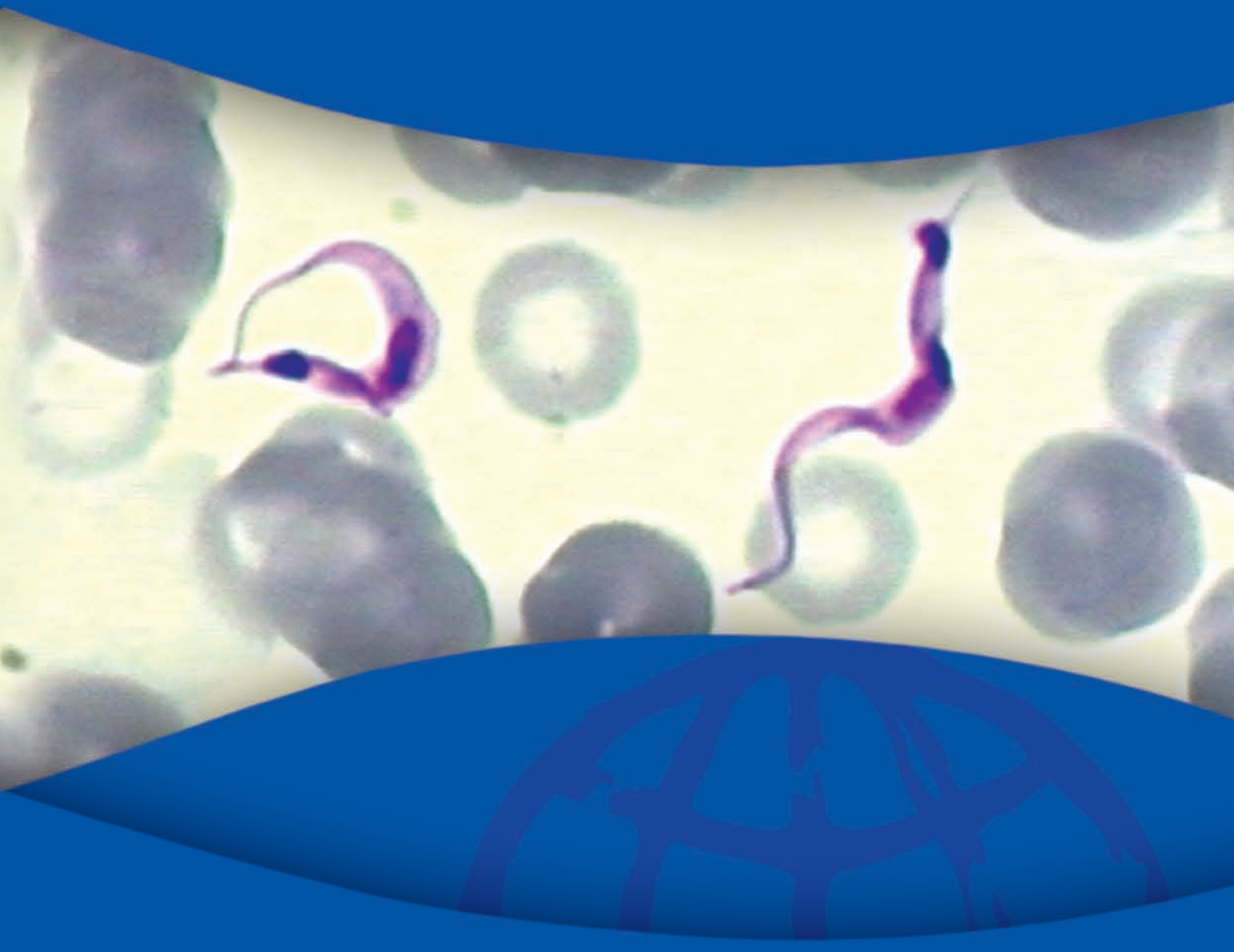
World Health Organization

Anti-*Trypanosoma cruzi* ASSAYS:
Operational Characteristics

Report 1



Diagnostics and Laboratory Technology

World Health Organization

Anti-*Trypanosoma cruzi* ASSAYS

Report 1

Diagnostics and Laboratory Technology

WHO Library Cataloguing-in-Publication Data

Anti-trypanosoma cruzi assays: operational characteristics.

1.Chagas disease - diagnosis. 2.Enzyme-linked immunosorbent assay. 3.Trypanosoma cruzi - immunology. I.World Health Organization.

ISBN 978 92 4 150029 6 **(NLM classification: WC 705)**

Cover image: Giemsa stained blood smear showing a trypomastigote developmental stage with Trypanosoma cruzi. Image provided courtesy of Gustavo Miranda Rocha and Tecia Carvalho from Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brasil.

Design and layout: L'IV Com Sàrl, Le Mont-sur-Lausanne

Printed in France

Contact: Dr G. Vercauteren, Essential Health Technologies - WHO - 20, Avenue Appia - 1211 Geneva 27- Switzerland

TABLE OF CONTENTS

1. INTRODUCTION

The World Health Organization (WHO), conscious of the need to advise Member States on the laboratory diagnosis of Chagas disease, provides objective assessments of the operational characteristics of commercially available assays for serological markers of Chagas disease. This programme is carried out by Hemocentro de São Paulo, Fundação PróSangue, Brazil, a WHO Collaborating Centre, and is coordinated by the Diagnostics and Laboratory Technology, Department of Essential Health Technologies, WHO. The programme provides information that is useful for decision-makers to allow them to make the most suitable choices when procuring diagnostics or establishing testing algorithms. The assessment focuses on the operational characteristics of these assays such as sensitivity, specificity and positive and negative predictive values. Additional information related to incubation temperatures, shelf-life and ease of use is also provided.

This report, documenting the operational characteristics of commercially available assays to detect antibodies to *Trypanosoma cruzi* in biological specimens, presents assessments of the following 23 assays carried out between October 2001 to May 2004.

Group 1: Enzyme immunoassays

- **CHAGAS-ELISA** (EBRAM Produtos Laboratoriais Ltda)
- **CHAGATEK ELISA** (Laboratorio Lemos – Polychaco)
- **Premier™ Chagas' IgG EIA** (Meridian Diagnostics Inc.)
- **TEST ELISA PARA CHAGAS *Ensayo de Segunda generación*** (BIOSChile Ingenería Genética S.A.)
- **BIOELISACRUZI®** (biolab-Mérieux S.A.)
- **HEMAGEN®** (Hemagen Diagnósticos Ltda)
- **CHAGAS-TEST IICS** (Instituto de Investigaciones en Ciencias de la Salud – Paraguay)
- **HBK 401 HEMOBIO CHAGAS** (EMBRABIO – Empresa Brasileira de Biotecnologia S.A.)
- **ABBOTT CHAGAS ANTIBODY EIA** (Abbott Laboratórios do Brasil Ltda)
- **Chagatest ELISA** (Wiener lab)
- **Bioelisa CHAGAS** (BIOKIT, S.A.)

Group 2: Agglutination and rapid assays

Group 2a: Agglutination assays

- **CHAGAS HAI IMUNOSERUM** (Laboratorio Lemos – Polychaco)
- **CHAGAS-HAI** (EBRAM Produtos Laboratoriais Ltda)
- **Imuno-HAI** (WAMA Diagnóstica)
- **CHAGAS HEMAGEN®** (Hemagen Diagnósticos Ltda)
- **HEMACRUZI®** (biolab-Mérieux S.A.)
- **SERODIA-Chagas** (FUJIREBIO INC.)
- **ID-Chagas Antibody Test** (DiaMed)

Group 2b: Rapid assays

- **Chagas STAT-PAK®** (Chembio Diagnostic Systems, Inc.)

Group 3: Confirmatory assays

- **IMUNOCRUZI®** (biolab-Mérieux S.A.)
- **HBK 740 IMUNOBLOT LINHAS anti-*T. cruzi*** (Innogenetics, Belgium)
- **TESA-blot** (biolab-Mérieux S.A.)
- **Radioimmunoprecipitation analysis [RIPA]** (in-house technique from University of Iowa)

This report provides data of evaluation of 19 anti-*Trypanosoma cruzi* test kits using defibrinated serum specimens (Groups 1 and 2); the operational characteristics of three additional assays and one in-house technique (Group 3) were reviewed. Copies of this report are available on request from Diagnostics and Laboratory Technology (DLT), Department of Essential Health Technologies, World Health Organization, 1211 Geneva 27, Switzerland. The report is also available on the DLT website http://www.who.int/diagnostics_laboratory.

2. BACKGROUND INFORMATIION

In August 1999, WHO initiated a programme to provide objective assessments of commercially available assays for the detection of antibodies to *Trypanosoma cruzi*, similar to that which has existed for HIV since 1988 and hepatitis B and hepatitis C

since 1998. This continuing programme is coordinated by Diagnostics and Laboratory Technology, WHO. As there is a lack of comparative performance data on commercially available test kits for the detection of *Trypanosoma cruzi* antibodies, the aim of the evaluation is to supply comparative data to those responsible for deciding which assays to use and potential assays users, in order that they apply their own selection criteria and choose the best assays for their particular situations.

Assays evaluated included Enzyme Immuno-assays (EIAs), agglutination assays and a rapid assay. There are many assays commercially available with suitability for screening large numbers of specimens per day (as would occur in blood transfusion settings) where the results are achieved in a matter of hours. Four confirmatory assays were evaluated: an indirect immunofluorescence assay, an immunoblot, a Western blot and radioimmunoprecipitation analysis (RIPA).

Whether testing is carried out in central laboratories or small health centres, it is necessary to identify the most appropriate assay for each particular circumstance. The need for reliable assays that can be used in situations with limited laboratory facilities continues to drive the development of simpler assays.

Assays that are simple in that they require little or no equipment, have few manipulation steps, can be read visually and are suitable for use on single or low numbers of specimens are appropriate for small rural health centres and primary care centres. Such assays can be carried out by less experienced staff; nonetheless, personnel should have a minimum of training with regard to the operation and interpretation of results, have undergone safety training and operate within the principles of quality assurance.

It is important that the necessary precautions are taken when taking the blood specimen for testing. It is essential that the site from which the specimen is taken be covered to reduce risk of infection and that proper waste disposal facilities for sharps be made available.

3. LABORATORY ASPECTS OF ANTI-*TRYPANOSOMA CRUZI* TESTING

3.1 A brief overview

Chagas disease is a major health problem in Latin America with 15 million people believed to be infected *(2)*. *Trypanosoma cruzi*, the causative agent of Chagas disease, is a protozoan parasite that is naturally transmitted by an insect vector. Due to successful vector control programmes, congenital transmission and transfusion of blood/blood products from infected donors have become the major routes of transmission. *Trypanosoma cruzi* infection is lifelong and can be split into two phases: the brief, mostly asymptomatic, acute phase, where there are large numbers of parasites in the blood; and the chronic phase, with low numbers of parasites in the blood, which may last for 10–30 years after which chronic manifestations such as cardiopathy and dilatation of the colon or oesophagus will develop in a significant percentage of individuals.

The nature of the antigens used in anti-*Trypanosoma cruzi* assays have a bearing on the specificity of the assay for individuals infected with related protozoan diseases such as leishmaniasis as these epitopes may cross-react with certain crude *Trypanosoma cruzi* antigens *(1)*. This is an important consideration for testing in populations where such related diseases are prevalent.

Direct detection of the parasite in the blood by microscopy, haemoculture, xenodiagnosis or nucleic acid detection is highly specific. However, these procedures are technically and operationally demanding, with a lack of sensitivity in the chronic stage when there are low numbers of parasites in the blood.

Hence, diagnosis of *Trypanosoma cruzi* infection is usually made on the basis of detection of antibodies to *Trypanosoma cruzi* antigens. Serological assays for detecting antibodies to *Trypanosoma cruzi* are generally classified as screening assays (sometimes referred to as first-line assays) or confirmatory assays (sometimes referred to as supplemental assays). First-line assays provide the presumptive identification of antibody-reactive specimens, and supplemental assays are used to confirm whether specimens found

reactive with a particular screening assay do indeed contain antibodies specific to *Trypanosoma cruzi*.

When a single screening assay is used for testing in a population with a very low prevalence of Chagas disease, the probability that a individual is infected when a reactive test result is obtained (i.e., the positive predictive value) is very low, since the majority of individuals with reactive results are not infected. This problem occurs even when an assay with high specificity is used. Accuracy can be improved if a second supplemental assay is used to retest all those specimens found reactive by the first assay. Those found non-reactive by the assay are considered negative for antibodies to *Trypanosoma cruzi* .

Specimens with low antibody titer are frequently drawn from individuals in endemic regions. In general, those specimens are difficult to confirm and give a definitive final status. The clinical significance of these specimens and associated potential risk of transmission by an individual presenting with low antibody titer is also little understood. These could represent individuals under spontaneous cure or treated by current antiparasitic medication *(3)* or cross-reactivity with other agents like *Leishmania(4)*.

3.2 Quality assurance

All laboratories carrying out serological and other assays for anti-*Trypanosoma cruzi* should have a well-functioning quality assurance programme. It is most important that quality assurance procedures be stringently applied so as to maximize the accuracy of the laboratory results. Procedures for detecting both technical laboratory and clerical errors must be included in all operating protocols. For example, procedures should be in place to guarantee that the correct results are communicated to the individuals seeking to know their Chagas disease status. External Quality Assessment Schemes (EQAS) are available for anti-*Trypanosoma cruzi* serology and it is recommended that laboratories participate in an EQAS, wherever possible, at least once a year to monitor their performance.

3.3 Safety

The testing of blood specimens should be performed in such a manner as to minimize occupational risk. Guidelines for good laboratory practice have been developed that, if followed, will ensure safety and keep laboratory accidents to a minimum. See *Laboratory Biosafety Manual, third edition,* World Health Organization, Geneva, 2004 (ISBN 92 4 154650 6) and the Global Alert and Response (GAR) section of the WHO website (http://www.who.int//ihr/biosafetypublications/en/index.html) for further information on laboratory biosafety and transport of infectious substances.

4. MATERIALS AND METHODS

4.1 Assays evaluated

Assays for these evaluations were kindly provided free of charge to WHO by four of the assay manufacturers (eight assays). The manufacturers were invited to visit the site at which the evaluations were to be conducted in order to demonstrate the test procedure and to ensure that the assays were performed correctly by the laboratory staff carrying out the evaluation. All of the assays were tested with serum specimens.

Group 1: EIAs

- **CHAGAS-ELISA** (EBRAM Produtos Laboratoriais
  Product code 5.000
  An Enzyme Linked Immunosorbent Assay (ELISA) for the detection of antibodies to inactivated *Trypanosoma cruzi* Y antigen in serum of patients. Results are read visually or in an ELISA plate reader.

- **CHAGATEK ELISA** (Laboratorio Lemos-Polychaco)
  Product code 380
  An ELISA for the detection of antibodies to purified *Trypanosoma cruzi* antigens in serum or plasma of patients. Results are read visually or in an ELISA plate reader.

- **Premier™ Chagas' IgG ELISA** (Meridian Diagnostics Inc.)
  Product code TCE100
  An ELISA for the detection of antibodies to purified *Trypanosoma cruzi* antigen in serum of patients. Results are read in an ELISA plate reader.

- **TEST ELISA PARA CHAGAS** ***Ensayo de Segunda generación*** (BIOSChile Ingeniería Genética S.A.)
  Product code not available
  An ELISA for the detection of antibodies to different strains of *Trypanosoma cruzi* – Y, Tulahuen and MNV2 – in the serum of patients. Results are read visually or in an ELISA plate reader.

- **BIOELISACRUZI®** (biolab-Mérieux S.A.)
  Product code 028.001
  An ELISA for the detection of antibodies to *Trypanosoma cruzi* Y antigen in serum or plasma of patients. Results are read visually or in an ELISA plate reader.

- **HEMAGEN®** (Hemagen Diagnósticos Ltda)
  Product code 66101-01
  An ELISA for the detection of antibodies to antigens extracted from the epimastigote and amastigote forms of *Trypanosoma cruzi* Y and CL in serum of patients. Results are read in an ELISA plate reader.

- **CHAGAS-TEST IICS** (Instituto de Investigaciones en Ciencias de la Salud – Paraguay)
  Product code not available
  An ELISA for the detection of antibodies to antigens from the epimastigote form of *Trypanosoma cruzi* Y in serum of patients. Results are read visually or in an ELISA plate reader.

- **HBK 401 HEMOBIO CHAGAS** (EMBRABIO – Empresa Brasileira de Tecnologia S.A.)
  Product code not available
  An ELISA for the detection of antibodies to *Trypanosoma cruzi* Y antigen in serum or plasma of patients. Results are read visually or in an ELISA plate reader.

- **ABBOTT CHAGAS ANTIBODY EIA** (Abbott Laboratórios do Brasil Ltda)
  Product code 7A07-26
  An Enzyme immunoassay for the detection of antibodies to *Trypanosoma cruzi* Y antigen in serum or plasma of patients. Beads coated in antigen are used , so any number of samples may be processed. The assay is conducted in individual tubes and wells of a reaction tray, and the final results read in a spectrophotometer.

- **Chagatest ELISA** (Wiener Lab.)
  Product code 1293254
  An ELISA for the detection of antibodies to recombinant antigens of *Trypanosoma cruzi* (Ag1, Ag2, Ag3, Ag13, Ag36 and SAPA) in serum or plasma of patients. Results are read in an ELISA plate reader.

- **Bioelisa CHAGAS** (BIOKIT, S.A.)
  Product code 3000-1236
  An ELISA for the detection of antibodies to recombinant antigens of *Trypanosoma cruzi* (TcD, TcE, PEP-2 and TcLo1) in serum or plasma of patients. Results are read in an ELISA plate reader.

Group 2: Agglutination and rapid assays

Group 2a: Agglutination assays

- **CHAGAS HAI IMUNOSERUM** (Laboratorio Lemos Polychaco)
  Product code 10200
  Sheep red blood cells are sensitized by binding *Trypanosoma cruzi* antigen to the red blood cell surface. Patient serum or plasma is added and if there are specific antibodies for the antigen in the serum a typical lattice will develop. If no specific antibodies are present the red blood cells will precipitate to form a button on the bottom of the well.

- **CHAGAS-HAI** (EBRAM Produtos Laboratoriais Ltda)
  Product code 200
  Bird red blood cells are sensitized by binding *Trypanosoma cruzi* Y antigen to the red blood cell surface. Patient serum is added and if there are specific antibodies for the antigen in the serum a typical lattice will develop. If no specific antibodies are present the red blood cells will precipitate to form a button on the bottom of the well.

- **Imuno-HAI** (WAMA Diagnóstica)
  Product code 34200-H
  Bird red blood cells are sensitized by binding purified *Trypanosoma cruzi* Y antigen to the red blood cell surface. Patient serum is added and if there are specific antibodies for the antigen in the serum a typical lattice will develop. If no specific antibodies are present the red blood cells will precipitate to form a button on the bottom of the well.

- **CHAGAS HEMAGEN®** (Hemagen Diagnósticos Ltda)
  Product code 6419
  Human red blood cells are sensitized by binding epimastigote and amastigote forms of *Trypanosoma cruzi* Y and CL antigens to the red blood cell surface. Patient serum is added and if there are specific antibodies for the antigen in the serum a typical lattice will develop. If no specific antibodies are present the red blood cells will precipitate to form a button on the bottom of the well.

- **HEMACRUZI®** (biolab-Mérieux S.A.)
  Product code 029.015
  Bird red blood cells are sensitized by binding purified *Trypanosoma cruzi* Y antigen to the red blood cell surface. Patient serum is added and if there are specific antibodies for the antigen in the serum a typical lattice will develop. If no specific antibodies are present the red blood cells will precipitate to form a button on the bottom of the well.

- **SERODIA-Chagas** (FUJIREBIO INC.)
  Product code not available
  This is a passive particle agglutination assay. Gelatin particle carriers are sensitized with inactivated *Trypanosoma cruzi* RF antigen. Patient serum or plasma containing specific antibodies will react with the coloured gelatin particles to form a smooth mat of agglutinated particles in the microtitre well. Negative reactions are characterised by a compact button formed by the settling of the nonagglutinated particles. Results are read visually or on a tray viewer with indirect lighting.

- **ID-Chagas Antibody Test** (DiaMed-ID Micro Typing System)
  Product code 020022
  The ID-Chagas test is a particle gel immunoassay. Red coloured particles are sensitised with three different synthetic peptides representing antigen sequences of Trypanosoma cruzi: Ag2, TcD and TcE. When these particles are mixed with serum or plasma containing specific antibodies, they agglutinate. The reaction mixture is centrifuged through a gel filtration matrix allowing free agglutinated particles to remain trapped on the top or distributed within the gel. The result can be read visually; positive results are indicated by red particles within or above the gel matrix in a tube, while negative results are indicated by the red particles at the bottom of the tube, beneath the gel matrix.

Group 2b: Rapid assay

- **Chagas STAT-PAK®** (Chembio Diagnostic Systems Inc)
  Product code CG101
  The Chembio Chagas STAT-PAK® is a rapid immunochromatographic test for the detection of antibodies to *Trypanosoma 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 test specimen is applied to the sample well. As the test specimen flows laterally across the membrane, the specific antibody binding protein dye conjugate binds to the human immunoglobulins in the specimen. Then, if the specimen contains any antibodies to *Trypanosoma cruzi*, the complex binds to the antigens on the solid phase in the test area producing a pink/purple line. In the absence of *Trypanosoma cruzi* antibodies there is no line in the test area. The Chembio Chagas STAT-PAK® also provides an integral control feature. The specimen continues to migrate along the membrane and produces a pink/purple line in the control zone, demonstrating that the reagents are functioning properly.

Group 3: Confirmatory assays

- **IMUNOCRUZI®** (biolab-Mérieux S.A.)
  Product code 022.002
  This is an indirect immnuofluorescence assay.

A glass slide is coated with antigen from *Trypanosoma cruzi* Y epimastigote form. Reactive sera will cause antibodies to attach to the antigens on the slide. Following addition of anti-human globulin that is conjugated to a fluorescent compound, results are read under a fluorescent microscope. Positive results are visualised by green fluorescence, whereas negative results show no fluorescence.

- **HBK 740 IMUNOBLOT LINHAS anti-*T.cruzi*** (EMBRABIO – Empresa Brasileira de Biotecnologia S.A.)
  Product code not available
  This is an immunoblot assay. *Trypanosoma cruzi* CRA, FRA, TcD, MAP, SAPA, Ag39 and Tc24 antigens are coated onto a nylon strip. If a person is infected, they will have antibodies to these viral proteins. When the serum or plasma sample is added, antibodies attach to the viral proteins and form a complex which is visible in a colour reaction. If a person is not infected, no antibodies will be present, so no reaction will occur.

- **TESA-blot** (biolab-Mérieux S.A.)
  Product code not available
  This is a Western blot utilising tripomastigote secreted and excreted antigen forms of *Trypanosoma cruzi*. The antigens are collected from the supernatant of infected cell cultures, separated by SDS-polyacrylamide gel electrophoresis, and transferred onto nitrocellulose membrane. To detect the antigen blotted on the membrane, serum or plasma is added and incubated with the membrane. If there are any antibodies present which are directed against one or more of the blotted antigens, those antibodies will bind to the protein(s). In order to detect the antibodies which have bound, anti-immunoglobulin antibodies coupled to a conjugate are added. A substrate is added which will react with the conjugate resulting in a visible band.

- **Radioimmunoprecipitation analysis [RIPA]** (in-house technique from University of Iowa)
  Product code not available.

4.2 WHO anti-*Trypanosoma cruzi* specimen reference panel

Ten blood transfusion centers in Latin America contributed to this programme by sending positive and negative plasma units, as defined by their own testing criteria, to Hemocentro de São Paulo. A total of 437 units/specimens were retained for the WHO anti-*T.cruzi* specimen reference panel. A summary of the specimens is shown in Table 1.

4.2.1 Plasma specimen treatment

The plasma units were converted to serum by the following defibrination process: 0.5 mL of a 0.2M $CaCl_2$ solution was mixed with 100 ml of plasma, and incubated at 37°C for 2 hours and then at 4°C for 24 hours. The plasma was centrifuged at 6000 × g for 30 minutes to separate the serum from the fibrin

Table 1. Description of plasma units and their original test results as submitted by each institution

Country of collection	Collecting institution	Assays used for screening of the blood units	Total
Argentina	Hospital de Pediatría	Chagatek ELISA + Serodia Chagas	45
Bolivia	Hospital Clínico "Viedma"	Chagatek ELISA + Chagas HAI Imunoserum	40
Brazil	FPS/HSP	Hemacruzi + Imunocruzi + Hemobio Chagas	146
Colombia	Instituto Nacional de Salud	IFI Freddy Chaparro	24
Ecuador	Cruz Roja Ecuatoriana	Chagatek ELISA+ CRE ELISA	26
El Salvador	Cruz Roja Salvadoreña	Chagas Anticorpos EIA + Hemobio Chagas	31
Honduras	Cruz Roja Hondureña	Chagas Anticorpos EIA + Hemobio Chagas	40
Mexico	Centro Nacional de Transfusión	Chagas Anticorpos EIA + Chagatest ELISA	10
Nicaragua	Cruz Roja Nicaragüense	Chagas Anticorpos EIA + Hemobio Chagas	31
Paraguay	Instituto de Investigación en Ciencias de la Salud	Chagas-Test IICS + IFI caseiro	44
Total			437

clot. To remove $CaCl_2$, the serum was dialyzed using a cellulose membrane that retains proteins of MW 12,000 kDa or greater (Sigma Cat. No. D-9652, Steinheim, Germany), and then filtered through a 5.0 µm-pore-size membrane (Sigma Cat. No. N-3771, Steinheim, Germany) to remove fibrin particles. Bronidox L (Henkel Chemicals, Dusseldorf, Germany) was added to a final concentration of 0.05%. A total of 10 aliquots of 1.5 mL each were prepared. The remaining serum was stored at -20°C until testing commenced.

4.2.2 Characterization of the panel specimens

The panel was characterized with the following four confirmatory assays:

- Indirect immunofluorescence assay (IF) IMUNOCRUZI® [biolab-Mérieux S.A, Rio de Janeiro, Brazil]
- INNO-LIA™ Chagas assay (IB) [Innogenetics, Ghent, Belgium]
- Western blot (WB) [TESA Blot – bioMérieux, Rio de Janeiro, Brazil]
- Radio immunoprecipitation assay (RIPA). This assay was performed at the University of Iowa as previously described*(5)*.

The final serological status of each specimen was defined as follows:

- anti-*T.cruzi* **Positive:** specimens positive in at least three out of four confirmatory assays
- anti-*T.cruzi* **Negative:** specimens negative in at least three confirmatory assays
- anti-*T.cruzi* **Inconclusive:** specimens positive in two out of four confirmatory assays.

Refer to Table 2 for further detail.

Table 2. Composition of WHO anti-*Trypanosoma cruzi* specimen reference panel

Anti-*Trypanosoma cruzi* positive	Anti-*Trypanosoma cruzi* negative	Total
168	262	430
39%	61%	100%

A total of seven specimens were considered inconclusive as a definitive reference result could not be made. These were excluded from the analysis; those results are described on Annex 2.

4.3 Laboratory testing

Testing was performed according to the manufacturers' instructions. Usually, one person carried out all the tests. The tests on initially reactive specimens were repeated. Specimens with discrepant results were repeated in duplicate. Two out of three results determined the overall test outcome.

4.4 Data analysis

4.4.1 Sensitivity, specificity, confidence intervals and predictive values of anti-*Trypanosoma cruzi* assays

The formula for calculation of sensitivity, specificity and predictive values is represented diagrammatically in Table 3.

Table 3. Calculation of sensitivity, specificity and predictive values

		True anti-*Trypanosoma cruzi* status[1]		
		+	–	
Results of assay under evaluation	+	**a True-positives**	b False-positives	a+b
	–	c False-negatives	**d True-negatives**	c+d
		a+c	b+d	

Sensitivity = a/(a+c) Positive predictive value = a/(a+b)
Specificity = d/(b+d) Negative predictive value = d/(c+d)
[1]: Status according to reference results

Sensitivity is the ability of the assay under evaluation to identify correctly specimens that contain *Trypanosoma cruzi* antibodies (where the reference result is positive). Thus, sensitivity is the number of true positive specimens recognized by the assay under evaluation as positive, divided by the number of specimens identified by the reference assays as positive, expressed as a percentage.

Specificity is the ability of the assay under evaluation to identify correctly specimens that do not contain antibody to *Trypanosoma cruzi* (where reference result is negative). Thus specificity is the number of

true negative specimens recognized by the assay under evaluation as negative, divided by the number of specimens identified by the reference assays as negative, expressed as a percentage.

95% confidence intervals are a means of determining whether sensitivity and specificity data calculated from results derived from laboratory-based evaluations carried out on a reasonable number of specimens i.e. a sample, will represent the total population. In order to calculate the exact 95% confidence intervals for binomial proportions, it is recommended that calculations be made from the F-distribution as the proportion is nearing close to 1.0 *(6)*.

Predictive values:

The **positive predictive value** (PPV) is the probability that when the test is reactive, the specimen does contain antibodies to *Trypanosoma cruzi*. This may be calculated in two ways:

1. using the simple formula a/(a+b) which will give an approximate value (see Table 3)
2. using the more precise formula which takes the prevalence of Chagas disease in the population into account.

$$PPV = \frac{(prevalence)(sensitivity)}{(prevalence)(sensitivity) + (1\text{-}prevalence)(1\text{-}specificity)}$$

The **negative predictive value** (NPV) is the probability that when a test result is negative, the specimen does not contain antibodies to *T.cruzi* (or is truly negative). This may be calculated two ways:

1. using the simple formula d/(c+d) which will give an approximate value (see Table 3)
2. using the more precise formula which takes the prevalence of Chagas disease in the population into account.

$$NPV = \frac{(1\text{-}prevalence)(specificity)}{(1\text{-}prevalence)(specificity) + (prevalence)(1\text{-}sensitivity)}$$

The probability that a test will accurately determine the true infection status of an individual being tested varies with the prevalence of *T.cruzi* infection in the population from which the individual comes. In general, the higher the prevalence of *T.cruzi* infection in the population, the greater the probability that an individual testing positive is truly infected (i.e., the greater the PPV). Thus, with increasing prevalence, the proportion of false-positive decreases; conversely, the likelihood that an individual showing negative test results is truly uninfected (i.e., the NPV), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of individuals testing false-negative. However, this effect only becomes apparent at prevalence of 80% and above.

4.4.2 Additional analyses

The technical aspects of the assays under evaluation were assessed by the technician who performed the testing. These assessments, along with other selected assay characteristics, contributed to an overall appraisal of the suitability of each assay for use in small laboratories. To enable comparison between assays, an arbitrary scoring system was used to rate specified assay characteristics.

5. ASSAY EVALUATIONS

Results from the test kits evaluated are divided into groups, as described in Section 1. Results from Group 1 assays (11 EIAs) are presented in Tables 1-6. Where necessary, tables are divided for ease of use (e.g., Table 1a contains information on the first six EIAs, and Table 1b on the remaining five EIAs). Results from Group 2 assays (7 agglutination assays and 1 rapid assay) are presented in Tables 7-12. Results from Group 3 assays (3 confirmatory assays) are presented in Tables 13-18. Tables 1, 7 and 13 summarize general characteristics of assays for Groups 1, 2 and 3, respectively. Results of the assays evaluated as compared to the reference tests are given in Tables 2, 8 and 14; Tables 3, 9 and 15 detail operational aspects. Technician appraisals of the test kits are summarized in Tables 4, 10 and 16. Factors in the calculation of ease of performance and suitability for use in small laboratories are listed in Tables 5 and 6 for Group 1, in Tables 11 and 12 for Group 2, and in Tables 17 and 18 for Group 3. Explanatory notes are provided under each table. Care should be taken when interpreting these results due to the relatively small sample size (n=430). Interpretation of sensitivity and specificity results should also take the 95% CI into account.

ASSAY EVALUATIONS

Group 1: Enzyme Immunoassays (EIAs

Table 1a. General characteristics and operational aspects: Group 1 EIAs, 1-6

NAME	CHAGAS – ELISA	CHAGATEK ELISA	Premier™ Chagas' IgG ELISA	TEST ELISA PARA CHAGAS Ensayo de Segunda generación	BIOELISACRUZI®	HEMAGEN®
Company	Ebram Produtos Laboratoriais Ltda	Laboratorio Lemos, Polychaco	Meridian Diagnostics, Inc.	BIOSChile Ingeniería Genética S.A.	biolab-Mérieux S.A.	Hemagen Diagnósticos Ltda
Assay type	Enzyme immunoassay	Enzyme immunoassay	Enzyme immunoassay	Enzyme immunoassay	Enzyme immunoassay	Enzyme immunoassay
Antigen type	*T. cruzi* Y inactivated antigen	*T. cruzi* purified antigen	*T. cruzi* purified antigen	Different strains of *T. cruzi* Y, Tulahuen and MNV2	*T. cruzi* Y antigen	Extracted from epimastigote and amastigote form of *T. cruzi* (strain Y and L)
Solid phase	Microplate (96 wells)	Microplate (96 wells)	Microplate (96 wells)	Microplate (96 wells)	Microplate (96 wells)	Microplate (96 wells)
Specimen type	Serum	Serum or plasma	Serum	Serum	Serum or plasma	Serum
Number of tests per kit (product code)	96 (5.000)	192 (380)	96 (TCE100)	96 (N/A)	192 (028.001)	192 (66101-01)
Lot numbers evaluated (expiry date)	0501 (7/2002) and 0803 (9/2002)	010306 (5/2002)	TCE100.013 (6/2002)	0730401 (5/2002)	0730401 (5/2002)	1CC0504 (8/2002)
Shelf life at (°C)	12 months (2-8)	N/D (2-8)	N/D (2-8)	12 months (2-8)	N/D (2-8)	N/D (2-8)
Volume of serum needed (µl) Final dilution of serum	10 1 : 10	10 1 : 21	10 1 : 21	20 1 : 11	5 1 : 201	10 1 : 26
Total time to perform the assay h. min	1.40	1.20	2.00	2.00	2.00	1.40
Reading	450 nm	450 nm	405 nm	450 nm	492/620 nm	450 nm
Indicative Price/ test US$ (as given at time of evaluation)	1.02 (96 tests)	0.85 (192 tests)	1.03 (96 tests)	1.80 (96 tests)	0.76 (192 tests)	1.89 (192 tests)

N/A: not applicable; N/D: not determined.

Table 1b. General characteristics and operational aspects: Group 1 EIAs, 7-11

NAME	CHAGAS – TEST *IICS*	HBK 401 HEMOBIO CHAGAS	ABBOTT CHAGAS ANTIBODY EIA	Chagatest ELISA	bioelisa CHAGAS
Company	Instituto de Investigaciones en Ciencias de la Salud – Paraguay	EMBRABIO – Empresa Brasileira de Biotecnologia S.A.	Abbott Laboratórios do Brasil Ltda	Wiener lab	BIOKIT, S.A.
Assay type	Enzyme immunoassay	Enzyme immunoassay	Enzyme immunoassay	Enzyme immunoassay	Enzyme immunoassay
Antigen type	Epimastigote form of *T. cruzi* Y	*T. cruzi* Y antigen	*T. cruzi* Y antigen	Recombinant antigens of *T.cruzi* (Ag1,2,3,SAPA,13 and 36)	Recombinant antigens of *T.cruzi* (TcD, TcE, PEP-2 and TcLo1)
Solid phase	Microplate (96 wells)	Microplate (96 wells)	Beads	Microplate (96 wells)	Microplate (96 wells)
Specimen type	Serum	Serum or plasma	Serum or plasma	Serum or plasma	Serum or plasma
Number of tests per kit (product code)	96 (N/A)	384 (N/A)	100 (7A07-26)	96 (1293254)	96 (3000-1236)
Lot numbers evaluated (expiry date)	395013 (10/2002)	ECA12L11 (6/2001)	72169QP (4/2001)	009544 (9/2001)	k-3801 (7/2002)
Shelf life at (°C)	N/D (2-8)	N/D (2-8)	4 months (2-8)	N/D (2-8)	N/D (2-8)
Volume of serum needed (µl) Final dilution of serum	10 1 : 51	5 1 : 201	10 1 : 40	10 1 : 21	10 1 : 21
Total time to perform the assay h. min	2.00	1.45	2.30	1.40	2.30
Reading	405 nm	450/630 nm	492/600 nm	450 nm	450/630 nm
Indicative Price/test US$ (as given at time of evaluation)	Not available (96 tests)	0.57 (192 tests)	1.86 (100 tests)	0.94 (96 tests)	2.25 (96 tests)

N/A: not applicable; N/D: not determined.

Table 2a. Comparison of the assays with reference results: Group 1 EIAs, 1-6

NAME		CHAGAS – ELISA	CHAGATEK ELISA	Premier™ Chagas' IgG ELISA	TEST ELISA PARA CHAGAS Ensayo de Segunda generación	BIOELISACRUZI®	HEMAGEN®
Final Sensitivity % (95 CI) n = 168		97.70 (94.0–99.3)	99.40 (96.7–100)	94.04 (89.3–97.1)	99.40 (91.2–98.1)	98.2 (94.9–99.6)	100 (97.8–100)
Specificity % (95 CI) n = 262		97.62 (95.1–99.2)	99.24 (97.3–99.9)	100 (98.6–100)	99.61 (97.9–100)	99.24 (97.3–99.9)	96.56 (93.6–98.4)
Indeterminate results %		0.68	0.92	0.46	0	1.83	0.46
Initial inter-reader variability %		N/A	N/A	N/A	N/A	N/A	N/A
PPV	0.1%	4	11.53	100	21	11	3
	5%	69	87.26	100	93	87	61
	10%	82	93.53	100	97	93	73
NPV	0.1%	99.99	99.99	99.99	99.99	99.99	100
	5%	98.87	99.96	99.68	99.96	99.90	100
	10%	99.72	99.68	99.34	99.93	99.80	100

CI: Confidence intervals; N/A: not applicable; NPV: negative predictive value; PPV: positive predictive value.

Table 2b. Comparison of the assays with reference results: Group 1 EIAs, 7-11

NAME		CHAGAS – TEST //CS	HBK 401 HEMOBIO CHAGAS	ABBOTT CHAGAS ANTIBODY EIA	Chagatest ELISA	bioelisa CHAGAS
Final Sensitivity % (95 CI) n = 168		97.02 (93.2–99.0)	100 (97.8–100)	99.40 (96.2–100)	98.80 (95.8–99.9)	100 (97.8–100)
Specificity % (95 CI) n = 262		99.60 (97.3–99.9)	99.62 (97.9–100)	98.09 (95.6 –99.4)	99.62 (97.9–100)	99.23 (97.3–99.9)
Indeterminate results %		0.23	0.23	0.69	1.606	0
Initial inter-reader variability %		N/A	N/A	N/A	N/A	N/A
PPV	0.1%	23.68	25.64	5.18	25.34	12.84
	5%	92.73	93.27	73.25	93.19	87.24
	10%	96.42	96.69	85.26	96.65	94.52
NPV	0.1%	99.99	100	99.99	99.99	100
	5%	99.84	100	100	100	100
	10%	99.66	100	99.93	99.86	100

CI: Confidence intervals; N/A: not applicable; NPV: negative predictive value; PPV: positive predictive value.

Table 3a. Detailed operational aspects: Group 1 EIAs, 1-6

NAME	CHAGAS – ELISA	CHAGATEK ELISA	Premier™ Chagas' IgG ELISA	TEST ELISA PARA CHAGAS Ensayo de Segunda generación	BIOELISACRUZI®	HEMAGEN®
Dimension (cm) of kit: w-l-h	9.8–14.1–8	15.2–16.5–10	15–20.5–8.5	12.5–20.4–9.5	12.3–18.5–10.5	17.6–21.5–9
Storage conditions (°C)	2–8	2–8	2–8	2–8	2–8	2–8
Incubation temperature (°C)	37	37	37 ± 2	37 ± 1	37	RT (18–26)
Reading endpoint stability (h.min)	0.30	0.20	0.60	N/D	N/D	N/D
Stability after dilution/ reconstitution/opening at (°C)						
- antigen	Exp.date (2-8)	Exp.date (2-8)	Exp.date (2-8)	Exp.date (2-8)	Exp.date (2-8)	6 months (2-8)
- controls	N/A	N/A	N/A	N/A	N/A	30 days (2-8)
- sample diluent	N/A	N/A	N/A	N/A	N/A	N/A
- conjugate	N/A	4 hrs (18–25)	N/A	N/A	15 days (4)	30 days (2-8)
- substrate	N/I	N/A	N/A	N/I	N/I	N/A
- wash buffer	1 year (2-8)	15 days	Exp. date (2-8)	N/I	4 weeks (4)	N/I
Number of sera per run minimum – maximum	1–91	1–93	1–90	1–91	1–90	1–93
Number of controls per test run	5	3	6	5	6	3
- negative	2	2	1	2	4	1
- cut-off/weak positive	0	0	3	2	0	0
- positive	2	1	1	1	2	1
- blank	1	0	1	0	0	1
internal control:						
reagent control	0	0	0	0	0	0
specimen addition control	0	0	0	0	0	0
Estimated time to perform one run: h. min	1.40	1.20	2.00	2.00	2.00	1.40
Equipment needed but not provided in the kit:[1]						
- washer	+	+	+	+	+	+
- incubator (water-bath)	+	+	+	+	+	+
- spectrophotometric reader	+	+	+	+	+	+
- refrigerator (storage)	+	+	+	+	+	+
- agitator , rocker	+/-	+/-	+/-	+/-	+/-	+/-
- aspiration device	-	-	-	-	-	-
- automatic pipette (µl)	+	+	+	+	+	+
- multichannel (µl)	+	+	+	+	+	+
- disposable tips	+	+	+	+	+	+
- dilution tubes/rack, microtiterplate	+	+	+	+	+	+
- distilled or deionised water	+	+	+	+	+	+
- plate covers	-	-	-	-	-	-
- graduated pipette; cylinder (ml)	+	+	+	+	+	+
- sulfuric acid/sodium hydroxide	-	-	-	-	-	-
- absorbent paper	+	+	+	+	+	+
- disinfectant	+	+	+	+	+	+
- gloves	+	+	+	+	+	+
- reagent trough	+	+	+	+	+	+
- timer	+	+	+	+	+	+
Definition of positive results	≥ NC+0.100 x 0.1	≥ NC+1.00	≥ reference serum	≥ 0.35 x (NC+PC)	≥ NC+0.250	≥ NC+0.250
Definition of grey zone or indeterminate result	Cutoff ± 10%	N/A	Cutoff ± 10%	Cutoff ± 10%	Cutoff ± 20%	N/A

[1] + : not provided in the kit but necessary to perform the test; - : provided in the kit or not necessary to perform the test; +/- : use is optional.

N/A: not applicable; NC: negative control; N/D: not determined; N/I: no information; PC: positive control; RT: room temperature.

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

Table 3b. Detailed operational aspects: Group 1 EIAs, 7-11

NAME	CHAGAS – TEST //CS	HBK 401 HEMOBIO CHAGAS	ABBOTT CHAGAS ANTIBODY EIA	Chagatest ELISA	bioelisa CHAGAS
Dimension (cm) of kit: w-l-h	14.1–20–13	20–30–12	16.7–20.7–9	10–18.2–14	14.3–16.5–9.7
Storage conditions (°C)	2–8	2–8	2–8	2–10	2–8
Incubation temperature (°C)	RT	37	38–42	37	37
Reading endpoint stability (h.min)	3.00	0.30	2.00	0.30	0.30
Stability after dilution/reconstitution/ opening at (°C)					
- antigen	Exp.date (2-8)	Exp. date (2-8)	Exp. date (2-8)	5 months (2-10)	Exp. date (2-8)
- controls	N/A	N/A	N/A	N/A	N/A
- sample diluent	3 months (2-8)	N/A	N/A	N/A	N/A
- conjugate	N/A	N/I	20 days (2-8)	N/A	15 days (2-8)
- substrate	3 months (2-8)	N/I	60 min. (25)	N/I	not stable
- wash buffer	N/A	4 weeks (25)	N/A	3 months (RT)	2 weeks (2-8)
Number of sera per run minimum – maximum	1–92	1–91	1–89	1–91	1–91
Number of controls per test run	4	5	7	5	5
- negative	2	3	3	3	3
- cut-off/weak positive	0	0	0	0	0
- positive	2	2	3	2	2
- blank	0	0	1	0	0
internal control:					
reagent control	0	0	0	0	0
specimen addition control	0	0	0	0	0
Estimated time to perform one run: h. min	2.00	1.45	2.30	1.40	2.30
Equipment needed but not provided in the kit:[1]					
- washer	+	+	+	+	+
- incubator (water-bath)	+	+	+	+	+
- spectrophotometric reader	+	+	+	+	+
- refrigerator (storage)	+	+	+	+	+
- agitator, rocker	+/-	+/-	+/-	+/-	+/-
- aspiration device	-	-	-	-	-
- automatic pipette (µl)	+	+	+	+	+
- multichannel (µl)	+	+	+	+	+
- disposable tips	+	+	+	+	+
- dilution tubes/rack, microtiterplate	+	+	-	+	+
- distilled or deionised water	+	+	+	+	+
- plate covers	-	-	-	-	-
- graduated pipette; cylinder (ml)	+	+	+	+	+
- sulfuric acid/sodium hydroxide	-	-	-	-	-
- absorbent paper	+	+	+	+	+
- disinfectant	+	+	+	+	+
- gloves	+	+	+	+	+
- reagent trough	+	+	+	+	+
- timer	+	+	+	+	+
Definition of positive results	≥ NC+0.200	≥ NC+0.300	≥ NC+(0.36 x PC)	≥ NC+0.300 x 0.1	≥ NC+0.300 x 0.1
Definition of grey zone or indeterminate result	N/A	N/A	N/A	Cutoff ±10%	N/A

[1] + : not provided in the kit but necessary to perform the test; - : provided in the kit or not necessary to perform the test; +/- : use is optional

N/A: not applicable; NC: negative control; N/I: no information; PC: positive control; RT: room temperature.

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

Table 4a. Technician's appraisal of the test kits: Group 1 EIAs, 1-6

NAME	Score	CHAGAS – ELISA	CHAGATEK ELISA	Premier™ Chagas' IgG ELISA	TEST ELISA PARA CHAGAS Ensayo de Segunda generación	BIOELISACRUZI®	HEMAGEN®
Number of steps in the test procedure: -1-2 steps -3-5 steps - >5 steps	 6 3 1	1	1	1	1	1	1
Clarity of kit instructions: - good - needs improvement	 2 1	1	1	1	1	1	1
Kit and reagent packaging and labelling: - good - needs improvement	 2 1	2	2	2	2	2	2
Total (out of possible 10)	10	4	4	4	4	4	4
Comments on the test kit		N/A	N/A	N/A	N/A	N/A	N/A

N/A: not applicable.

Table 4b. Technician's appraisal of the test kits: Group 1 EIAs, 7-11

NAME	Score	CHAGAS – TEST //CS	HBK 401 HEMOBIO CHAGAS	ABBOTT CHAGAS ANTIBODY EIA	Chagatest ELISA	bioelisa CHAGAS
Number of steps in the test procedure: -1-2 steps -3-5 steps - >5 steps	 6 3 1	1	1	1	1	1
Clarity of kit instructions: - good - needs improvement	 2 1	1	1	2	1	2
Kit and reagent packaging and labelling: - good - needs improvement	 2 1	1	2	2	2	2
Total (out of possible 10)	10	3	4	5	4	5
Comments on the test kit		N/A	N/A	N/A	N/A	N/A

N/A: not applicable.

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

Table 5a. Calculation of ease of performance: Group 1 EIAs, 1-6

NAME	CHAGAS – ELISA	CHAGATEK ELISA	Premier™ Chagas' IgG ELISA	TEST ELISA PARA CHAGAS Ensayo de Segunda generación	BIOELISACRUZI®	HEMAGEN®
Need to prepare:[1]						
-antigen	1	1	1	1	1	1
-substrate	1	1	0	0	0	1
-wash solution	0	0	0	0	0	0
-conjugate	1	0	1	1	1	0
-predilution of serum	1	1	0	1	0	0
Stability after dilution/opening: (expiry date = 1; less = 0)						
-antigen	1	1	1	1	1	1
-controls	1	1	1	1	1	1
-sample diluent	1	1	1	1	1	1
-conjugate	1	1	1	1	1	0
-substrate	1	1	0	0	0	0
-wash buffer	1	1	1	1	0	1
Sufficient reagents (yes =0; no = 1)	1	1	1	1	1	1
Wash step required (yes =0; no = 1)	0	0	0	0	0	0
Item needed but not provided in the kit:						
-reagent trough	0	0	0	0	0	0
-automatic /multichannel pipette	0	0	0	0	0	0
-dilution – tubes, rack/microtiter plate	0	0	0	0	0	0
-distilled or deionised water	0	0	0	0	0	0
-plate covers	1	1	1	1	1	1
-graduated pipette, cylinder	0	0	0	0	0	0
-sulfuric acid/sodium hydroxide	1	1	1	1	1	1
Technician's appraisal of the test kit[2] (rating out of 10)	4	4	4	4	4	4
Total (out of possible 30)	17	16	14	15	13	13
Ease of performance: -less easy < 20 -easy 20 ≤ x ≤ 25 -very easy > 25	Less easy	Less easy	Less easy	Less easy	Less easy	Less easy

[1] 1 : positive rating: reagent needs no preparation; item provided in the kit; 0 : negative rating: reagent needs preparation; item not provided in the kit
[2] See Table 4a.

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

Table 5b. Calculation of ease of performance: Group 1 EIAs, 7-11

NAME	CHAGAS – TEST //CS	HBK 401 HEMOBIO CHAGAS	ABBOTT CHAGAS ANTIBODY EIA	Chagatest ELISA	bioelisa CHAGAS
Need to prepare:[1]					
-antigen	1	1	1	1	1
-substrate	0	0	0	0	0
-wash solution	1	0	1	0	0
-conjugate	1	0	0	1	0
-predilution of serum	0	0	0	1	1
Stability after dilution/opening: (expiry date = 1; less = 0)					
-antigen	1	1	1	1	1
-controls	0	1	1	1	1
-sample diluent	0	1	1	1	1
-conjugate	1	0	0	1	0
-substrate	0	0	0	0	0
-wash buffer	1	1	1	1	0
Sufficient reagents (yes =0; no = 1)	1	1	1	1	1
Wash step required (yes =0; no = 1)	0	0	0	0	0
Item needed but not provided in the kit:					
-reagent trough	0	0	0	0	0
-automatic /multichannel pipette	0	0	0	0	0
-dilution – tubes, rack/microtiter plate	0	0	1	0	0
-distilled or deionised water	0	0	0	0	0
-plate covers	1	1	1	1	1
-graduated pipette, cylinder	0	0	0	0	0
-sulfuric acid/sodium hydroxide	1	1	1	1	1
Technician's appraisal of the test kit[2] (rating out of 10)	3	4	5	4	5
Total (out of possible 30)	12	12	15	15	13
Ease of performance: -less easy < 20 -easy 20 ≤ x ≤ 25 -very easy > 25	Less easy	Less easy	Less easy	Less easy	Less easy

[1] 1 : positive rating: reagent needs no preparation; item provided in the kit; 0 : negative rating: reagent needs preparation; item not provided in the kit
[2] See Table 4b.

Table 6a. Suitability for use in small laboratories: Group 1 EIAs, 1-6

NAME	Score	CHAGAS – ELISA	CHAGATEK ELISA	Premier™ Chagas' IgG ELISA	TEST ELISA PARA CHAGAS Ensayo de Segunda generación	BIOELISACRUZI®	HEMAGEN®
Sensitivity - 100% - 98–100% - <98%	 5 3 0	0	3	0	3	3	5
Specificity - >98% - 95–98% - <95%	 5 3 0	3	5	5	5	5	3
Incubation temperature - room temperature - other than room temperature	 3 1	1	1	1	1	1	3
Shelf-life - >1 year - > 6 months < 1 year - < 6 months	 3 2 1	2	1	1	2	1	1
Storage at - ambient temperature possible (opened kit) - ambient temperature possible (unopened kit) - 2-8°C required	 5 2 1	1	1	1	1	1	1
Price per test (US$) - < 1.0 - < 2.0 - > 2.0	 3 2 1	2	3	2	2	3	2
Ease of performance - very easy - easy - less easy	 5 3 1	3	3	1	3	1	1
Rapidity of performance for one specimen - < 10 min - 10–30 min - > 30 min	 3 2 1	1	1	1	1	1	1
Washer/agitator - not needed - needed	 3 1	1	1	1	1	1	1
Reading - visual: inter-reader variability ≤ 3% : inter-reader variability > 3% - reading equipment	 5 3 1	1	1	1	1	1	1
Total (out of possible 40)		15	20	14	20	18	19
Suitability for use in small laboratories: - less suitable < 23 - suitable 23 ≤ x ≤ 30 - very suitable > 30		Less suitable	Less suitable	Less suitable	Less suitable	Less suitable	Less suitable

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

Table 6b. Suitability for use in small laboratories: Group 1 EIAs, 7-11

NAME	Score	CHAGAS – TEST //CS	HBK 401 HEMOBIO CHAGAS	ABBOTT CHAGAS ANTIBODY EIA	Chagatest ELISA	bioelisa CHAGAS
Sensitivity - 100% - 98–100% - <98%	 5 3 0	0	3	3	3	5
Specificity - >98% - 95–98% - <95%	 5 3 0	5	5	5	5	5
Incubation temperature - room temperature - other than room temperature	 3 1	3	1	1	1	1
Shelf-life ->1 year - > 6 months < 1 year - < 6 months	 3 2 1	1	1	1	1	1
Storage at - ambient temperature possible (opened kit) - ambient temperature possible (unopened kit) - 2-8°C required	 5 2 1	1	1	1	1	1
Price per test (US$) - < 1.0 - < 2.0 - > 2.0	 3 2 1	1	3	2	3	1
Ease of performance - very easy - easy - less easy	 5 3 1	1	1	1	3	1
Rapidity of performance for one specimen - < 10 min - 10–30 min - > 30 min	 3 2 1	1	1	1	1	1
Washer/agitator - not needed - needed	 3 1	1	1	1	1	1
Reading - visual: inter-reader variability ≤ 3% : inter-reader variability > 3% - reading equipment	 5 3 1	1	1	1	1	1
Total (out of possible 40)		15	18	17	20	18
Suitability for use in small laboratories: - less suitable < 23 - suitable 23 ≤ x ≤ 30 - very suitable > 30		Less suitable	Less suitable	Less suitable	Less suitable	Less suitable

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

GROUP 2: AGGLUTINATION AND RAPID ASSAYS

Table 7. General characteristics and operational aspects: Group 2, Agglutination and rapid assays

NAME	CHAGAS HAI IMUNOSERUM	CHAGAS – HAI	Imuno-HAI	CHAGAS HEMAGEN®	HEMACRUZI®	SERODIA – Chagas	ID-Chagas Antibody Test	Chagas STAT-PAK®
Company	Laboratorio Lemos, Polychaco	Ebram Produtos Laboratoriais Ltda	WAMA Diagnóstica	Hemagen Diagnósticos Ltda	biolab – Mérieux S.A	FUJIREBIO INC.)	Dia-Med-ID Micro Typing System	Chembio Diagnostic Systems, Inc.
Assay type	Indirect hemag-glutination	Indirect hemag-glutination	Indirect hemag-glutination	Indirect hemag-glutination	Indirect hemag-glutination	Passive particle agglutination	Particle gel immunoassay	Immunochroma-tographic
Antigen type	*T. cruzi* antigen	*T. cruzi Y* antigen	*T. cruzi* Y purified antigen	Epimastigote and amastigote form of *T. cruzi* Y and CL	*T. cruzi* Y antigen	*T. cruzi* RF inactivated antigen	Three different peptides Ag2, TcD and TcE (4)	*T. cruzi* recombinant antigens
Solid phase	Sheep red cells	Bird red cells	Bird red cells	Human red cells	Bird red cells	Gelatin particles	Polymer particles	Nitrocellulose membrane
Specimen type	Serum or plasma	Serum	Serum	Serum	Serum	Serum or plasma	Serum or plasma	Serum, plasma, whole blood
Number of tests per kit (product code)	480 (10200)	96 (200)	200 (34200-H)	384 (6419)	480 (Cod. 029.015)	100 (N/A)	288 (020022)	20 (CG-101)
Lot numbers evaluated (expiry date)	011701 (1/2002)	0621 (9/2002)	108019AC (12/2002)	ICI0502 (1/2003)	H10651I (10/2002)	WM10401 (10/2002)	50460 (1/2002) 031001120 (2/2002)	CG051904/1 (30/04/2006) CG090304/1 (31/08/2006)
Shelf life upon manufacture at (°C)	N/A (2-8)	12 months (2-8)	16 months (2-8)	N/A (2-8)	N/A (2-8)	18 months (2-10)	N/A (2-8)	N/D (8-30)
Volume of serum needed (µL) Final dilution of serum	10 1 : 8	10 1 : 32	10 1 : 32	10 1 : 26	10 1 : 40	25 1 : 32	5 no dilution	5 (serum/plasma) 10 (whole blood) + 6 drops (240µl) of sample diluent
Total time to perform the assay: h. min.	1.50	1.20	1.20 to 2.20	1.50	1.20	2.20	0.20	0.20
Reading	Plate format V, visual	Plate format V, visual	Plate format V, visual	Plate format V, visual	Plate format V, visual	Plate format U, visual	Visual	Visual
Indicative Price/test US$ (as given at time of evaluation)	0.33 (480 tests)	0.66 (96 tests)	0.23 (200 tests)	0.29 (384 tests)	0.14 (480 tests)	1.77 (100 tests)	0.85 (288 tests)	N/D

N/A: not available; N/D: not determined.

Table 8. Comparison of the assays with reference results: Group 2, Agglutination and rapid assays

NAME		CHAGAS HAI IMUNOSERUM	CHAGAS – HAI	Imuno-HAI	CHAGAS HEMAGEN®	HEMACRUZI®	SERODIA – Chagas	ID-Chagas Antibody Test	Chagas STAT-PAK®
Final Sensitivity % (95 CI) n = 168		97.60 (94.0–99.3)	88.09 (82.2–92.6)	100 (97.2–100)	92.26 (87.1–95.8)	99.40 (96.7–100)	100 (97.2–100)	97.02 (93.2–99.0)	93.0* (87.9–96.5)
Specificity % (95 CI) n = 262		78.62 (77.2–83.4)	59.92 (53.7–65.9)	95.80 (92.6–97.9)	89.31 (84.9–92.8)	97.33 (94.6–98.9)	97.70 (95.1–99.2)	99.62 (97.9–100)	95.3 (91.5–97.7)
Indeterminate results %		0.46– 0.69	1.14	0.23	3.20	1.14	0.46	2.97	1.86
Initial inter-reader variability %		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/D
PPV	0.1%	0	0	2	1	4	4	20	2
	5%	19	11	55	31	66	69	93	51
	10%	34	20	72	49	81	82	97	68
NPV	0.1%	99.99	99.98	100	99.99	99.99	100	99.99	99.99
	5%	99.84	98.96	100	99.96	99.96	100	99.84	99.61
	10%	99.66	97.84	100	99.93	99.93	100	99.66	99.18

CI: Confidence intervals; N/A: not applicable; ND: not determined; NPV: negative predictive value; PPV: positive predictive value.

* Note: The evaluation of the Chagas STAT-PAK (Chembio Diagnostics Systems, INC) was performed later and it was not possible to test the entire WHO Chagas Specimen Reference Panel using the assay. An abbreviated panel with a total of 212 anti-*Trypanosoma cruzi* positive specimens and 158 anti-Trypanosoma cruzi negative specimens was used to determine these performance characteristics.

Table 9. Detailed operational aspects: Group 2, Agglutination and rapid assays

NAME	CHAGAS HAI IMUNO-SERUM	CHAGAS – HAI	Imuno-HAI	CHAGAS HEMAGEN®	HEMACRUZI®	SERODIA – Chagas	ID-Chagas Antibody Test	Chagas STAT-PAK®
Dimension (cm) of kit: w-l-h	9.7–21.2–8	9–13.2–6	14.5–20–13.5	17.6–21.5–9	12.3–18.5–10.5	9.8–13.3–6.5	6.2–8.3–5.2 16.8–17.6–11.3	N/D
Storage conditions (°C)	2–8	2–8	2–8	2–8	2–8	2–10	2–8 18–25	8–30
Incubation temperature (°C)	RT (20–25)	RT	RT	RT	RT (25)	RT (15–30)	RT (18–25)	RT
Reading endpoint stability (h.min)	24.00	N/A	2.00	N/A	N/A	N/A	N/A	0
Stability after dilution/ reconstitution/ opening at (°C)	N/D	N/D	N/D	2–8	2–8	2–10	2–8 18–25	N/D
- antigen	Exp. date (2-8)	Exp. date (2-8)	Exp. date (2-8)	7 days (2-8)	Exp. date (2-8)	7 days (2-10)	Exp. date (2-8)	Exp. date (8-30)
- controls	N/A	N/A	N/A	30 days (2-8)	N/A	N/A	N/A	N/A
- sample diluent	N/I	N/I	N/I	N/A	4 weeks (2-8)	N/A	N/A	N/A
- conjugate	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
- substrate	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
- wash buffer	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Number of sera per run minimum – maximum	1–94	1–95	1–94	1–94	1–94	1–30	1–286	1–20
Number of controls per test run	2	1	2	2	2	1	2	2
- negative	1	0	1	1	1	0	1	1
- cut-off/weak positive	0	0	0	0	0	0	0	0
- positive	1	1	1	1	1	1	1	1
- blank	0	0	0	0	0	0	0	0
internal control:								
reagent control	0	0	0	0	0	0	0	0
specimen addition control	0	0	0	0	0	0	0	0

N/A: not available; N/D: not determined; N/I: no information; RT: room temperature.

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

Table 9. (continued) Detailed operational aspects: Group 2, Agglutination and rapid assays

NAME	CHAGAS HAI IMUNO-SERUM	CHAGAS – HAI	Imuno-HAI	CHAGAS HEMA–GEN®	HEMA–CRUZI®	SERO DIA – Chagas	ID-Chagas Anti-body Test	Chagas STAT-PAK®
Estimated time to perform one run: h. min	1.50	1.20	1.20 to 2.20	1.50	1.20	2.20	0.20	0.20
Equipment needed but not provided in the kit:[1]								
- washer	-	-	-	-	-	-	-	-
- incubator (water-bath)	+	+	-	-	-	-	-	-
- spectrophotometric reader	-	-	-	-	-	-	-	-
- refrigerator (storage)	+	+	+	+	+	+	+	-
- agitator, rocker	+	+	+	+	+	+	+	-
- aspiration device	-	-	-	-	-	-	-	-
- automatic pipette (µl)	+	+	+	+	+	+	+	+
- multichannel (µl)	+	+	+	+	+	+	-	-
- disposable tips	+	+	+	+	+	+	+	+
- dilution tubes/rack, microtiterplate	+	+	+	+	+	+	-	-
- distilled or deionised water	+	+	+	+	+	+	-	-
- plate covers	+	+	+	+	+	+	-	-
- graduated pipette; cylinder (ml)	+	+	+	+	+	+	-	-
- sulfuric acid/sodium hydroxide	-	-	-	-	-	-	-	-
- absorbent paper	+	+	+	+	+	+	+	-
- disinfectant	+	+	+	+	+	+	+	+
- gloves	+	+	+	+	+	+	+	+
- reagent trough	+	+	+	+	+	+	+	+
- timer	+	+	+	+	+	+	+	+
Definition of positive results	Hemag-glutination	Hemag-glutination	Hemag-glutination	Hemag-glutination	Hemag-glutination	Particle Agglutination	Red line on the surface	Two pink/purple colored lines, one in the control area and one in the Test area
Definition of grey zone or indeterminate result	N/A	N/A	N/A	N/A	N/A	Ring shape with a smooth round outer margin	Particles dispersed within the gel	No distinct pink/purple line visible in the Control area

[1] + : not provided in the kit but necessary to perform the test; - : provided in the kit or not necessary to perform the test; +/- : use is optional.

N/A: not applicable.

Table 10. Technician's appraisal of the test kits: Group 2, Agglutination and rapid assays

NAME	Score	CHAGAS HAI IMUNOSERUM	CHAGAS – HAI	Imuno-HAI	CHAGAS HEMAGEN®	HEMACRUZI®	SERODIA – Chagas	ID-Chagas Antibody Test	Chagas STAT-PAK®
Number of steps in the test procedure: -1-2 steps -3-5 steps - >5 steps	6 3 1	3–6	3	3	3	3	3	3	6
Clarity of kit instructions: - good - needs improvement	2 1	1	1	1	1	2	1	2	2
Kit and reagent packaging and labelling: - good - needs improvement	2 1	1	2	2	2	2	2	2	2
Total (out of possible 10)	10	5–8	6	6	6	7	6	7	10
Comments on the test kit		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

N/A: not applicable.

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

Table 11. Calculation of ease of performance: Group 2, Agglutination and rapid assays

NAME	CHAGAS HAI IMUNOSERUM	CHAGAS – HAI	Imuno-HAI	CHAGAS HEMAGEN®	HEMACRUZI®	SERODIA – Chagas	ID-Chagas Antibody Test	Chagas STAT-PAK®
Need to prepare:[1]								
-antigen	1	1	1	0	1	0	1	1
-substrate	1	1	1	1	1	1	1	1
-wash solution	1	1	1	1	1	1	1	1
-conjugate	1	1	1	1	1	1	1	1
-predilution of serum	0	0	0	0	0	0	1	1
Stability after dilution/opening: (expiry date = 1; less = 0)								
-antigen	1	1	1	0	1	0	1	0
-controls	1	1	1	0	1	1	1	1
-sample diluent	1	1	0	1	0	1	1	1
-conjugate	1	1	1	1	1	1	1	1
-substrate	1	1	1	1	1	1	1	1
-wash buffer	1	1	1	1	1	1	1	1
Sufficient reagents (yes =0; no = 1)	1	1	1	1	1	1	1	0
Wash step required (yes =0; no = 1)	1	1	1	1	1	1	1	1
Item needed but not provided in the kit:								
-reagent trough	0	0	0	0	0	0	0	0
-automatic/ multichannel pipette	0	0	0	0	0	0	0	0
-dilution – tubes, rack/microtiter plate	0	0	0	0	0	0	0	0
-distilled or deionised water	0	0	0	0	0	0	0	0
-plate covers	0	0	0	0	0	0	0	0
-graduated pipette, cylinder	0	0	0	0	0	0	0	0
-sulfuric acid/ sodium hydroxide	0	0	0	0	0	0	0	0
Technician's appraisal of the test kit[2] (rating out of 10)	5-8	6	6	6	7	6	7	10
Total (out of possible 30)	17–20	18	17	15	18	16	20	21
Ease of performance: -less easy < 20 -easy 20 ≤ x ≤ 25 -very easy > 25	Easy	Less easy	Less easy	Less easy	Less easy	Less easy	Easy	Easy

[1] 1 : positive rating: reagent needs no preparation; item provided in the kit; 0 : negative rating: reagent needs preparation; item not provided in the kit. For Chagas STAT-PAK®, conjugate, substrate, and wash buffer are not part of the kit
[2] See Table 10.

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

Table 12. Suitability for use in small laboratories: Group 2, Agglutination and rapid assays

NAME	Score	CHAGAS HAI IMUNOSERUM	CHAGAS – HAI	Imuno-HAI	CHAGAS HEMAGEN®	HEMACRUZI®	SERODIA – Chagas	ID-Chagas Antibody Test	Chagas STAT-PAK®
Sensitivity - 100% - 98–100% - <98%	 5 3 0	3	0	5	0	3	5	0	0
Specificity - >98% - 95–98% - <95%	 5 3 0	0	0	3	0	3	5	5	3
Incubation temperature - room temperature - other than room temperature	 3 1	3	3	3	3	3	3	3	3
Shelf-life ->1 year - > 6 months < 1 year - < 6 months	 3 2 1	1	2	3	1	1	1	1	3
Storage at - ambient temperature possible (opened kit) - ambient temperature possible (unopened kit) - 2–8°C required	 5 2 1	1	1	1	1	1	1	1	5
Price per test (US$) - < 1.0 - < 2.0 - > 2.0	 3 2 1	3	3	3	3	3	2	3	2
Ease of performance - very easy - easy - less easy	 5 3 1	3	3	3	1	3	1	3	3
Rapidity of performance for one specimen - < 10 min - 10–30 min - > 30 min	 3 2 1	1	1	1	1	1	1	2	2
Washer/agitator - not needed - needed	 3 1	3	3	3	3	3	3	3	3
Reading - visual: inter-reader variability ≤ 3% : inter-reader variability > 3% - reading equipment	 5 3 1	5	5	5	5	5	5	5	5
Total (out of possible 40)		23	21	30	18	26	27	26	29
Suitability for use in small laboratories: - less suitable < 23 - suitable 23 ≤ x ≤ 30 - very suitable > 30		Less suitable	Less suitable	Suitable	Less suitable	Suitable	Suitable	Suitable	Suitable

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

GROUP 3: CONFIRMATORY ASSAYS

Table 13. General characteristics and operational aspects: Group 3 Confirmatory Assays

NAME	IMUNOCRUZI®	HBK 740 IMUNOBLOT LINHAS anti – *T.cruzi*	TESA – blot
Company	biolab – Mérieux S.A.	EMBRABIO – Empresa Brasileira de Biotecnologia S.A.	biolab – Mérieux S.A.
Assay type	Indirect Imunofluorescence	Immunoblot	Western Blot
Antigen type	*T. cruzi* Y epimastigote form	CRA(cytoplasmic repetitive antigen), FRA(flagellar repetitive antigen), TcD homologue (clone13), MAP (microtubule associated protein), SAPA(shed acute phase antigen, Ag 39 (116 to 140 D antigen) and recombinant protein homologous to Tc24.	Tripomastigote excreted and secreted antigen (TESA)
Solid phase	Glass slide	Nylon strip	Nitrocellulose membrane
Specimen type	Serum	Serum or plasma	Serum or plasma
Number of tests per kit (product code)	100 (022.002)	20 (N/A)	24 (N/A)
Lot numbers evaluated (expiry date)	B91293v (3/2001)	LUA12C14 (3/2001)	LE 0102 1-31 2/2002
Shelf life at (°C)	N/D (2-8)	12 months (2-8)	N/D (2-8)
Volume of serum needed (µL) Final dilution of serum	10 1 : 20	10 1 : 101	10 1 : 101
Total time to perform the assay h. min	1.50	18.00 ± 2.00	4.00
Reading	Visual Fluorescent microscope	Visual	Visual
Indicative Price/test US$ (as given at time of evaluation)	0.19 (100 tests)	20.00 (20 tests)	N/(24 tests)

N/D: not determined

Table 14. Comparison of the assays with reference results; Group 3 Confirmatory Assays

NAME		IMUNOCRUZI®	HBK 740 IMUNOBLOT LINHAS anti – *T.cruzi*	TESA – blot
Final sensitivity % (95 CI) n = 168		Confirmatory	Confirmatory	Confirmatory
Specificity % (95 CI) n = 262		Confirmatory	Confirmatory	Confirmatory
Indeterminate results %[1]		2.06	1.83	0
Initial inter-reader variability %		N/D	N/D	N/D
PPV	0.1%	N/A	N/A	N/A
	5%	N/A	N/A	N/A
	10%	N/A	N/A	N/A
NPV	0.1%	N/A	N/A	N/A
	5%	N/A	N/A	N/A
	10%	N/A	N/A	N/A

[1] Indeterminate results % determined according to the manufacturer's instructions for use.

CI: Confidence intervals; N/A: not applicable; N/D: not determined; NPV: negative predictive value; PPV: positive predictive value.

Table 15. Detailed operational aspects: Group 3 Confirmatory Assays

NAME	IMUNOCRUZI®	HBK 740 IMUNOBLOT LINHAS anti – *T. cruzi*	TESA – blot
Dimension (cm) of kit: w-l-h	3–6–9.6	16.5–18–14.2	12.4–18.5–10.3
Storage conditions (°C)	2–8	2–8	2–8
Incubation temperature (°C)	37	Room temperature	Room temperature (20–25)
Reading endpoint stability (h.min)	24.00	Months	Months
Stability after dilution/ reconstitution/opening at (°C)	2–8	2–8	2–8
- antigen	Exp. date (-20)	Exp. date (2–8)	Exp. date (2–8)
- controls	N/A	N/A	N/A
- sample diluent	N/A	N/A	N/A
- conjugate	N/I	N/I	N/A
- substrate	N/A	N/I	N/I
- wash buffer	2 months (2–8)	N/I	N/I
Number of sera per run minimum – maximum	1–98	1–18	1–22
Number of controls per test run	2	2	2
- negative	1	1	1
- cut-off/weak positive	0	0	0
- positive	1	1	1
- blank	0	0	0
internal control:			
reagent control	0	0	0
specimen addition control	0	0	0
Estimated time to perform one run: h. min	1.50	18.00 ± 2.00	4.00
Equipment needed but not provided in the kit:[1]			
- washer	-	-	-
- incubator (water-bath)	+	-	-
- spectrophotometric reader	-	-	-
- refrigerator (storage)	+	+	+
- agitator , rocker	+/-	+	+
- aspiration device	-	+	+
- automatic pipette (µl)	+	+	+
- multichannel (µl)	-	-	-
- disposable tips	+	+	+
- dilution tubes/rack, microtiterplate	+	-	-
- distilled or deionised water	+	+	+
- plate covers	-	-	-
- graduated pipette; cylinder (ml)	+	+	+
- sulfuric acid/sodium hydroxide	-	-	-
- absorbent paper	+	+	+
- disinfectant	+	+	+
- gloves	+	+	+
- reagent trough	+	+	+
- timer	+	+	+
Definition of positive results	Green fluorescence	At least 2 bands appeared and the sum of their intensities was greater than2.5	Band present at 120–200kDa
Definition of grey zone or indeterminate result	Green with no fluorescence	2 or more bands with a sum of intensities greater than 1 but less than or equal to 2.5	N/A

[1] + : not provided in the kit but necessary to perform the test; - : provided in the kit or not necessary to perform the test; +/- : use is optional.

N/A: not available; N/I: no information.

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

Table 16. Technician's appraisal of the test kits: Group 3 Confirmatory Assays

NAME	Score	IMUNOCRUZI®	HBK 740 IMUNOBLOT LINHAS anti – *T.cruzi*	TESA – blot
Number of steps in the test procedure: - 1-2 steps - 3-5 steps - >5 steps	 6 3 1	3	1	1
Clarity of kit instructions: - good - needs improvement	 2 1	1	1	1
Kit and reagent packaging and labelling: - good - needs improvement	 2 1	2	2	2
Total (out of possible 10)	10	6	4	4
Comments on the test kit		N/A	N/A	N/A

N/A: not available.

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

Table 17. Calculation of ease of performance: Group 3 Confirmatory Assays

NAME	IMUNOCRUZI®	HBK 740 IMUNOBLOT LINHAS anti – *T.cruzi*	TESA – blot
Need to prepare:[1]			
-antigen	0	1	1
-substrate	1	0	0
-wash solution	0	0	0
-conjugate	0	0	0
-predilution of serum	0	1	1
Stability after dilution/opening: (expiry date = 1; less = 0)			
-antigen	1	1	1
-controls	1	1	1
-sample diluent	1	1	1
-conjugate	0	0	1
-substrate	1	0	0
-wash buffer	1	1	1
Sufficient reagents (yes =0; no = 1)	1	1	1
Wash step required (yes =0; no = 1)	0	0	0
Item needed but not provided in the kit:			
-reagent trough	0	0	0
-automatic/multichannel pipette	0	0	0
-dilution – tubes, rack/microtiter plate	0	0	0
-distilled or deionised water	0	0	0
-plate covers	0	0	0
-graduated pipette, cylinder	0	0	0
-sulfuric acid/sodium hydroxide	0	0	0
Technician's appraisal of the test kit[2] (rating out of 10)	6	4	4
Total (out of possible 30)	13	11	12
Ease of performance: -less easy < 20 -easy 20 ≤ x ≤ 25 -very easy > 25	Less easy	Less easy	Less easy

[1] 1 : positive rating: reagent needs no preparation; item provided in the kit; 0 : negative rating: reagent needs preparation; item not provided in the kit
[2] See Table 16.

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

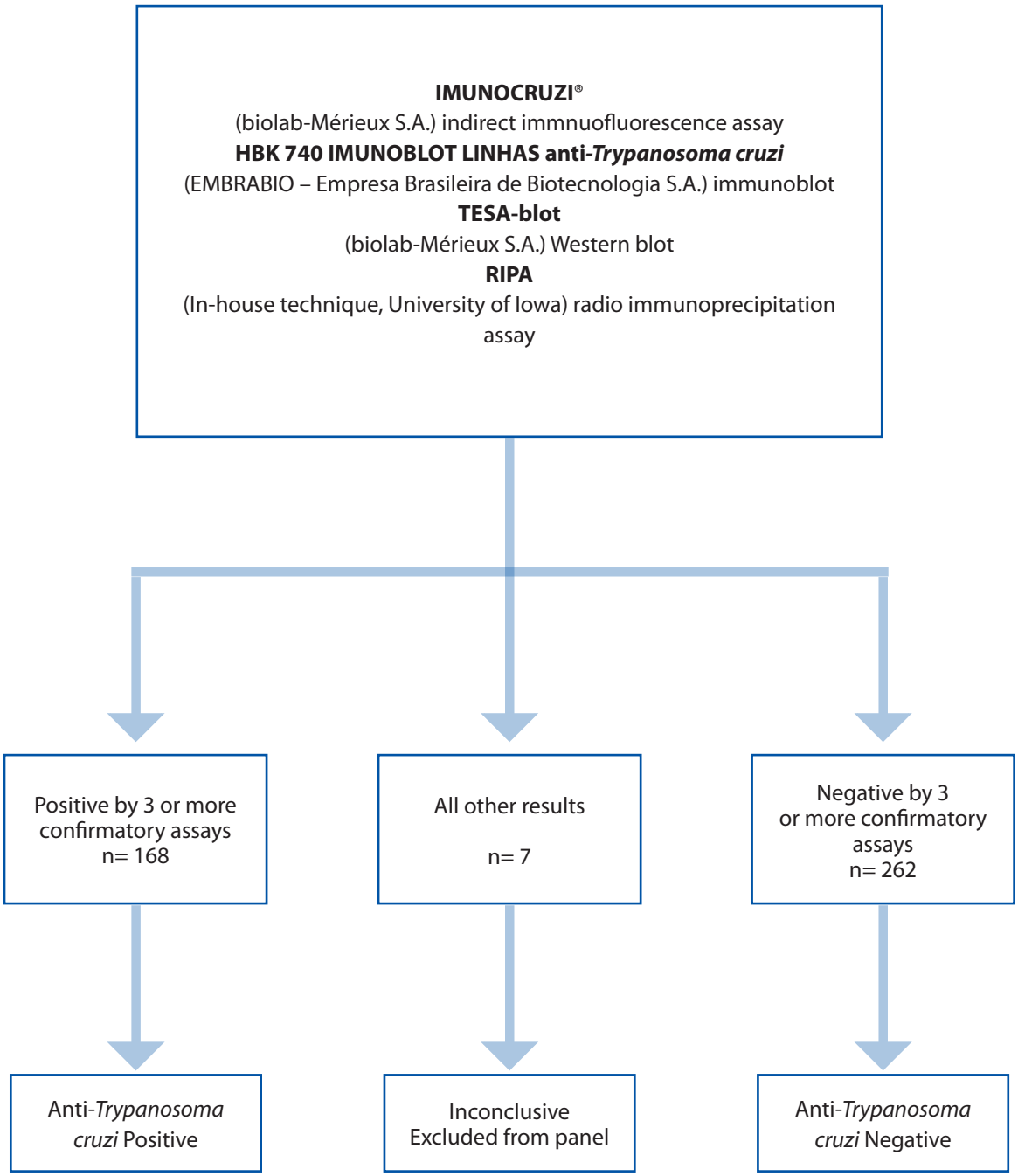
Table 18. Suitability for use in small laboratories: Group 3 Confirmatory Assays

NAME	Score	IMUNOCRUZI®	HBK 740 IMUNOBLOT LINHAS anti – *T. cruzi*	TESA – blot
Sensitivity				
- 100%	5			
- 98–100%	3	5	5	5
- <98%	0			
Specificity				
- >98%	5			
- 95–98%	3	0	0	0
- <95%	0			
Incubation temperature				
- room temperature	3	1	3	3
- other than room temperature	1			
Shelf-life				
->1 year	3			
- > 6 months < 1 year	2	1	2	1
- < 6 months	1			
Storage at				
- ambient temperature possible (opened kit)	5			
- ambient temperature possible (unopened kit)	2	1	1	1
- 2-8°C required	1			
Price per test (US$)				
- < 1.0	3			
- < 2.0	2	3	1	1
- > 2.0	1			
Ease of performance				
- very easy	5			
- easy	3	1	1	1
- less easy	1			
Rapidity of performance for one specimen				
- < 10 min	3			
- 10–30 min	2	1	1	1
- > 30 min	1			
Washer/agitator				
- not needed	3	3	1	1
- needed	1			
Reading				
- visual: inter-reader variability ≤ 3%	5			
: inter-reader variability > 3%	3	5	5	5
- reading equipment	1			
Total (out of possible 40)		21	20	19
Suitability for use in small laboratories:				
- less suitable < 23				
- suitable 23 ≤ x ≤ 30		Less suitable	Less suitable	Less suitable
- very suitable > 30				

Note: *These criteria are primarily technical and while an assay may be regarded as "technically" suitable for use in laboratories with limited facilities or where small numbers of samples are routinely tested, the sensitivity and specificity of the assay are over-riding factors in determining the suitability of an assay for use in any laboratory.*

ANNEX 1

ALGORITHM FOR CHARACTERIZATION OF THE WHO ANTI-*TRYPANOSOMA CRUZI* SPECIMEN REFERENCE PANEL



ANNEX 2

SPECIMENS THAT WERE EXCLUDED FROM ANALYSIS DUE TO INCONCLUSIVE RESULTS BY THE CONFIRMATORY ASSAYS

Specimen number	Country of origin	IFA result	Imunoblot result	Western blot result	RIPA result
231	Argentina	negative	positive	positive	negative
229	Argentina	negative	negative	positive	negative
227	Argentina	negative	positive	positive	negative
224	Argentina	inconclusive	inconclusive	positive	negative
222	Paraguay	inconclusive	inconclusive	positive	positive
218	Bolivia	inconclusive	negative	positive	negative
217	Bolivia	1/40	negative	positive	negative

ANNEX 3

LIST OF ASSAY MANUFACTURERS' ADDRESSES

Abbott Laboratórios do Brasil Ltda
Rua Michigan, 735-São Paulo, SP 69-5106/R2, Brazil
Tel: +55 11 536 7000;
website: www.abbottbrasil.com.br

BIOKIT, S.A.
08186 Llissà d'Amunt, Barcelona , Spain
Tel: +34 93 860 9000; Fax: +34 93 860 9017;
website: www.biokit.com

biolab-Mérieux S.A
Estrada do Mapuá, 491 Jacarepaguá, CEP 22710-261, Rio de Janeiro, Brazil
Tel: +55 21 2444 1400; Fax: +55 21 2445 6025;
website: www.biomerieux.com

BIOSChile Ingeniería Genética S.A.
Av. Marathón, 1943 Santiago, Chile
Tel: +56 2 238 1878; Fax: +56 2 239 4250;
website: www.bioschile.cl

DiaMed
1785 Cressier sur Morat, Switzerland
Tel: +41 26 674 5111; Fax: +41 26 674 5445;
website: www.diamed.com

EBRAM Produtos Laboratoriais Ltda.
Rua Júlio de Castilhos, 500 Belenzinho, São Paulo, SP-CEP 03059-000, Brazil
Tel: +55 11 291 2811; Fax: +55 11 608 4096;
website: www.ebram.com

EMBRABIO – Empresa Brasileira de Biotecnologia S.A.
Av. Ermano Marchetti, 826-Lapa, 05038-000, São Paulo, Brazil
Tel: +55 11 3611 5901; Fax: 55 11 3611 5902;
website: www.embrabio.com

FUJIREBIO INC.
2-62-5, Nihombashi-hamacho 2-Chome Chuo-ku, Tokyo 103-0007, Japan
website: www.fujirebio.co.jp

Hemagen Diagnósticos Ltda.
Rua Diogo Moreira nº 222, Pinheiros São Paulo, SP-CEP 05423-01, Brazil
Tel: +55 11 3819 5222; Fax: +55 11 3816 7623;
website: www.hemagen.com

Instituto de Investigaciones en Ciencias de la Salud
Rio de la Plata y Lagerenza, Asunción, Paraguay
Tel: +595 21 421 312 / +595 21 424520
Fax: +595 21 480185;
website: www.una.py/iics

Laboratório Lemos – Polychaco
Santiago del Estero, 1162-C1075AAX, Buenos Aires, Argentina
Tel: +54 11 4304 2204 Fax: +54 11 4305 0929;
website: www.dmed.com.br

Meridian Diagnostics, Inc.
3471 River Hills Drive, Cincinnati, Ohio 45244, USA
Tel: +1 513 271 3700; +1 800 543 1980;
Fax: +1 513 271 0124; website: www.meridianbioscience.com

Wiener lab.
Riobamba 2944, 2000 Rosario, Argentina
Tel: +54 341 432 9191; Fax: +54 341 432 5554;
website: www.wiener-lab.com.ar

WAMA Diagnóstica
Rua Rui Barbosa, 1077 – Centro, CEP 13560-330, São Carlos, SP, Brazil
Tel: +55 16 272.7711; Fax: +55 16 272.6468;
website: www.wamadiagnostica.com.br

7. ADDITIONAL READING

Additional reading may be obtained by visiting the website of the Diagnostics and Laboratory Technology team at www.who.int/diagnostics_laboratory. In addition to general information on diagnostics, there are WHO composite reports that detail the performance and operational characteristics of a number of commercially available assays for HIV, HCV and HBV and details about the WHO Test Kit Bulk Procurement Scheme.

8. ACKNOWLEDGEMENTS

We would like to thank Dr. E. Vinelli, National Blood Transfusion Service, Tegucigalpa, Honduras; Dr. Mauricio Beltrán, Instituto Nacional de Salud, Santa Fe de Bogotá, Colombia; Dr. Graciela Velázquez, Instituto Nacional de Investigaciones en Ciencia de la Salud, Asunción, Paraguay; Dr. María del Carmen Zambrana, Hospital Viedma, Cochabamba, Bolivia; Dr. Dalton de Alencar Chamone, Fundação Pró-Sangue Hemocentro de São Paulo, São Paulo, Brazil; Dr. Ana del Pozo, Hospital de Pediatría "Professor Dr. J.P. Garrahan", Buenos Aires, Argentina; Dr. Marco Herdoiza Holguín, Cruz Roja Ecuatoriana, Quito, Ecuador for supplying specimens to the WHO anti-*Trypanosoma cruzi* Specimen Reference Panel.

We acknowledge the four companies, Abbott Laboratórios, BIOLAB, EMBRABIO and Instituto de Investigaciones en Ciencias de la Salud, for supplying the test kits free of charge.

NOTE

Other assays for diagnosis of Chagas disease were available at the time of this evaluation but due to the nature, design and capacity of this prospective study, it was not possible to include them all in the evaluation.

9. REFERENCES

1. Gorlin J, Rossmann S, Robertson G, Stallone F, Hirschler N, Nguyen KA, Gilcher R, Fernandes H, Alvey S, Ajongwen P, Contestable P, Warren H. Evaluation of a new Trypanosoma cruzi antibody assay for blood donor screening. Transfusion, 2008; 48(3):531-40. Epub 2007 Dec 7

2. Tarleton RL, Reithinger R, Urbina JA, Kitron U, Gürtler RE. The challenges of Chagas Disease--grim outlook or glimmer of hope. PLoS Med. 2007; 4(12):e332

3. Viotti R, Vigliano C, Lococo B, Bertocchi G, Petti M, Alvarez MG, Postan M,Armenti A. Long-term cardiac outcomes of treating chronic Chagas disease with benznidazole versus no treatment: a nonrandomized trial. Ann Intern Med. 2006; 144(10):724-34

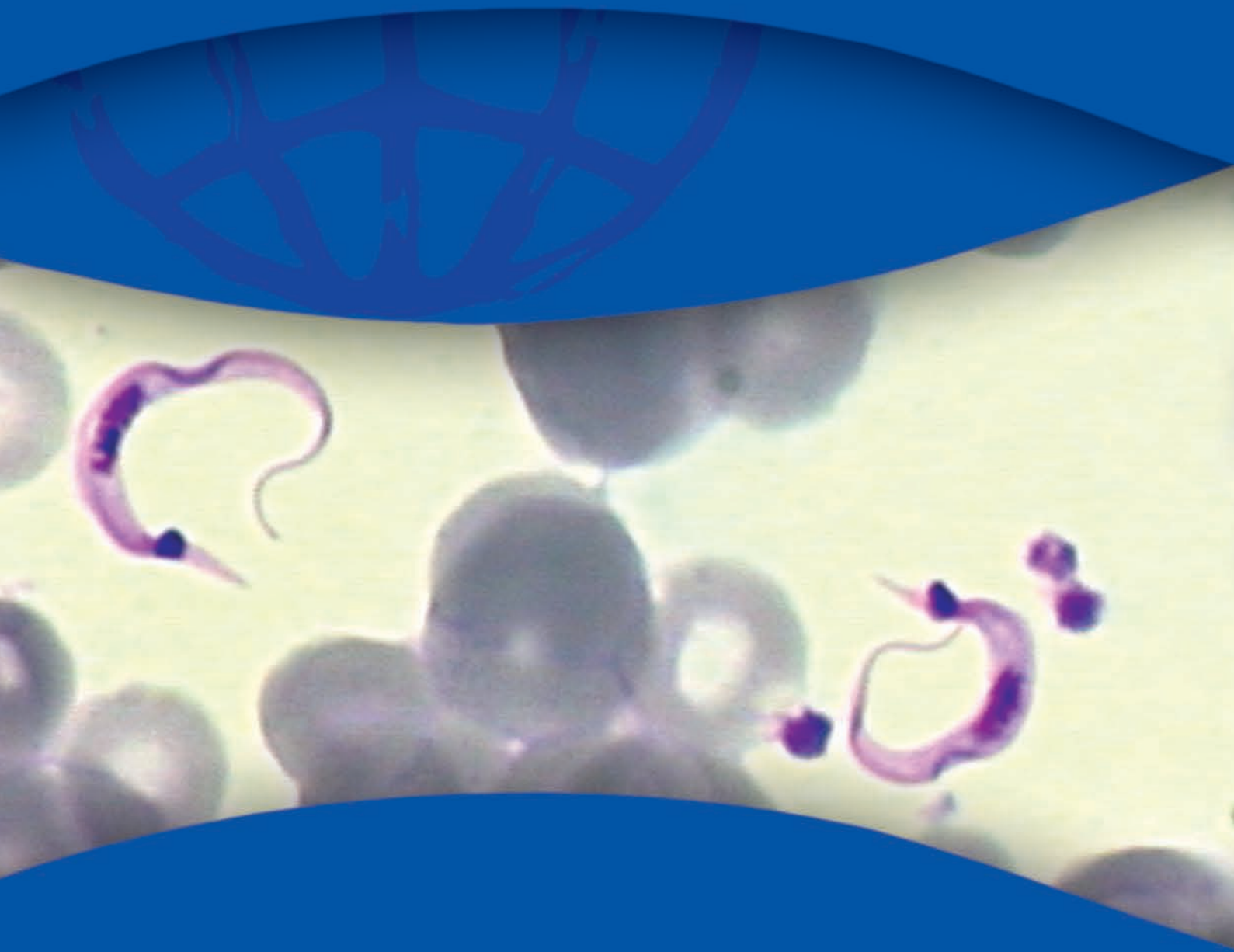
4. Caballero ZC, Sousa OE, Marques WP, Saez-Alquezar A, Umezawa ES. Evaluation of serological tests to identify Trypanosoma cruzi infection in humans and determine cross-reactivity with Trypanosoma rangeli and Leishmania spp. Clin VaccineImmunol. 2007; 14(8):1045-9

5. Kerndt PR, Waskin HA, Kirchhoff LV, Steurer F, Waterman SH, Nelson JM, Gellert GA, Shulman IA. Prevalence of antibody to Trypanosoma cruzi among blood donors in Los Angeles, California. Transfusion. 1991; 31(9):814-8

6. Armitage P, Berry G, Matthews J. Statistical methods in medical research, 4th ed. Oxford, Blackwell Scientific Publications, 2002.

CONTACT

Department of Essential Health Technologies
World Health Organization
20 Avenue Appia
CH-1211 Geneva 27
Switzerland
E-mail: diagnostics@who.int
www.who.int/diagnostics_laboratory



978 92 4 150029 6