Elecsys Chagas

cobas®

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07028164119	07028164500	300	**cobas e** 801

English

System information

Short name	ACN (application code number)
CHAGAS	10146

Intended use

In vitro diagnostic test for the qualitative determination of antibodies to Trypanosoma cruzi (T. cruzi, the causative agent of the Chagas disease) in human serum and plasma. This assay is indicated as an aid in the diagnosis of infection with Trypanosoma cruzi. This assay is also indicated as a donor screening test to detect antibodies to Trypanosoma cruzi in serum or plasma specimens from individual human blood donors. It may also be used to screen individual organ donors when specimens are obtained while the donor's heart is still beating.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the **cobas e** 801 immunoassay analyzer.

Note: Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit Catalogue Number: 07028164190 for the Elecsys Chagas. The last 3 digits -190 have been replaced by -119 for logistic purposes.

Summary

Chagas disease (also known as American trypanosomiasis) is caused by the flagellated protozoan parasite Trypanosoma cruzi.[2] The parasite is usually transmitted by hematophagous triatomine insects (family of Reduviidae) in endemic areas but also through infected blood components, organ transplantations, congenitally from mother to infant, ingestion of contaminated food and in laboratory accidents.[2,3]

T. cruzi is found in the Americas, except for isolated cases in which infected persons have carried the parasites to non-endemic regions (e.g. Far East, Australia, Europe).[4,5,6] It is estimated that 6-7 million people are infected worldwide, predominantly in Latin America and 20-30 % of these develop symptomatic, potentially life-threatening Chagas disease.[6,7]

The natural history of the infection is characterized by an acute and a chronic phase. The acute phase lasts 8 to 12 weeks, during which most patients remain asymptomatic or develop nonspecific symptoms.[5] Patients develop a strong immune response to a variety of T. cruzi antigens, and a decrease of parasite levels can be observed. The chronic phase begins once parasitemia falls below detectable levels by microscopy (in the absence of antitrypanosomal therapy), and infection mostly appears asymptomatic but lifelong. Diagnosis of Chagas disease is usually made by serology, biopsy or PCR. A positive serology is considered as a sign of active T. cruzi infection or past exposure. Serologically positive asymptomatic patients are capable of transmitting the parasite to the vector insect and directly to other individuals via blood components, organ donation, or to the fetus transplacentally.[8]

Determination of specific antibodies to T. cruzi is used to identify blood of individuals infected with T. cruzi. The Elecsys Chagas assay uses recombinant antigens representing FCaBP, FRA and Cruzipain for the determination of antibodies to T. cruzi.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 18 µL of sample, biotinylated recombinant T. cruzi-specific antigens and T. cruzi-specific recombinant antigens labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack (M, R1, R2) is labeled as CHAGAS.

M Streptavidin-coated microparticles, 1 bottle, 14.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 T. cruzi-Ag~biotin, 1 bottle, 16.7 mL:
Biotinylated T. cruzi-specific antigens (recombinant, E. coli) > 100 µg/L; MES[b)] buffer 50 mmol/L, pH 6.5; preservative.

R2 T. cruzi-Ag~$Ru(bpy)_3^{2+}$, 1 bottle, 16.7 mL:
T. cruzi-specific antigens (recombinant, E. coli) labeled with ruthenium complex > 100 µg/L; MES buffer 50 mmol/L, pH 6.5; preservative.

b) MES = 2-(N-Morpholino) ethane sulfonic acid

CHAGAS Cal1 Negative calibrator 1, 1 bottle of 1.0 mL:
Human serum, non-reactive for anti-T. cruzi antibodies; buffer; preservative.

CHAGAS Cal2 Positive calibrator 2, 1 bottle of 1.0 mL:
Human serum, reactive for anti-T. cruzi antibodies; buffer; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

All human material should be considered potentially infectious.
The calibrators (CHAGAS Cal1, CHAGAS Cal2) have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.
The serum containing anti-T. cruzi IgG (CHAGAS Cal2) was sterile filtrated.
The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[9,10]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents (M, R1, R2) in the kit are ready-for-use and are supplied in **cobas e** packs.

Calibrators

The calibrators are supplied ready-for-use in bottles compatible with the system.

Unless the entire volume is necessary for calibration on the analyzer, transfer aliquots of the ready-for-use calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C for later use.

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the **cobas e** pack	
unopened at 2-8 °C	up to the stated expiration date
on the **cobas e** 801 analyzer	16 weeks

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Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	16 weeks
on **cobas e** 801 analyzer at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA, K_3-EDTA, ACD, CPD, CP2D, CPDA and Na-citrate plasma.

Plasma tubes containing separating gel can be used.

Criterion: Correct assignment of positive and negative samples. Samples with a COI (cutoff index) ≥ 1.0: ± 20 % recovery; samples with a COI < 1.0: ± 0.20 COI recovery.

Stable for 7 days at 20-25 °C, 14 days at 2-8 °C, 12 months at -20 °C (± 5 °C). The samples may be frozen 6 times.

The sample types listed were tested with a selection of sample collection tubes or systems that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates and thawed samples before performing the assay.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.
The performance of the Elecsys Chagas assay has not been established with cadaveric samples or body fluids other than serum and plasma.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 07092571190, PreciControl Chagas, 16 x 1.0 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- **cobas e** 801 analyzer

Accessories for the **cobas e** 801 analyzer:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cups, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibrators:

Place the calibrators in the sample zone.

Read in all the information necessary for calibrating the assay.

Calibration

Calibration frequency: Calibration must be performed once per reagent lot using CHAGAS Cal1, CHAGAS Cal2 and fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Range for the electrochemiluminescence signals (counts) for the calibrators:
Negative calibrator (CHAGAS Cal1): 400-2500
Positive calibrator (CHAGAS Cal2): 12000-300000

Quality control

For quality control, use PreciControl Chagas.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of CHAGAS Cal1 and CHAGAS Cal2. The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (signal sample/cutoff).

Interpretation of the results

Numeric result	Result message	Interpretation/ further steps
COI < 1.0	Non-reactive	Negative for antibodies to T. cruzi
COI ≥ 1.0	Reactive	Positive for antibodies to T. cruzi

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Limitations - interference
The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.62 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 287 nmol/L or ≤ 70 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
Albumin	≤ 7.0 g/dL
IgG	≤ 7.0 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1.0 g/dL

Criterion: Samples with a COI ≥ 1.0: ± 20 % recovery; samples with a COI < 1.0: ± 0.20 COI recovery.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

The high-dose hook effect does not lead to false negative results in the Elecsys Chagas assay.

A negative test result does not completely rule out the possibility of an infection with T. cruzi.

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, special drugs used in Chagas disease therapy were tested. No interference with the assay was found.

Special drugs

Drug	Concentration tested mg/L
Benznidazole	≤ 360
Nifurtimox	≤ 720

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

The Elecsys Chagas assay has a high dilution sensitivity. Avoid any sample cross-contamination during sample pre-analytics.

Limits and ranges
Detection limit: ≤ 10 WHO mIU/mL

The stated sensitivity was determined by reading off the anti-T. cruzi antibody concentration corresponding to the signal of the cutoff value from standard curves obtained by serial dilution of the 1st WHO Chagas Antibody Reference Panel (1st Int. Std. for Chagas (TcI) antibody in human plasma NIBSC 09/188 + 1st Int. Std. for Chagas (TcII) antibody in human plasma NIBSC 09/186) in human serum negative for antibodies to T. cruzi.

Expected values
The prevalence of IgG antibodies to T. cruzi varies considerably depending upon geographical location and upon the population studied.

Specific performance data
Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 801 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS[c], negative	0.085	0.001	1.4	0.001	1.6
HS, weakly positive	1.13	0.011	1.0	0.018	1.6
HS, high positive	9.31	0.135	1.4	0.221	2.4
PC[d] Chagas 1	0.084	0.001	1.2	0.001	1.7
PC Chagas 2	3.44	0.031	0.9	0.057	1.7

c) HS = human serum

d) PC = PreciControl

Analytical specificity
A total of 594 samples with other infectious diseases from endemic and non-endemic regions were tested with the Elecsys Chagas assay and the following results were found:

Disease	N	Non-reactive	Reactive
EBV	26	26	0
Leishmaniosis	241	241	0
Malaria	204	203	1
Dengue	87	87	0
Syphilis	19	19	0
Toxoplasmosis	15	15	0
African trypanosomiasis (T. brucei)	2	2	0
Total	594	593	1

6 additional samples (Dengue: 5/Leishmaniosis: 1) were excluded from the table. These samples were from Chagas endemic regions and were found reactive in the Elecsys Chagas assay as well as in at least one additional Chagas antibody assay.

Relative sensitivity
The relative sensitivity was evaluated in a total of 674 samples from Chagas-infected patients in different stages of the disease obtained from a reference center in Spain and from Chagas endemic regions in Latin America. All samples were either predefined as positive by PCR (n = 158) or considered reactive in at least 3 serological methods (n = 516).
135 samples from the Chagas positive serology characterized group additionally included clinical information to establish the disease stage.

All Chagas positive samples were found reactive.
The resulting overall relative sensitivity was 100 % (95 % confidence interval, two-sided: 99.45-100 %).

Relative specificity
Relative specificity was evaluated using cohorts of fresh or frozen serum/plasma specimens from blood donors (n = 14681), hospitalized patients (n = 517) and pregnant women (n = 313) collected at four blood donation centers in Italy, Germany, Colombia and Argentina. The blood donation cohort of 9635 samples from Europe included 5244 samples from blood donors coming from a Mediterranean Leishmania endemic region. 5046 samples were collected from blood donors from Latin America.

Samples found to be reactive for anti-T. cruzi antibodies in at least one method were confirmed by two reference centers according to their diagnostic algorithms. Relative specificity from these cohorts is summarized in the table below.

Cohort	N	Relative specificity, % (95 % CI[e]), two-sided)
Blood donors	14681[f]	99.90 (99.83-99.94)

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Cohort	N	Relative specificity, % (95 % CI[e)], two-sided)
Pregnant women	313	100 (98.83-100)
Hospitalized patients	517	100 (99.29-100)

e) CI = confidence interval

f) 8 samples were confirmed reactive and were excluded from the specificity calculation.

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References

2 Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. Lancet 2010;375:1388-1402.

3 Pan American Health Organisation. Chagas Disease (American Trypanosomiasis).

4 Gascon J, Bern C, Pinazzo MJ, Chagas disease I Spain, the United States and other non-endemic countries. Acta Trop 2010;115:22-27.

5 Bern C, Chagas disease. N Engl J Med 2015. 373: 456-466.

6 Chagas Disease in Latin America: an epidemiological update based on 2010 estimates. Wkly Epidemiol Rec 2015;90:33-43.

7 http://www.who.int/mediacentre/factsheets/fs340/en/ (Accessed February 16, 2016)

8 Maguire JH, Trypanosoma. 2nd ed. Philadelphia: Lippincott, Williams & Wilkins; 2004.

9 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

10 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number