Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please send an e-mail to: ocod@fda.hhs.gov and include 508 Accommodation and the title of the document in the subject line of your e-mail.

Alinity s

Chagas Reagent Kit

Trypanosoma cruzi (E coli, Recombinant) Antigen

en

Chagas
06P08
G92073R03
B6P0R0

Read Highlighted Changes: Revised March 2022.

REF 06P0850

Instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from these instructions.

For laboratory professional use only.

NAME

Alinity s Chagas Reagent Kit

Trypanosoma cruzi (*E coli*, Recombinant) Antigen

INTENDED USE

The Alinity s Chagas assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of antibodies to *Trypanosoma cruzi* (the causative agent of Chagas disease) in human serum and plasma specimens on the Alinity s System.

The Alinity s Chagas assay is intended to screen individual human donors, including volunteer donors of whole blood and blood components, and other living donors for the presence of antibodies to *Trypanosoma cruzi*. The assay is also intended for use in testing serum and plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing serum specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens.

SUMMARY AND EXPLANATION OF THE TEST

Chagas disease or American Trypanosomiasis is caused by the parasite *Trypanosoma cruzi* (*T cruzi*).[1]

It is estimated that 6 million to 7 million people globally have Chagas disease (most in Latin America).[2]

There are 3 morphologic forms in the life cycle of *T cruzi*: epimastigote (multiplying form found in the midgut of insect vectors); amastigote (multiplying intracellular form in mammalian hosts); and trypomastigote (nondividing extracellular form in mammalian blood and insect feces).[3] The majority of *T cruzi* proteins are expressed in all 3 morphologic forms.[4]

T cruzi is a species that is genetically heterogeneous and currently divided into 6 consensus groups (*T cruzi* I-VI). Human infection of individuals in North, Central and South America (above the Amazon basin) is typically associated with the *T cruzi* I lineage. Human infections in the Southern Cone of South America are commonly associated with the *T cruzi* II, *T cruzi* V, and *T cruzi* VI lineages.[3, 5]

Trypanosomiasis is primarily transmitted to humans by hematophagous triatomine insects. Other transmission modes include transfusion of blood products, organ transplantation, and congenital infection and oral ingestion of contaminated food. In the Americas, the vector-borne transmission route is still the prevailing means for new human infections.[3]

Most infected persons, after a mild acute phase, enter the lifelong indeterminate phase that is characterized by a lack of symptoms, low parasitemia levels, and antibodies to a variety of *T cruzi* antigens.[3] Approximately 10% to 30% of persons with chronic *T cruzi* infections, however, develop cardiac or gastrointestinal dysfunction as a consequence of the persistent presence of the parasite.[1] The acute phase (which lasts approximately 2 months after infection) can be confirmed directly through observation of the presence of parasites or indirectly through the presence of IgM and IgG antibodies. In the chronic phase, parasites are usually rare, and laboratory diagnosis relies mainly on serological tests. Antibodies are present in more than 98% of infected individuals.[6]

The Alinity s Chagas assay is based on recombinant proteins FP3, FP6, FP10, and TcF. In aggregate, these 4 hybrid recombinant proteins represent at least 14 distinct antigenic regions that broadly represent all 3 morphologic forms. Moreover, these recombinant proteins also contain antigens recognized by antibodies present in persons with acute *T cruzi* infection as well as those with chronic Chagas disease. The epitopes represented by these proteins are used to detect antibodies to *T cruzi* for blood screening and diagnostic applications.[1, 7-9]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is an automated two-step immunoassay for the qualitative detection of antibodies to *T cruzi* in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, *T cruzi* recombinant antigen (FP3, FP6, FP10, and TcF) coated paramagnetic microparticles and assay diluent, are combined and incubated. The antibodies to *T cruzi* present in the sample bind to the recombinant antigen coated microparticles. The mixture is washed. Anti-human IgG acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLU). There is a direct relationship between the amount of antibodies to *T cruzi* in the sample and the RLU detected by the system optics.

The presence or absence of antibodies to *T cruzi* in the sample is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration.

For additional information on system and assay technology, refer to the Alinity s System Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity s Chagas Reagent Kit 06P08

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	06P0850
Tests per cartridge	500
Number of cartridges per kit	5
Tests per kit	2500
MICROPARTICLES	27.0 mL
CONJUGATE	26.5 mL
ASSAY DILUENT	30.3 mL

MICROPARTICLES *T cruzi* recombinant antigens (FP3, FP6, FP10, and TcF) coated microparticles in TRIS buffer. Minimum concentration: 0.04% solids. Preservatives: ProClin 300 and ProClin 950.

CONJUGATE Murine anti-human IgG acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizer and surfactant. Minimum concentration: 0.05 µg/mL. Preservatives: antimicrobial agents.

ASSAY DILUENT TRIS buffer with protein (bovine) stabilizer. Preservatives: ProClin 300 and ProClin 950.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Performance characteristics of this product have not been established for laboratory diagnosis of *T cruzi* infection.

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials and all consumables contaminated with potentially infectious materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are suspected of containing, or are contaminated with infectious agents.[10-13]

The following warnings and precautions apply to: MICROPARTICLES / ASSAY DILUENT	
	
WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
H402*	Harmful to aquatic life.
H412	Harmful to aquatic life with long lasting effects.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P273	Avoid release to the environment.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EC 1272/2008 (CLP) has been implemented.

The following warnings and precautions apply to: CONJUGATE	
Contains polyethylene glycol octylphenyl ether (Triton X-405).	
H402*	Harmful to aquatic life.
H412	Harmful to aquatic life with long lasting effects.
Prevention	
P273	Avoid release to the environment.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EC 1272/2008 (CLP) has been implemented.

Follow local chemical disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.

For the most current hazard information, see the product Safety Data Sheet.

Safety Data Sheets are available at www.transfusion.abbott or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the Alinity s System Operations Manual, Section 8.

Reagent Handling

- Do not invert reagent cartridges.
- Upon receipt, reagent cartridges can be used immediately or stored in an upright position.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity s System Operations Manual, Section 7.

Reagent Storage

- Do not freeze.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2 to 8°C	Until expiration date	Store in upright position.
Opened	2 to 15°C	15 days after opening*	Store in upright position. Discard after 15 days. If cartridge does not remain upright during storage off the system, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.

* Includes time on board the system.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 15°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, refer to the Alinity s System Operations Manual, Section 5.

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when a calibration error occurs or a control value is out of the specified range. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, refer to the Alinity s System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The Alinity s Chagas Assay File must be installed on the Alinity s System prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the Alinity s System Operations Manual, Section 2.

For information on printing assay parameters, refer to the Alinity s System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the Alinity s System Operations Manual.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Other specimen types and anticoagulants have not been verified with this assay.

Specimen Types	Anticoagulants
Serum (including serum separator tubes)	Not Applicable
Plasma	Dipotassium EDTA (including plasma preparation tubes)
	Tripotassium EDTA
	Lithium heparin (including plasma separator tubes)
	Sodium citrate
	Sodium heparin
	ACD-A
	ACD-B
	CP2D
	CPD
	CPDA-1

- Liquid anticoagulants may have a dilution effect resulting in lower S/CO values for individual specimens.
- Performance has not been established for the use of plasmapheresis specimens.
- Performance has not been established for the use of umbilical cord blood or bodily fluids such as urine, saliva, semen, amniotic fluid, cerebrospinal fluid, or pleural fluid.
- Performance has been established for the use of cadaveric serum specimens (including specimens collected post-mortem, non-heart-beating) that have been collected up to 24 hours after death.[14] Follow general standards and/or regulations for collection, storage, and handling.
- Performance has not been established for the use of cadaveric plasma specimens.
- Testing of cadaveric serum specimens from patients with plasma dilution due to transfusions of > 2000 mL of blood or colloids within 48 hours, or > 2000 mL of crystalloids within 1 hour (or any combination thereof) prior to collection of the specimens has not been verified.
- The system does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used with the assay.

Specimen Conditions

- Do not use:
  - heat-inactivated specimens
  - pooled specimens
  - grossly hemolyzed specimens
  - specimens with obvious microbial contamination
  - specimens with fungal growth
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

Failure to follow the specified centrifugation procedure may give erroneous or inconsistent results.

- Clear, nonhemolyzed specimens should be used when possible. Specimens containing visible particulate matter may give erroneous or inconsistent test results.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Prior to centrifugation, previously frozen specimens must be mixed gently and thoroughly after thawing.
- All specimens must be centrifuged between 30 000 - 75 000 g-minutes.
- All specimens must be tested or retested within 48 hours of initial centrifugation. After 48 hours, these specimens need to be recentrifuged between 30 000 - 75 000 g-minutes.

The acceptable time and force ranges that meet this criterion are listed in the table below.

Centrifugation Time (Minutes)	RCF (x g)	g-Minutes
10	3000	30 000
15	2000 - 3000	30 000 - 45 000
20	1500 - 3000	30 000 - 60 000
25	1300 - 3000	32 500 - 75 000

Convert rpm to RCF as follows: $RCF = 1.12 \times r_{max} (rpm/1000)^2$

Convert RCF to rpm as follows:

$$rpm = 1000 \times \sqrt{\frac{RCF}{1.12 \times r_{max}}}$$

RCF - The relative centrifugal force generated during centrifugation.

rpm - The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).

Centrifugation Time - The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.

r_{max} - Radius of the rotor in millimeters. The radius measured is dependent on whether the rotor is a fixed angle rotor or a swinging bucket rotor. This value is typically provided with the rotor by the manufacturer. For the fixed angle rotor, r_{max} is the measure of the distance from the rotor axis (center) to the bottom of the specimen tube in the rotor or rotor adapter. For the swinging bucket rotor, r_{max} is the measure of the distance from the rotor axis (center) to the bottom of the specimen tube in the rotor adapter or bucket at full extension.

NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius (r_{max}) should be manually measured in millimeters and the RCF calculated.

g-minutes - The unit of measure for the product of RCF (× g) and centrifugation time (minutes).

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Living Donor Serum/ Plasma	Room temperature (15 to 30˚C)	7 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	2 to 8˚C	14 days	Specimens may be stored on or off the clot, red blood cells, or separator gel.
	-20˚C or colder	3 months	Remove serum or plasma from the clot, red blood cells, or separator gel.

- Living donor specimens stored at -20˚C or colder for greater than the maximum storage time may be used for informational purposes (*e.g.*, lookback testing, discordant sample testing, clinical and validation testing) and must not be used for releasing patient results or for patient management.

- Storage at a combination of 15 to 30˚C and 2 to 8˚C may not exceed 14 days (inclusive of shipping time) and cannot exceed the maximum durations listed in the table above.
- Performance has not been established for living donor specimens that have undergone more than 6 freeze/thaw cycles.

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Cadaveric Serum	Room temperature (15 to 30˚C)	3 days	If specimens are not processed directly after initial centrifugation, it is recommended to remove the supernatant from the clot, red blood cells or separator gel until further processing.
	2 to 8˚C	14 days	If specimens are not processed directly after initial centrifugation, it is recommended to remove the supernatant from the clot, red blood cells or separator gel until further processing.
	-20˚C or colder	3 months	If specimens are not processed directly after initial centrifugation, it is recommended to remove the supernatant from the clot, red blood cells or separator gel until further processing.

- Performance has not been established using cadaveric specimens stored at -20˚C or colder for greater than 3 months.
- Storage at a combination of 15 to 30˚C and 2 to 8˚C may not exceed 14 days (inclusive of shipping time) and cannot exceed the maximum durations listed in the table above.
- Performance has not been established for cadaveric specimens that have undergone more than 6 freeze/thaw cycles.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

PROCEDURE

Materials Provided

06P08 Alinity s Chagas Reagent Kit

Materials Required but not Provided

- Alinity s Chagas Assay File
- 06P0803 Alinity s Chagas Calibrator Kit
- 06P0820 Alinity s Chagas Assay Control Kit
- 06P0824 Alinity s Chagas Release Control Kit
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity s Concentrated Wash Buffer

For information on materials required for operation of the system, refer to the Alinity s System Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the Alinity s System Operations Manual, Section 9.

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity s System Operations Manual, Section 5.

- Primary tubes may be on board the system for up to 10 hours.
- If using primary or aliquot tubes, refer to the Alinity s System Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- Maximum number of replicates sampled from the same sample cup: 10

  ≤ 3 hours on the reagent and sample manager:
  - Sample volume for first test: 240 µL
  - Sample volume for each additional test from same sample cup: 40 µL

  > 3 hours on the reagent and sample manager:
  - Replace with a fresh aliquot of sample.
- Refer to the Alinity s Chagas Calibrator Kit, Assay Control Kit, and/or Release Control Kit package inserts for preparation and usage.
- For general operating procedures, refer to the Alinity s System Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity s System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Calibration

For instructions on performing a calibration, refer to the Alinity s System Operations Manual, Section 5.

Three replicates of Alinity s Chagas Calibrator 1 are automatically tested by the system. The calibrator must be priority loaded.

Each assay control must be tested to evaluate the assay calibration.

Once a calibration is accepted and stored, it may be used for 14 days. During this time, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.
- Daily quality control results are outside of quality control limits used to monitor and control system performance.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

Assay Controls

The Alinity s Chagas Assay Controls must be tested once every 24 hours when the system is being used.

Assay control values must be within the ranges specified in the Alinity s Chagas Assay Control Kit package insert. When the assay control values are within range, sample results are generated, and a valid release control result is required to release test results. If an assay control value is not within range, sample results are not generated for in-process or scheduled samples. For troubleshooting information, refer to the Alinity s System Operations Manual, Section 10.

Release Controls

The Alinity s Chagas Release Control must be tested in order to release test results.

The release control is tested at user-defined intervals. For configuring the release control, refer to the Alinity s System Operations Manual, Section 2. For manually ordering the release control, refer to the Alinity s System Operations Manual, Section 5.

The release control must meet specifications defined in the Alinity s Chagas Release Control Kit package insert in order to validate the system functionality and release test results. If the release control does not meet specifications, refer to the Alinity s System Operations Manual, Section 10, for additional information.

Other Controls

Additional controls may be tested at operator's discretion in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy. For additional information on configuring customer controls, refer to the Alinity s System Operations Manual, Section 2.

Invalidate controls: Additional controls may be tested anywhere within a run as an invalidate control. Specifications may be assigned to invalidating controls. If an invalidate control fails to meet assigned specifications, no sample results are calculated or provided by the system. When an invalidate control meets assigned specifications, sample processing continues, and a valid release control result is required to release test results.

Non-validating controls: Additional controls may be tested anywhere within a run as a non-validating control. Specifications may be assigned to non-validating controls. A valid release control result is required to release test results. If the user-assigned specifications for the non-validating control(s) are not met and the release control specifications are met, there will be no effect on sample processing. In this case, reactive sample results must not be considered invalid.

Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[15]

RESULTS

Calculation

The Alinity s System calculates results for the Alinity s Chagas assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control.

Cutoff RLU = Calibrator 1 Mean RLU x 0.85

The cutoff RLU is stored for each reagent lot calibration.

S/CO = Sample RLU/Cutoff RLU

Interpretation of Results

The cutoff is 1.00 S/CO.

Initial Results

Initial Result (S/CO)	Interpretation	Retest Procedure
< 1.00	Nonreactive	No retest required. Specimen considered negative for antibodies to *T cruzi*.
≥ 1.00	Reactive	Retest in duplicate.

Final Interpretation

Retest Results (S/CO)	Final Results	Final Interpretation
Both results < 1.00	Nonreactive	Specimen considered negative for antibodies to *T cruzi*.
One or both results ≥ 1.00	Repeatedly Reactive	Specimen should be further tested by supplemental methods.

Supplemental methods should follow appropriate FDA recommendations and regulations for specimens found to be repeatedly reactive.

Customers outside the US must follow their country's government recommendations and regulations for specimens found to be repeatedly reactive.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity s System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- Potential interference has not been evaluated for substances other than those described in the **SPECIFIC PERFORMANCE CHARACTERISTICS - Interference** section of this package insert.
- False reactive results can be expected with any test kit. Falsely elevated results may be observed due to non-specific interactions (refer to the **SPECIFIC PERFORMANCE CHARACTERISTICS** section of this package insert).
- Reactivity of the Alinity s Chagas assay may be observed with specimens obtained from individuals infected with *Trypanosoma brucei*, *Leishmania*, or *Plasmodium vivax* (refer to the **SPECIFIC PERFORMANCE CHARACTERISTICS** section of this package insert).
- Although the association of infectivity and the presence of antibodies to *T cruzi* is strong, it is recognized that presently available methods for the detection of antibodies to *T cruzi* are not sensitive enough to detect all potentially infectious units of blood or possible cases of *T cruzi* infection. A nonreactive test result does not exclude infection.

Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert for specimen limitations.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Reproducibility

A study was performed based on guidance from CLSI EP15-A2.[16] Testing was conducted using 3 lots of the Alinity s Chagas Reagent Kit, Calibrator Kit, Assay Control Kit, and Release Control Kit. Panel members and controls were tested twice a day for 5 days in replicates of 4 at 3 sites.

		Mean	Within-Run		Between-Run		Between-Day		Within-Laboratory[a]		Between-Site		Between-Lot		Reproducibility[b]	
Sample	**N**	**S/CO**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Low *T cruzi* Antibody	360	1.79	0.051	2.8	0.015	0.9	0.019	1.1	0.056	3.2	0.000	0.0	0.170	9.5	0.180	10.1
High *T cruzi* Antibody	359[c]	8.15	0.236	2.9	0.000	0.0	0.119	1.5	0.264	3.2	0.190	2.3	0.343	4.2	0.473	5.8
Positive Control	360	2.82	0.086	3.0	0.031	1.1	0.023	0.8	0.094	3.3	0.030	1.1	0.172	6.1	0.201	7.1
Negative Control	360	0.02	0.001	NA	0.000	NA	0.000	NA	0.001	NA	0.000	NA	0.006	NA	0.006	NA

%CV = Coefficient of Variation expressed as a percentage; N = Number of Replicates; NA = Not Applicable: %CVs are not meaningful when S/CO approaches zero; SD = Standard Deviation

[a] Includes within-run, between-run, and between-day variability.

[b] Includes within-run, between-run, between-day, between-site, between-lot and the site-lot interaction variability.

[c] One replicate was missing due to a pressure monitoring error.

Specificity

A total of 6828 fresh serum specimens and 8976 fresh plasma specimens from volunteer whole blood donors were collected at 3 distinct blood centers. The specimens were collected from donors that had not been screened on a previous donation using an FDA-licensed test for antibodies to *T cruzi* (*i.e.*, first time donors). The initial and repeat reactive rates for the serum specimens were 0.09% (6/6828) and 0.09% (6/6828), respectively. The initial and repeat reactive rates for the plasma specimens were 0.04% (4/8976) and 0.04% (4/8976), respectively. Repeatedly reactive specimens were further tested using an *anti-T cruzi* supplemental assay. Based on supplemental test results, 3 specimens were positive, 3 specimens were negative, and 4 specimens were indeterminate.

Specificity based on assumed zero prevalence of antibody to *T cruzi* in whole blood donors was estimated in this study to be 99.98% (15 787/15 790) with a 95% confidence interval of 99.94% to 100.00%.

Specimen Category	**Number Tested**	**IR (% of Total) (95% CI)**	**RR (% of Total) (95% CI)**	**Number Positive by Supplemental Testing (% of RR)**	**Specificity (%)[a] (95% CI)**
Volunteer Blood Donors - Serum	6828	6 (0.09) (0.03 - 0.19)	6 (0.09) (0.03 - 0.19)	2 (33.33)	99.97 (6820/6822) (99.89 - 100.00)
Volunteer Blood Donors - Plasma	8976	4 (0.04) (0.01 - 0.11)	4 (0.04) (0.01 - 0.11)	1 (25.00)	99.99 (8967/8968) (99.94 - 100.00)
Total Donors	**15 804**	**10 (0.06) (0.03 - 0.12)**	**10 (0.06) (0.03 - 0.12)**	**3 (30.00)**	**99.98 (15 787/15 790) (99.94 - 100.00)**

IR = Initially Reactive; RR = Repeatedly Reactive; CI = Confidence Interval

[a] Based on supplemental test results for the 10 repeatedly reactive specimens, 3 specimens were positive (2 blood donor serum and 1 blood donor plasma), 4 specimens were indeterminate (2 blood donor serum and 2 blood donor plasma), and 3 specimens were negative (2 blood donor serum and 1 blood donor plasma); all 7 repeatedly reactive specimens found to be either positive or indeterminate by supplemental testing were excluded from the specificity calculations. Seven additional Alinity s Chagas nonreactive specimens (2 blood donor serum and 5 blood donor plasma) were positive (1) or indeterminate (6) by supplemental testing; all 7 specimens were excluded from the specificity calculations.

For total donors, the IR rate not reactive on retest was estimated to be 0.00% (0/15 794) with a 95% confidence interval of 0.00% to 0.02%.

IR Rate Not Reactive on Retest = 100% × (Number of IR − Number of RR)/(Number Tested − Number of RR)

Sensitivity

A total of 320 specimens from the categories shown in the table below were tested using the Alinity s Chagas assay at 3 clinical sites. Sensitivity was estimated to be 100.00% (320/320) with a 95% confidence interval of 98.85% to 100.00% for preselected positive specimens.

			Alinity s Chagas		
Specimen Category	**Number Tested**	**Number Positive**	**Number RR (% of Total)**	**Number RR that were Positive (% of RR)**	**Sensitivity (%) (95% CI)**
Preselected *T cruzi* Parasite Positive[a]	112	112	112 (100.00)	112 (100.00)	100.00 (112/112) (96.76 - 100.00)
Preselected *T cruzi* Serology Positive[b]	208	208	208 (100.00)	208 (100.00)	100.00 (208/208) (98.24 - 100.00)
Total	**320**	**320**	**320 (100.00)**	**320 (100.00)**	**100.00 (320/320) (98.85 - 100.00)**

RR = Repeatedly Reactive; CI = Confidence Interval

[a] Preselected *T cruzi* parasite positive specimens were determined by historical diagnosis (xenodiagnosis or hemoculture) or prospective xenodiagnosis.

[b] Preselected *T cruzi* serology positive specimens were determined to have *T cruzi* antibody by 2 different serological tests. Specimens were obtained from South America (85), Mexico (9) and the United States (114).

An additional 615 specimens from individuals from Chagas endemic areas were tested using the Alinity s Chagas assay and a commercially available anti-*T cruzi* assay at 3 clinical sites.

			Alinity s Chagas		
Specimen Category	Number Tested	Number Positive	Number RR (% of Total)	Number RR that were Positive (% of RR)	Sensitivity (%) (95% CI)
Individuals from Chagas Endemic Areas[a]	615	148[b]	147 (23.90)	146 (99.32)	98.65 (146/148) (95.20 - 99.84)

RR = Repeatedly Reactive; CI = Confidence Interval

[a] Specimens were obtained from the endemic countries of Argentina (170), Brazil (48), Guatemala (149), Mexico (100), Panama (50), and Peru (98).

[b] Out of 148 specimens positive by supplemental testing, 146 specimens were repeatedly reactive, and 2 specimens were nonreactive using the Alinity s Chagas assay.

Other Specimen Conditions or Disease States

A total of 256 specimens from individuals with other specimen conditions or disease states unrelated to *T cruzi* infection were evaluated. Of the 256 specimens, 13 were repeatedly reactive using the Alinity s Chagas assay and a commercially available anti-*T cruzi* assay, and positive by supplemental testing.

Specimen Category	Number Tested	IR (% of Total)	RR (% of Total)	Number Positive by Supplemental Testing (% of Repeatedly Reactive)
Other Specimen Conditions or Disease States[a]	256	17 (6.64)	17 (6.64)	13 (76.47)[b]

IR = Initially Reactive; RR = Repeatedly Reactive

[a] The specimens included the following: Leishmaniasis Positive (30), Anti-HTLV I/II Positive (10), Anti-HCV Positive (10), Anti-HIV-1/HIV-2 Positive (10), HBV Positive (10), Malaria *P vivax* Positive (23), Malaria *P falciparum* positive (19), *T brucei* Positive (10), Anti-*T pallidum* Positive (10), Rheumatoid Factor Positive (10), Anti-ds DNA Positive (10), Pregnant Females (14), Multiparous Females (10), Hyper IgG/IgM (10), Influenza Vaccine Recipient (10), HAMA Positive (10), *E coli* Infection (10), Heterophilic Antibody Positive (10), Anti-*T gondii* Positive (10), and Anti-nuclear Antibody Positive (10), Fungal (Yeast) Infection (10).

[b] Ten *T brucei* Positive and 3 Malaria *P vivax* Positive specimens were positive by supplemental testing. Three Leishmaniasis Positive specimens were repeatedly reactive using the Alinity s Chagas assay and indeterminate by supplemental testing. One Leishmaniasis Positive specimen was repeatedly reactive using the Alinity s Chagas assay and negative by supplemental testing.

Interference

Potentially Interfering Endogenous Substances

A study was performed based on guidance from CLSI EP07-A2.[17]

No interference was observed using the Alinity s Chagas assay from potentially interfering substances at the levels shown below.

Potentially Interfering Substance	Interferent Level
Conjugated Bilirubin	≤ 20 mg/dL
Unconjugated Bilirubin	≤ 20 mg/dL
Hemoglobin	≤ 500 mg/dL
Triglycerides	≤ 3000 mg/dL
Total Protein	≤ 12 g/dL

In addition, a negative and positive control were spiked with biotin to a concentration of 4250 ng/mL. No interference was observed using the Alinity s Chagas assay.

The effect of potentially interfering substances has only been evaluated for those listed in this package insert.

PERFORMANCE CHARACTERISTICS OF CADAVERIC SPECIMEN TESTING

Reproducibility

Twenty-three cadaveric donor serum specimens and 23 living donor serum specimens were spiked with human plasma reactive for antibodies to *T cruzi* to create low-level reactive specimens.

Each specimen was tested once per day for 6 days using each of 3 lots of the Alinity s Chagas Reagent Kit. Total %CV values were determined.

			Total[a]	
Specimen Category	Number of Replicates	Mean S/CO	SD	%CV
Cadaveric[b]	414	3.39	0.192	5.7
Living Donor	414	3.38	0.207	6.1

[a] Total variability contains within-specimen, between-lot and lot-specimen interaction variance components.

[b] Cadaveric serum specimens were collected up to 10.0 hours after death.

Specificity

Specificity was determined by testing 55 cadaveric serum specimens and 55 living donor serum specimens. Each specimen was tested once using each of 3 lots of the Alinity s Chagas Reagent Kit.

Specimen Category	Lot	Nonreactive	Repeatedly Reactive	Specificity (%) (95% CI)
Cadaveric[a]	Lot 1	55	0	100.00 (93.51 - 100.00)
	Lot 2	55	0	100.00 (93.51 - 100.00)
	Lot 3	55	0	100.00 (93.51 - 100.00)
Living Donor	Lot 1	55	0	100.00 (93.51 - 100.00)
	Lot 2	55	0	100.00 (93.51 - 100.00)
	Lot 3	55	0	100.00 (93.51 - 100.00)

CI = Confidence Interval

[a] Cadaveric serum specimens were collected up to 23.7 hours after death.

Analytical Sensitivity

Cadaveric serum specimens and living donor serum specimens were spiked with human plasma reactive for antibodies to *T cruzi* to create low-level reactive specimens. Each specimen was tested once, within 24 hours of spiking, using each of 3 lots of the Alinity s Chagas Reagent Kit. All specimens were reactive on all 3 reagent lots.

Specimen Category	Lot	Number of Specimens	Mean S/CO	Sensitivity (%) (95% CI)
Cadaveric[a]	Lot 1	54	3.24	100.00 (93.40 - 100.00)
	Lot 2	54	3.47	100.00 (93.40 - 100.00)
	Lot 3	54	3.38	100.00 (93.40 - 100.00)
Living Donor	Lot 1	55	3.20	100.00 (93.51 - 100.00)
	Lot 2	55	3.45	100.00 (93.51 - 100.00)
	Lot 3	55	3.36	100.00 (93.51 - 100.00)

CI = Confidence Interval

[a] Cadaveric serum specimens were collected up to 23.7 hours after death.

Cadaveric Specimen Storage

Cadaveric specimen storage was determined by testing a minimum of 12 low-level reactive specimens, prepared by spiking nonreactive cadaveric serum specimens to a target S/CO value near the cutoff with human plasma reactive for anti-*T cruzi*, and a minimum of 12 nonreactive cadaveric serum specimens. Each specimen was tested at Day 0, and then subjected to either 2 to 8°C storage for 14 days, room temperature (15 to 30°C) storage for 3 days, -20°C or colder storage for 3 months, or 6 freeze/thaw cycles. Nonreactive specimens were evaluated by calculating the differences between the mean S/CO of Day 0 and the mean S/CO of each storage condition and related timepoint. Reactive specimens were evaluated by calculating the percent differences between the mean S/CO of Day 0 and the mean S/CO of each storage condition and related timepoint. There were no changes to the interpretation; the data demonstrate that cadaveric serum specimens can be stored at the following conditions when tested using the Alinity s Chagas assay.

Storage Condition	Timepoint	Nonreactive Specimens Upper Limit of 2-sided 95% CI of Differences	Reactive Specimens Lower Limit of 2-sided 95% CI of % Differences
Room Temperature (15 to 30°C)[a]	3 days	0.00 S/CO	-11.9%
2 to 8°C[a]	14 days	0.00 S/CO	-3.2%
-20°C or colder[b]	3 months	0.00 S/CO	-3.5%
Freeze/Thaw[a]	6 cycles	0.00 S/CO	-12.8%

CI = Confidence Interval

[a] Cadaveric serum specimens were collected up to 41.8 hours after death.

[b] Cadaveric serum specimens were collected up to 14.5 hours after death.

BIBLIOGRAPHY

1. Chang CD, Cheng KY, Jiang LX, et al. Evaluation of a prototype *Trypanosoma cruzi* antibody assay with recombinant antigens on a fully automated chemiluminescence analyzer for blood donor screening. *Transfusion* 2006;46:1737-1744.
2. World Health Organization. Chagas disease (American trypanosomiasis). http://www.who.int/en/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)/. Updated April 2021. Accessed February 10, 2022.
3. Bern C, Kjos S, Yabsley M, et al. *Trypanosoma cruzi* and Chagas' Disease in the United States. *Clin Micro Reviews* 2011;24(4):655–681.
4. Paba J, Santana J, Teixeira A, et al. Proteomic analysis of the human pathogen *Trypanosoma cruzi*. *Proteomics* 2004;4(4):1052-1059.
5. Zingales B, Andrade S, Briones, M, et al. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. *Mem Inst Oswaldo Cruz* 2009;104:1051-1054.
6. World Health Organization. WHO Consultation on International Biological Reference Preparations for Chagas Diagnostic Tests. https://www.who.int/bloodproducts/ref_materials/WHO_Report_1st_Chagas_BRP_consultation_7-2007_final.pdf. Published July 2007. Accessed February 10, 2022.
7. Hoft DF, Kim KS, Otsu K, et al. *Trypanosoma cruzi* expresses diverse repetitive protein antigens. *Infect Immun* 1989;57(7):1959-1967.
8. Cheng KY, Cheng CD, Salbilla VA, et al. Immunoblot assay using recombinant antigens as a supplemental test to confirm the presence of antibodies to *Trypanosoma cruzi*. *Clin Vaccine Immunol* 2007;14(4):355-361.
9. Houghton RL, Benson DR, Reynolds LD, et al. A multi-epitope synthetic peptide and recombinant protein for the detection of antibodies to *Trypanosoma cruzi* in radioimmunoprecipitation-confirmed and consensus-positive sera. *J Infect Dis* 1999;179:1226-1234.
10. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
11. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories.* 6th ed. Washington, DC: US Government Printing Office; June 2020.
12. World Health Organization. *Laboratory Biosafety Manual.* 4th ed. Geneva: World Health Organization; 2020.
13. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition.* CLSI Document M29-A4. Wayne, PA: CLSI; 2014.
14. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for Industry Recommendations for Obtaining a Labeling Claim for Communicable Disease Donor Screening Tests Using Cadaveric Blood Specimens from Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps), November 2004. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm091374.pdf. Accessed February 10, 2022.
15. Westgard JO. *Basic QC Practices.* 3rd ed. Madison, WI: Westgard Quality Corporation; 2010.
16. Clinical and Laboratory Standards Institute (CLSI). *User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition.* CLSI Document EP15-A2. Wayne, PA: CLSI; 2005.
17. Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition.* CLSI Document EP07-A2. Wayne, PA: CLSI; 2005.

Note for number formatting:

- A space is used as thousands separator (example: 10 000 specimens).
- A period is used to separate the integer part from the fractional part of a number written in decimal form (example: 3.12%).

Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
ASSAY DILUENT	Assay Diluent
CONJUGATE	Conjugate
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRODUCT OF GERMANY	Product of Germany
REF	List Number
SN	Serial number

Abbott GmbH
Max-Planck-Ring 2
65205 Wiesbaden
Germany
+49-6122-580

CE
0123

DISTRIBUTED IN THE USA BY

Abbott Laboratories
Abbott Park, IL 60064 USA

Customer Service: Contact your local representative or find country-specific contact information at www.transfusion.abbott

For customers in the European Union: if, in the course of using this device, you have reason to believe that a serious incident has occurred, report it to the manufacturer and to your national authority.

A summary of safety and performance (SSP) for this device is available at https://ec.europa.eu/tools/eudamed. This is the SSP location after the launch of European Database on Medical Devices. Search for the device using the UDI-DI provided on the outer packaging of the device.

US License No. 2095

Revised March 2022.