml concentrate conjugate, 1 x 70 ml conjugate diluent, 1 x 120 ml sample diluent, 3 x 100 ml concentrate washing solution, 5 x 14 ml substrate buffer, 1 x 1.5 ml chromogen, 1 x 5 ml positive control, 1 x 7 ml negative control, 1 resealable bag and adhesive seals.

Material required not provided

- Distilled or deionised water.
- Multichannel pipettes and micropipettes (10 µl, 100 µl, 200 µl) and disposable tips.
- Incubator at 37°C ± 1°C.
- Timer.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 630 nm is advisable.
- Manual or automated wash system.
- Stopping solution (5-plates kit): 1N sulphuric acid. 2N or 4N sulphuric acid could also be used.

Sample collection

Use fresh serum or plasma (EDTA). Other anticoagulants should be evaluated before use. Samples can be stored at 2-8°C for 3 days. For longer periods, samples should be frozen (-20°C). Avoid repeated freezing and thawing. Specimens showing visible particulate matter should be clarified by centrifugation. Serum or plasma samples should not be heat inactivated, since that may cause incorrect results.

Automatic processing

Automated or semi-automated assay may be used with different instruments. It is very important to validate any automated system to demonstrate that results obtained for samples are equivalent to the ones obtained using manual assay. It is recommended that the user validate periodically the instrument. If there is any difficulty in the setting of Biokit automatic processors, please contact your distributor.

PROCEDURE (See procedural flow chart)

Previous operations

Allow all the reagents to reach room temperature (20-25°C) before running the assay.
Gently mix all liquid reagents before use.
Dilute the concentrate washing solution 1/10 with distilled or deionised water. For one plate, mix 50 ml of the concentrate solution with 450 ml of water. If less than a whole plate is used, prepare the proportional volume of solution.
Dilute the concentrate conjugate 1/51 with the conjugate diluent according to table 1. For the 1 plate packaging, if the entire plate is to be used, add 300 µl of concentrate conjugate to the bottle containing 15 ml of conjugate diluent. **Mix gently**.

TABLE 1

Strips used	1	2	4	6	8	10	12
Conjugate diluent ml	1.0	2.0	4.0	6.0	8.0	10.0	12.0
Concentrate conjugate µl	20	40	80	120	160	200	240

Assay procedure

1. Use only the number of strips required for the test. Reserve 6 wells for blank and controls. Pipette to the rest of the wells 200 µl of sample diluent and 10 µl of each sample to the designated wells.

2. Transfer 200 µl of negative control to 3 wells and 200 µl of positive control to 2 wells. DO NOT DILUTE CONTROLS. THEY ARE READY TO USE. Leave a well empty for the blank.

3. Cover the plate with an adhesive seal, **mix gently** and incubate for 1 hour at 37°C.

4. Remove and discard the adhesive plate cover. Aspirate the contents of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing 3 more times. Ensure that each column of wells soak for at least 15 seconds before the next aspiration cycle. After the last washing blot the microplate on absorbent tissue to remove any excess liquid from the wells.

5. Transfer 100 µl of diluted conjugate into each well of the microplate, except the blank.

6. Cover the plate with the adhesive seal and incubate for 30 minutes at 37°C.

7. During the last 5-10 minutes of this incubation prepare the substrate-chromogen solution. If the entire plate is used add 280 µl of chromogen (TMB) to the bottle containing the substrate buffer (14 ml) and **mix well**. If the entire plate is not used, follow table 2. The final solution should be colourless; discard if it becomes blue.

   TABLE 2

Strips required	1	2	4	6	8	10	12
Substrate buffer ml	1.0	2.0	4.0	6.0	8.0	10.0	12.0
Chromogen (TMB) µl	20	40	80	120	160	200	240

NOTE: The TMB is dissolved in DMSO. As the melting point of the DMSO is 18°C, the chromogen solution should be allowed to reach a temperature of 20-25°C, and be **well mixed** before use. A yellowish colour is normal for the chromogen solution.

8. Remove and discard the adhesive plate cover. Aspirate and wash the plate as in step 4.

9. Add 100 µl of substrate-TMB solution to each well.

10. Incubate uncovered for 30 minutes at room temperature (20-25°C).

11. Stop the reaction by adding 100 µl of stopping solution in the same sequence and time intervals as for the substrate-TMB.

12. Blank the reader at 450 nm with the blank well and read the absorbance of each well, within 30 minutes. It is recommended to read in bichromatic mode using a 620 - 630 nm reference filter.

Quality control
Results of an assay are valid if the following criteria are accomplished:

1. Substrate blank.
   Absorbance value must be less than or equal to 0.100.

2. Negative control mean (NCx).
   Absorbance of individual negative control values must be less than or equal to 0.200 after subtracting the blank. If one value is outside this range, discard this value and recalculate the mean. If two values are outside range, the run should be repeated.

   Example:

Negative control	Absorbance
1	0.045
2	0.043
3	0.041
Total	0.129

   NCx = 0.129/3 = 0.043
   None of the values in this example has to be discarded.

3. Positive control mean (PCx).
   The positive control mean absorbance must be equal to or greater than 0.600 after subtracting the blank. If the mean is less than 0.600 the run should be repeated.

   Example:

Positive control	Absorbance
1	1.536
2	1.551
Total	3.087

   PCx = 3.087/2 = 1.544

Results

1. Calculate the cut-off value by adding 0.300 to the mean absorbance of the negative control.

   Cut-off = NCx + 0.300

   Example: NCx = 0.043 Cut-off = 0.043 + 0.300 = 0.343

2. Divide the sample absorbance by the cut-off value.

   Positive: ratio absorbance/cut-off ≥ 1.0
   Negative: ratio absorbance/cut-off < 0.9
   Equivocal: ratio absorbance/cut-off ≥ 0.9 < 1.0

Interpretation of results

A positive result indicates infection with *T. cruzi*. The clinical history of the patient must be taken in consideration.

Limitations of the procedure

Samples with positive or equivocal result must be reanalysed in duplicate. If the result is repeatedly positive or equivocal, the sample should be further investigated with other methods.
Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.
As in all sensitive immunoassays, there is the possibility that non-repeatable positive results occur.
A negative result does not exclude the possibility of exposure or infection with *T. cruzi*.

Performance characteristics

Evaluations

The performance of **bioelisa CHAGAS** has been evaluated in several studies against other commercial assays by testing samples from routine blood donors and samples previously classified as positive or negative for *T. cruzi* antibodies.

- In an evaluation in a blood bank, 3024 donor samples were tested in parallel with the routine methods used for screening of Chagas antibodies (EIA, HA and IFA). From these, 21 samples were reactive with the **bioelisa CHAGAS**. Seven of them were confirmed as true positives. Therefore, the sensitivity obtained was 100% and the specificity 99.5%, considering all initially reactive samples in the screening.

- In another blood bank, 2723 samples were assayed in parallel with the routine methods used for screening of Chagas antibodies (EIA, HA and IFA). From these, 72 samples were reactive with the **bioelisa CHAGAS**. Two of them were confirmed as true positives. Therefore, the sensitivity obtained was 100% and the specificity in the screening was 97.4%.

- In a third blood bank, 2655 donor samples were tested in parallel with the routine methods used for screening of Chagas antibodies (EIA, HA and IFA). From these, 57 samples were reactive with the **bioelisa CHAGAS**. Ten of them were confirmed as true positives. In this evaluation the sensitivity obtained was 100% and the specificity in the screening was 98.2%.

- A panel of 498 samples classified as positive (18) or negative (480) was assayed. The sensitivity obtained was 100% and the specificity 98.3%.

- A panel of 115 samples previously classified as true positive was tested. The results obtained for all samples were positive, with 100% sensitivity.

- 796 samples characterised as true negative were assayed, 4 of which showed reaction with the **bioelisa CHAGAS**. In this study the specificity obtained was 99.5%.

Precision

Intra-assay reproducibility:
The coefficients of variation obtained for the absorbance values of a positive sample assayed in 24 replicates were 2.5%, 4.2% and 3.1% in three lots studied.

Inter-assay reproducibility:
Three positive samples of distinct levels were tested in 3 different assays. The coefficients of variation obtained for the ratios absorbance/cut-off of the 3 samples were 4.6%, 3.5% and 8.7%.

bioelisa: Troubleshooting guide

Problem	Possible causes	*Solution*
1. Controls out of validation.	1a. Incorrect temperature, timing or pipetting.	*Check procedure. Repeat assay.*
	1b. Improper preparation of reagents, error of dilution, reagents not well mixed.	*Check procedure. Repeat assay.*
	1c. Cross-contamination of controls.	*Pipette carefully. Do not interchange caps. Repeat assay.*
	1d. Incorrect reading filter.	*Check that the wavelength of the filter used is 450 nm. If no reference filter of 620-630 nm is used, absorbance increases approximately 0.050.*
	1e. Interference in the optical pathway.	*Check the reader. Clean or dry the bottom of microplate. Check for air bubbles. Repeat reading.*
	1f. Used components from different lots.	*Do not use components from different lots as they are adjusted for each batch released.*
	1g. Expired reagents.	*Check the kit expiry date. Use a non-expired kit.*
2. No colour or only a light colour developed at the end of the assay.	2a. One or more reagents not added or added in wrong sequence.	*Check procedure. Repeat assay.*
	2b. Inactive conjugate: improper conservation.	*Check for contamination. Recheck procedure. Repeat assay.*
	2c. Inactive microplate: improper conservation.	*Always keep unused strips in the resealable plastic bag, very well closed, with the desiccant bag inside. Repeat assay.*
	2d. Inactive substrate: improper conservation or dilution, cross-contamination with the stopping solution.	*Always use freshly prepared mixture of substrate buffer and TMB. Recheck procedure. Repeat assay.*

bioelisa: Troubleshooting guide

Problem	Possible causes	*Solution*
3. Too much colour in all microplate wells.	3a. Contaminated, oxidised or improperly prepared substrate.	*Check that substrate is colourless, discard if blue. Make sure that TMB is completely liquid before using. Make sure that TMB is well mixed in the substrate buffer. Use acid washed or disposable containers. Repeat assay.*
	3b. Contaminated or improperly prepared reagents.	*Check for contamination: turbid aspect. Check dilutions. Repeat assay.*
	3c. Contaminated washing solution (1x).	*Check the quality of distilled or deionized water used for dilution. Repeat assay.*
	3d. Insufficient washing or washing not consistent: filling volume and/or aspiration insufficient or not uniform. Insufficient number of washing cycles, contaminated device.	*Check the washing device. Fill wells with washing solution close to the top, aspirate completely.* ***Increase the number of wash cycles****. After washing, blot the inverted microplate on tissue paper.*
	3e. Using of a washing solution from other manufacturer.	*Use only* ***biokit*** *washing solution.*
	3f. Improper dilution of samples.	*Check procedure. Repeat assay.*
4. Poor reproducibility or high number of non repeatable reactive samples.	4a. Washing problems.	*See 3c, 3d, 3e.*
	4b. Uncalibrated pipettes or tips not well fitted. Improper pipetting.	*Use only calibrated pipettes, with well fitted tips and pipette carefully, without bubbles and splashing. Repeat assay.*
	4c. Reagents too cold or not well mixed before using.	*Equilibrate reagents to room temperature and mix thoroughly before using.*
	4d. Air currents over the microplate during incubations.	*Keep the microplate protected from air currents.*
	4e. Too long time for addition of samples and/or reagents. Inconsistency in time intervals. Air bubbles.	*Develop consistent and uniform technique.*
	4f. Interference in the optical pathway.	*See 1e.*