Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please call 800-835-4709 or 240-402-8010, extension 1. CBER Consumer Affairs Branch or 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.

en

ABBOTT ESA Chagas
8L34

G10297R09
B8L340

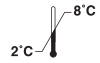
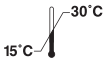
Read Highlighted Changes
Revised October 2024

Trypanosoma cruzi (*E coli*, Recombinant) Antigen

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

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

Key to Symbols

ISO 15223 Symbols	
	Caution
	Consult instructions for use
	Manufacturer
	Store at 2-8˚C
	Store at 15-30˚C
	Use by/Expiration date
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
REF	List Number

Other Symbols	
CONCENTRATED WASH BUFFER	Concentrated Wash Buffer
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTROL −	Negative Control
CONTROL +	Positive Control
DO NOT FREEZE	Do not Freeze
INCUBATION TRAYS	Incubation Trays
PRODUCT OF USA	Product of USA
SPECIMEN DILUENT	Specimen Diluent
STRIPS	Strips
SUBSTRATE TABLETS	Substrate Tablets
WARNING: SENSITIZER	May cause an allergic reaction

US License No. 43

Abbott

NAME AND INTENDED USE

ABBOTT ESA Chagas is an *in vitro* enzyme strip assay intended for the qualitative detection of antibodies to *Trypanosoma cruzi (T cruzi)* in human serum and plasma specimens. The assay is intended for use as an additional, more specific test on human serum or plasma specimens found to be repeatedly reactive using a licensed screening test for antibodies to *T cruzi*.

SUMMARY AND EXPLANATION OF THE TEST

Chagas disease or American Trypanosomiasis is caused by the parasite *T cruzi*. 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).[1] The majority of *T cruzi* proteins are expressed in all 3 morphologic forms. ABBOTT ESA Chagas is based on recombinant proteins FP10, FP6, FP3, and TcF.[2-5] 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 epitopes recognized by antibodies present in persons with acute *T cruzi* infections as well as those with chronic Chagas disease.[6]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

ABBOTT ESA Chagas is a multi-step enzyme strip assay.

- Four individually prepared *T cruzi* recombinant antigens (FP10, FP6, FP3, and TcF), 3 onboard controls (2 onboard visual calibrators [human IgG], and 1 onboard sample addition control [anti-human IgG]) have been applied separately as discrete lines across strips that are composed of nitrocellulose membrane laminated onto a plastic support. These 4 *T cruzi* recombinant antigens are also used in ABBOTT PRISM Chagas in which they are coated onto the surface of microparticles. Licensed screening assays use the combined reactivity of their representative antigens to give a single composite signal. In ABBOTT ESA Chagas, the reactivity of each recombinant antigen is evaluated individually, resulting in a more specific test. The calibrators (H-CAL and L-CAL) are used to interpret results of the assay and indicate that conjugate was added to the strip. The control indicates that a sample was added to the strip.
- The strips are incubated with sample (either plasma, serum, or ABBOTT ESA Chagas Positive or Negative Control) and specimen diluent in the trough of the incubation tray. During incubation, *T cruzi* antibodies present in the sample bind to the antigen(s) on the strips.
- After this first incubation is complete, the strips are washed with a 1x Wash Buffer. Then a goat anti-human:alkaline phosphatase conjugate is added to the strips and incubated. The conjugate binds antibody to *T cruzi* that is present.
- After the second incubation is complete, the strips are washed and the enzyme substrate (BCIP/NBT) is added and incubated.
- After incubation with the substrate, the strips are washed and dried. For each sample, color intensity at the location of each recombinant antigen is individually graded against the onboard calibrators to interpret the assay results (see **Interpretation of Results**).

REAGENTS

ABBOTT ESA Chagas Kit (REF 8L34-68)

- **STRIPS** 1 Bottle (30 strips) *T cruzi* (*E coli*, recombinant) Antigen Coated Strips: Each strip contains 1 goat anti-human IgG specimen control band, 2 human IgG calibrator bands, and 4 individual bands coated with *T cruzi* antigens. Minimum concentrations for the antigen bands: FP10: 2.5 µg/mL, FP6: 0.35 µg/mL, FP3: 0.2 µg/mL, and TcF: 37.5 µg/mL.
- **CONJUGATE** 1 Bottle (38 mL) Anti-human (Goat):Alkaline Phosphatase Conjugate in TRIS buffer with protein stabilizers and detergent. Minimum concentration: 0.1 µg/mL. Preservative: 0.15% ProClin 950.
- **CONTROL −** 1 Bottle (0.25 mL) Negative Control is recalcified, human plasma. Preservative: 0.1% sodium azide and 0.15% ProClin 950.
- **CONTROL +** 1 Bottle (0.25 mL) Positive Control is recalcified, human plasma containing antibodies to *T cruzi*, including mouse/human chimeric monoclonal antibody. Minimum activity: 1+. Preservative: 0.1% sodium azide and 0.15% ProClin 950.
- **SPECIMEN DILUENT** 1 Bottle (38 mL) Specimen Diluent containing TRIS buffer with protein stabilizers and surfactants. Preservative: 0.15% ProClin 950.
- **CONCENTRATED WASH BUFFER** 1 Bottle (38 mL) Concentrated Wash Buffer containing TRIS buffer and detergent. Preservative: 0.15% ProClin 950.
- **SUBSTRATE TABLETS** 10 BCIP/NBT Substrate Tablets (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium).
- **INCUBATION TRAYS** 10 Incubation Trays.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use
- **The performance characteristics of this product have not been established for the laboratory diagnosis of *T cruzi* infection.**
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

⚠ **CAUTION:** This product contains human-sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human-sourced materials should be considered potentially infectious. It is recommended that these reagents, human specimens, and all consumables contaminated with potentially infectious materials be 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.[7-10] These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where specimens and reagents are handled.
- Clean and disinfect all spills of specimens or reagents using an appropriate disinfectant such as 0.1% sodium hypochlorite, or other suitable disinfectant.[11,12]
- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local, state, and federal regulations.[13,14]
- The human plasma used in the Negative Control has been tested and found to be nonreactive for antibodies to *T cruzi*, HBsAg, HIV-1Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.
- The human plasma used in the positive control contains antibodies to *T cruzi*, including mouse/human chimeric monoclonal antibody. The human plasma used in the Positive Control has been tested and found to be reactive for *T cruzi* and nonreactive for HBsAg, HIV-1Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.

The following warnings and precautions apply to the: **CONJUGATE**, **CONCENTRATED WASH BUFFER** and **SPECIMEN DILUENT**

WARNING	Contains methylisothiazolone.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
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.

The following warnings and precautions apply to the: CONTROL –	
	
WARNING	Contains methylisothiazolone and sodium azide.
H317	May cause an allergic skin reaction.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
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.

The following warnings and precautions apply to the: CONTROL +	
	
WARNING	Contains methylisothiazolone and sodium azide.
H317	May cause an allergic skin reaction.
H402	Harmful to aquatic life.
EUH032	Contact with acids liberates very toxic gas.
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.

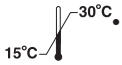
- Follow local chemical disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.
- For the most current hazard information, see the product Safety Data Sheet.
- Safety Data Sheets are available at www.transfusion.abbott or contact your local representative.

Handling Precautions

- **Use caution to avoid microbial and chemical contamination of samples, reagents, and equipment.**
- Use disposable pipette tips for all pipetting steps.
- Use a new pipette tip for each specimen.
- Do not interchange bottles or bottle caps between reagents.
- Do not use kits beyond the expiration date.
- **DO NOT INTERCHANGE REAGENTS OR STRIPS BETWEEN KIT LOTS.** Kit components and strips from kits with different lot numbers must not be used together.
- Treat negative and positive controls as potentially infectious.
- Use only the ABBOTT ESA Chagas Positive and Negative Controls provided with the kit.
- Use accurately calibrated equipment.
- Distilled or deionized water (Clinical and Laboratory Standards Institute [CLSI] clinical laboratory reagent water or better)[15] must be used for preparation of the 1x Wash Buffer and substrate. Store water, 1x Wash Buffer, and substrate in nonmetallic containers.
- Use forceps to hold the strip within the identification portion avoiding contact with the nitrocellulose membrane.
- Do not cut strips.
- Do not reuse strips or reaction troughs.
- **Do not allow strips to dry out during the procedure, prior to completion of color development.**
- To prevent fading, keep developed strips out of strong light (eg, direct sunlight).

Storage Instructions

- When stored and handled as directed, reagents are stable until the expiration date.
- Store ABBOTT ESA Chagas Kit at 2-8°C. Do not freeze.
- Allow all reagents to reach room temperature (15-30°C) before use, and return unused reagents to 2-8°C storage after use. Store unused strips in the closed original container.



- Store the 1x Wash Buffer at 15-30°C and use within 7 days of preparation.
- Store the substrate solution at 15-30°C and use within 4 hours of preparation.

Indications of Instability or Deterioration of Reagents

Changes in the physical appearance of the reagents supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed (eg, obvious changes in reagent color or cloudiness, which may be associated with microbial contamination), they should not be used.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

- Serum (including serum collected in serum separator tubes), plasma collected in EDTA, potassium oxalate, sodium citrate, ACD-A, ACD-B, CP2D, CPD, or CPDA-1 anticoagulants, or plasma collected from segmented tubing may be used with ABBOTT ESA Chagas. Follow the manufacturer's specimen collection instructions for serum and plasma collection tubes.

  CAUTION: Do not use specimens collected in heparin.

Specimen Conditions

- This assay was designed and validated for use with individual human serum and plasma specimens. This assay has not been validated for use with pooled specimens.
- Serum from heparinized patients may be incompletely coagulated. Draw the specimen prior to heparin therapy or after heparin therapy is discontinued and activated partial thromboplastin time (aPTT) levels return within normal range.
- Do not use heat-inactivated specimens.
- Do not use specimens with obvious microbial contamination or gross lipemia.
- Performance has not been established using plasmapheresis or cadaveric specimens, umbilical cord blood, or body fluids such as urine, saliva, semen, amniotic fluid, cerebrospinal fluid, or pleural fluid. These specimens should not be tested using ABBOTT ESA Chagas.
- Clear, nonhemolyzed specimens should be used when possible. Specimens containing visible particulate matter may give erroneous or inconsistent test results and must be centrifuged prior to testing.

Potential Interfering Substances

- No qualitative performance differences were observed when a minimum of 26 nonreactive donor specimens and 27 reactive donor specimens, which were created by spiking with *T cruzi* antibody to low-level reactivity, were spiked with potentially interfering substances, creating samples with artificially elevated levels of bilirubin (≤ 20 mg/dL), hemoglobin in plasma (≤ 500 mg/dL), red blood cells (≤ 0.4% v/v), triglycerides (≤ 3000 mg/dL), or protein (≤ 12 g/dL).
- In an additional study, 20 specimens from patients with elevated levels of endogenous hemoglobin in whole blood (16.2 to 18.1 g/dL), 19 specimens with elevated levels of endogenous triglycerides (1009 to >10 450 mg/dL), 18 specimens with elevated levels of endogenous total protein (9.1 to 11.2 g/dL), and 20 specimens with elevated levels of endogenous bilirubin (5.1 to 11.2 mg/dL) were spiked with *T cruzi* antibody to target a low level of reactivity. All of these specimens prior to spiking were negative. All of the spiked specimens remained positive.

Preparation for Analysis

FAILURE TO FOLLOW THE SPECIFIED CENTRIFUGATION PROCEDURE MAY GIVE ERRONEOUS OR INCONSISTENT TEST RESULTS.

Nonfrozen specimens must be centrifuged such that g-minutes are between 30 000 and 75 000, and then may be stored at 2-8°C for up to 7 days. After 7 days, specimens need to be recentrifuged such that g-minutes are between 30 000 and 75 000.

Previously frozen specimens must be mixed gently and thoroughly after thawing and centrifuged such that g-minutes are between 30 000 and 75 000.

A refrigerated or nonrefrigerated centrifuge is acceptable for use. The acceptable time and force ranges that meet the criterion of Nonfrozen and Previously frozen specimens are described in the following table.

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

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 (ie, 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 (x g) and centrifugation time (minutes).

Storage and Shipping

- After collection, specimens may be stored at 30°C or colder for up to 7 days, 2-8°C for up to 14 days, or frozen at -20°C or colder for up to 2 months (inclusive of shipping time). Storage at a combination of 2-8°C and 30°C or colder may not exceed 14 days.
- Prior to freezing, the serum or plasma should be removed from the clot or red blood cells to avoid hemolysis.
- Specimens stored at -20°C or colder for greater than 2 months may be used for informational purposes (eg, lookback testing, discordant sample testing, clinical and validation testing).
- When shipping specimens, package and label specimens in compliance with applicable regulations covering the transport of clinical specimens and infectious substances.
- Thirty nonreactive and 30 low-level reactive specimens showed no qualitative performance differences when subjected to 6 freeze/thaw cycles. However, some specimens that have undergone multiple freeze/thaw cycles, or have been stored frozen for prolonged periods, may give erroneous or inconsistent test results.

PROCEDURE

Materials Provided

- REF 8L34-68 ABBOTT ESA Chagas Kit

Materials Required but not Provided

- Distilled or Deionized Water (CLSI clinical laboratory reagent water or better)[15]
- 1 mL Pipette
- 20 µL Pipette
- Repeater Pipette (eg, 25 mL Eppendorf)
- Microfuge or small centrifuge
- Bi-directional Orbital Rocker capable of maintaining 20-35 rpm
- Nonmetallic containers with caps
- Vacuum-powered aspirator with trap
- Forceps

Reagent Preparation

Before use, bring all reagents to room temperature (15-30°C).

Determine the number of specimens to be tested and the number of strips required. Each run requires the following number of strips:

- one strip for the positive control
- one strip for the negative control
- one strip for each test specimen
- **NOTE:** Each tray holds 8 strips.

1x Wash Buffer

- The 1x Wash Buffer may be prepared before starting the **ABBOTT ESA Chagas Procedure** and must be used within 7 days.
- Gently invert the Concentrated Wash Buffer several times to ensure a homogeneous solution. Avoid foaming.
- Each strip requires 6 mL of 1x Wash Buffer. Make a 1:10 dilution of Concentrated Wash Buffer by adding 1 volume of Concentrated Wash Buffer to 9 volumes of distilled or deionized water in a nonmetallic container with cap and mix thoroughly by gently inverting to ensure a homogeneous solution. Avoid foaming.
- Use the table below as a guide for preparing the 1x Wash Buffer. When processing a number of strips not specified in the table, refer to the next highest maximum number of strips to be processed. For example, for 7 strips, use volumes for processing 8 strips.

Maximum Number of Strips to be Processed	Volume of Concentrated Wash Buffer (mL)	Volume of Distilled or Deionized Water (mL)	Total Volume of Prepared 1x Wash Buffer (mL)*
3	2	18	20
6	4	36	40
8	5	45	50
10	7	63	70
15	10	90	100
20	13	117	130
25	16	144	160
30	19	171	190

* Provides sufficient quantity in slight excess of the required minimum to assure an uninterrupted test procedure.

Substrate

The substrate will be prepared during the **ABBOTT ESA Chagas Procedure** in Step 14.

- **Use the substrate within 4 hours of preparation.**
- Each strip requires 1 mL of substrate. Dissolve 1 substrate tablet in 20 mL of distilled or deionized water in a nonmetallic container with cap. Swirl container gently to obtain a homogeneous solution. Avoid foaming.
- Use the table below as a guide for preparing substrate.

Number of Strips to be Processed	Number of Substrate Tablets	Volume of Distilled or Deionized Water (mL)*
3 - 19	1	20
20 - 30	2	40

* Provides sufficient quantity in slight excess of the required minimum to assure an uninterrupted test procedure.

ABBOTT ESA Chagas Procedure

Initial Preparation

1. Read **Handling Precautions** before performing the assay procedure.
2. Allow all test materials to reach room temperature (15-30˚C) before performing the assay. Mix reagents thoroughly by **gently** inverting each component (except strips and substrate tablets) several times to ensure a homogeneous solution. Avoid foaming.

   The entire assay procedure is performed at room temperature (15-30˚C).
3. If required, centrifuge specimens (excluding the ABBOTT ESA Chagas Negative and Positive Controls) as described in the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section.
4. If not already prepared, prepare 1x Wash Buffer.
5. Using forceps, remove each strip from the container and place it with the filled rectangle facing down into each reaction trough of the incubation tray. Check to ensure that there is only 1 strip in each trough.

   Each run requires the following number of strips:

   - one strip for the positive control
   - one strip for the negative control
   - one strip for each test specimen

   NOTE: Each tray holds 8 strips.
6. Prepare a record to identify the numbers on the strips (ID) with the corresponding specimen identification numbers.
7. Place each incubation tray on the rocker.

Specimen Incubation

8. Add 1 mL of specimen diluent to each reaction trough containing a strip. **Use forceps to gently submerge each strip in specimen diluent, touching the strip at the ID end.**
9. Incubate each tray on the rocker at 20-35 rpm for 5-7 minutes to allow the strips to become saturated with specimen diluent. After incubation, remove the incubation tray(s) from the rocker.

   NOTE: The motion of the diluted specimen or controls over the strips, generated by the rocker, is important in achieving optimum performance of the assay. Periodically check to ensure that a rocking motion is maintained throughout the incubations. Improper functioning of the rocker, which may affect antibody binding, will invalidate the test results and require that the assay be repeated.
10. Deliver 20 µL of each specimen or each control to a single reaction trough containing strip and specimen diluent. When dispensing specimen or control, the pipette tip should be below the surface of the specimen diluent.
11. Cover the incubation tray(s). Incubate each tray on the rocker at 20-35 rpm for 2 hours ± 5 minutes.

    After incubation, remove the incubation tray(s) from the rocker. Remove the tray cover(s) and aspirate the liquid (at the strip ID end) from each reaction trough.
12. Wash the strips by adding 1 mL of 1x Wash Buffer to each reaction trough containing a strip. Return the incubation tray(s) to the rocker and incubate at 20-35 rpm for 4-5 minutes.

    After incubation, remove the incubation tray(s) from the rocker and aspirate the liquid (at the strip ID end) from each reaction trough.

    Repeat this step two more times (3 washes total).

Conjugate Incubation

13. After washing the strips, add 1 mL of conjugate to each reaction trough containing a strip.
14. Cover the incubation tray(s). Incubate each tray on the rocker at 20-35 rpm for 1 hour ± 5 minutes.

    Prepare the substrate during the conjugate incubation period.

    After incubation, remove the incubation tray(s) from the rocker. Remove the tray cover(s) and aspirate the liquid (at the strip ID end) from each reaction trough.
15. Wash the strips by adding 1 mL of 1x Wash Buffer to each reaction trough containing a strip. Return the incubation tray(s) to the rocker and incubate at 20-35 rpm for 4-5 minutes.

    After incubation, remove the incubation tray(s) from the rocker and aspirate the liquid (at the strip ID end) from each reaction trough.

    Repeat this step two more times (3 washes total).

Substrate Incubation

16. After aspiration of 1x Wash Buffer, add 1 mL of substrate to each reaction trough containing a strip.
17. Cover the incubation tray(s). Incubate each tray on the rocker at 20-35 rpm for 10 ± 1 minute. Color will develop during the 10-minute incubation period.

    After incubation, remove the incubation tray(s) from the rocker. Remove the tray cover(s) and aspirate the liquid (at the strip ID end) from each reaction trough.
18. Wash the strips by adding 1 mL of distilled or deionized water to each reaction trough containing a strip. Return the incubation tray(s) to the rocker and incubate at 20-35 rpm for 4-5 minutes.

    After incubation, remove the incubation tray(s) from the rocker and aspirate the liquid (at the strip ID end) from each reaction trough.

    Repeat this step two more times (3 water washes total).

Final Preparation

19. Using forceps, transfer strips to a paper towel with the filled rectangle facing up and aligned in a single direction. Gently blot dry with a paper towel. Then allow the strips to air dry for at least 30 minutes.
20. Interpret the results within 24 hours and 30 minutes of removal from the reaction trough. Record the results for each specimen and control as described in **QUALITY CONTROL PROCEDURES** and **RESULTS**.

QUALITY CONTROL PROCEDURES

The ABBOTT ESA Chagas Positive and Negative Controls must be included in each run of test specimens. A run may include any number of specimens, strips, or incubation trays. The controls are not run with each incubation tray.

Each strip includes onboard controls that must be evaluated before assessment of recombinant antigen bands. Human IgG low-level calibrator (L-CAL) and human IgG high-level calibrator (H-CAL) are used to interpret results of the assay. The anti-human IgG specimen addition control (SPM-CTL) is included to indicate that a specimen was added to the strip.

If the negative and/or positive controls do not meet all of the criteria below, then the control result and associated test results are invalid. The samples must be retested.

Negative Control

Onboard Controls

- Must display clearly distinguishable onboard L-CAL and H-CAL bands.
- The L-CAL band (at the visual cutoff, 1+) must be clearly lighter (less intense) than the H-CAL band (at scoring of 3+).
- Must display a clearly distinguishable SPM-CTL band, with color intensity greater than the L-CAL (2+ or greater).

Antigen Bands

- Must not display a visible band at any of the four recombinant antigen locations.

The strips MUST NOT display uneven background color or colored deposits across any portion of seven possible visible bands. Some uneven background color or colored deposits between the bands or at the ends of the strip are acceptable.

Positive Control

Onboard Controls

- Must display clearly distinguishable onboard L-CAL and H-CAL bands.
- The L-CAL band (at the visual cutoff, 1+) must be clearly lighter (less intense) than the H-CAL band (at scoring of 3+).

- Must display a clearly distinguishable SPM-CTL band, with color intensity greater than the L-CAL (2+ or greater).

Antigen Bands

- Must display a clearly distinguishable band at the locations of recombinant antigens FP10, FP6, and TcF, with color intensities for each recombinant antigen greater than the L-CAL (2+ or greater).
- Must display a clearly distinguishable band at the location of recombinant antigen FP3 band with color intensity of +/- or greater.

The strips MUST NOT display uneven background color or colored deposits across any portion of seven possible visible bands. Some uneven background color or colored deposits between the bands or at the ends of the strip are acceptable.

RESULTS

Specimen Strip Validity Criteria

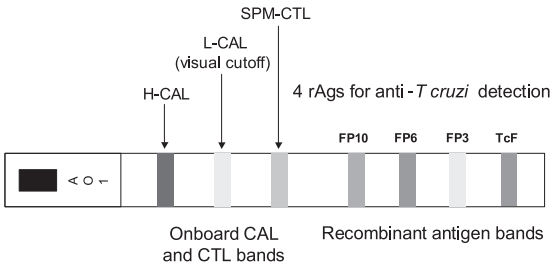
If a specimen strip does not meet all of the criteria below, then the associated **test result is invalid** and the specimen **must be retested** in a new run.

- Must display clearly distinguishable onboard L-CAL and H-CAL bands.
- The L-CAL band (at the visual cutoff, 1+) must be clearly lighter (less intense) than the H-CAL band (at scoring of 3+).
- Must display a clearly distinguishable SPM-CTL band, with color intensity greater than the L-CAL (2+ or greater).

The strips MUST NOT display uneven background color or colored deposits across any portion of seven possible visible bands. Some uneven background color or colored deposits between the bands or at the ends of the strip are acceptable.

Reading Results

For valid strips, reactivity of test specimens against individual *T cruzi* recombinant antigens (rAgs) is visually graded by comparing the color intensity of each of the four *T cruzi* antigens against the color intensity of the L-CAL (visual cutoff, intensity of 1+) and the H-CAL (intensity of 3+) on the strip incubated with the test specimen. The identity and location of the rAgs coated on the strips are shown below.



NOTE: A01 (on the strip above) is an example of the printed strip ID.

Visual color intensities **on each strip incubated with test specimen** should be graded against the L-CAL and H-CAL bands on the same strip, not against strips incubated with the negative or the positive control.

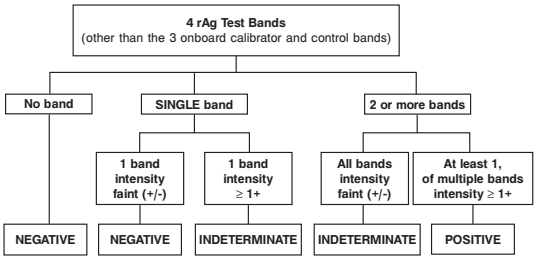
The intensity of reactivity against *T cruzi* rAgs is visually graded against the intensities of the onboard L-CAL and H-CAL as follows:

REACTION BAND INTENSITY	VISUAL GRADE
Absent	-
Faint or less than the L-CAL	+/-
Equal to the L-CAL	1+
Greater than the L-CAL but less than the H-CAL	2+
Equal to the H-CAL	3+
Greater than the H-CAL	4+

Occasionally, a strip may have a dark background. If the L-CAL and H-CAL onboard calibrator bands are distinguishable from the background (ie, darker than the background, with the H-CAL band darker than the L-CAL), the strip is interpretable and the intensity of the antigen bands should be compared to the onboard calibrators as described in this section.

Interpretation of Results

A NEGATIVE, INDETERMINATE, or POSITIVE interpretation for a specimen is based on the reaction pattern of the rAg bands present on the strip. For valid strips, the following criteria should be used to interpret the result.



Interpretation criteria are summarized in the following tables.

Antigen Band Pattern	Result
• No antigen bands visible **or** • A **SINGLE** antigen band, having a +/- intensity	NEGATIVE
• A **SINGLE** antigen band, having an intensity of 1+ or greater **or** • Two or more bands, all having a +/- intensity	INDETERMINATE
• Two or more bands, with **at least 1 band** having an intensity of 1+ or greater	POSITIVE

Specimens with INDETERMINATE test results must be retested once and may need to be recentrifuged. Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section to determine if recentrifugation is needed.

Initial Result	Retest Result	Interpretation
POSITIVE	No retest required	POSITIVE: Antibodies to *T cruzi* detected, indicative of a *T cruzi* infection
INDETERMINATE	Retest is POSITIVE	POSITIVE: Antibodies to *T cruzi* detected, indicative of a *T cruzi* infection
	Retest is INDETERMINATE Retest is NEGATIVE	INDETERMINATE: Antibodies to *T cruzi* may or may not be present. These individuals, especially those with risk factors[a], may be retested after 6 months using a freshly drawn specimen.
NEGATIVE	No retest required	NEGATIVE: Antibodies to *T cruzi* not detected

[a] Identifiable risk factors include: born in a Chagas endemic area or parent born in an endemic area, etc.

LIMITATIONS OF THE PROCEDURE

- This assay was designed and validated for use with individual human serum and plasma specimens. This assay has not been validated for use with pooled specimens.
- **Do not use specimens collected in heparin.**
- Serum from heparinized patients may be incompletely coagulated. Draw the specimen prior to heparin therapy or after heparin therapy is discontinued and activated partial thromboplastin time (aPTT) levels return within normal range.
- False-reactive test results can be expected with any test kit. False-reactive test results have been observed due to nonspecific interactions. Refer to the **SPECIFIC PERFORMANCE CHARACTERISTICS** section of this package insert for assay performance characteristics.
- Some specimens that have undergone multiple freeze/thaw cycles or have been stored frozen for prolonged periods may result in erroneous or inconsistent test results.
- All specimens must be centrifuged according to the **Preparation for Analysis** section of this package insert prior to running the assay.

- Performance has not been established using plasmapheresis or cadaveric specimens, umbilical cord blood, or body fluids such as urine, saliva, semen, amniotic fluid, cerebrospinal fluid, or pleural fluid. These specimens should not be tested using ABBOTT ESA Chagas.
- Additional testing for Leishmania should be considered for individuals with indeterminate results with ABBOTT ESA Chagas who have identifiable risk factors for leishmaniasis.
- Do not use heat-inactivated specimens.
- Do not use specimens with obvious microbial contamination or gross lipemia.
- Do not use serum or plasma specimens with obvious gross hemolysis (dark red to black). No qualitative performance differences were observed when specimens were spiked with 500 mg/dL of hemoglobin. No qualitative performance differences were observed for specimens with up to 1510 mg/dL endogenous levels of hemoglobin.
- The **ABBOTT ESA Chagas Procedure** and **RESULTS** must be closely followed when testing serum or plasma specimens for the presence of antibodies to *T cruzi*.
- Avoid microbial contamination of reagents by carefully following handling precautions within this package insert.
- A test result that is negative does not exclude the possibility of exposure to or infection with *T cruzi*.

SPECIFIC PERFORMANCE CHARACTERISTICS

Clinical Reproducibility

Reproducibility was determined at the clinical testing sites with ABBOTT ESA Chagas by testing a 6-member panel. Panel Members 1 and 2 were *T cruzi* antibody-negative specimens. Panel Member 3 was a *T cruzi* antibody-positive specimen diluted 1:45 with recalcified negative plasma to create an indeterminate banding pattern close to the visual cutoff (targeting at least 2 bands with intensity of +/-). Panel Members 4, 5, and 6 were *T cruzi* antibody-positive specimens. Each panel member was tested once per day over 3 days with each of 3 reagent lots at 4 clinical sites, with 1 technician at each site.

There were 36 strips tested for each of the 6 panel members with 4 antigen bands per strip for a total of 864 antigen band readings. 99.19% (857/864) of the antigen band readings were within one level of intensity of the band reading reported by the majority of strip readers. 0.81% (7/864) of the antigen band readings for the 3 positive panel members were two levels of intensity higher than the band reading reported by the majority of strip readers.

Table I shows the percent agreement for the strip interpretation for each of the panel members. Eight (8) out of 36 strips for the indeterminate Panel Member 3 were interpreted as negative.

This study shows acceptable reproducibility performance for ABBOTT ESA Chagas.

Table I
ABBOTT ESA Chagas Reproducibility

Panel Member	Expected Reactivity	n	ABBOTT ESA Chagas POS	IND	NEG	% Agreement
1	Negative	36	0	0	36	100.00
2	Negative	36	0	0	36	100.00
3	Indeterminate	36	0	28	8	77.78
4	Positive	36	36	0	0	100.00
5	Positive	36	36	0	0	100.00
6	Positive	36	36	0	0	100.00

POS = Positive, IND = Indeterminate, NEG = Negative

Clinical Specificity in US Blood Donors

A total of 330 serum and plasma specimens from United States (US) blood donors were tested with ABBOTT ESA Chagas (Table II). The specimens were presumed negative for antibodies to *T cruzi* based on a *T cruzi* antibody licensed enzyme-linked immunosorbant assay (*T cruzi* antibody licensed ELISA). In this study, 327 out of 330 specimens (99.1%, with a 95% confidence interval of 97.4% to 99.8%) were negative by ABBOTT ESA Chagas, 3 (0.9%) were indeterminate, and none were positive. Of the 3 specimens with indeterminate ABBOTT ESA Chagas results, 2 were negative with a laboratory-developed *T cruzi* radioimmune precipitation assay (*T cruzi* RIPA), not licensed by the FDA, and the other specimen was not tested by *T cruzi* RIPA. There were no false positives with ABBOTT ESA Chagas.

In an ABBOTT ESA Chagas post-marketing study, an additional 300 serum and plasma specimens from United States (US) blood donors were tested with ABBOTT ESA Chagas (Table II). The specimens were presumed negative for antibodies to *T cruzi* based on ABBOTT PRISM Chagas. In this study, 297 out of 300 specimens (99.0%, with a 95% confidence interval of 97.1% to 99.8%) were negative by ABBOTT ESA Chagas, 3 (1.0%) were indeterminate, and none were positive. Of the 3 specimens with indeterminate ABBOTT ESA Chagas results, all 3 were negative with *T cruzi* RIPA. There were no false positives with ABBOTT ESA Chagas.

Table II
Specificity of ABBOTT ESA Chagas with Specimens from US Blood Donors

Screening Assay	Donors	Number Tested	ABBOTT ESA Chagas POS	IND	NEG
T cruzi ELISA Nonreactive	Plasma	165	0	0	165
	Serum	165	0	3	162
	Total	**330**	**0**	**3**	**327**
ABBOTT PRISM Chagas Nonreactive	Plasma	66	0	0	66
	Serum	234	0	3	231
	Total	**300**	**0**	**3**	**297**

POS = Positive, IND = Indeterminate, NEG = Negative

Specificity with Specimens from Individuals with Medical Conditions or Containing Potentially Interfering Substances

Specificity of ABBOTT ESA Chagas was evaluated internally at Abbott by testing 618 frozen serum and plasma specimens collected from individuals with medical conditions unrelated to *T cruzi* infection or containing potentially interfering substances (Table III). Specimens that were positive or indeterminate with ABBOTT ESA Chagas were tested further with *T cruzi* RIPA (shown in brackets).

Table III
Specificity of ABBOTT ESA Chagas with Specimens from Individuals with Medical Conditions Unrelated to *T cruzi* Infection

Category	Total	ABBOTT ESA Chagas POS	IND	NEG
Leishmaniasis[a]	58	1 [0-0-1-0]	24 [2-0-2-20]	33 [0-0-0-33]
Malaria Positive[b]	32	0 [0-0-0-0]	3 [0-0-3-0]	29 [0-0-0-29]
Syphilis Serologic Positive	16	0 [0-0-0-0]	1 [0-0-1-0]	15 [0-0-0-15]
Other Medical Conditions Unrelated to *T cruzi* Infection and Specimens Containing Potentially Interfering Substances[c]	512	0 [0-0-0-0]	17[d] [3-1-13-0][e]	495 [0-0-0-495]
Total	**618**	**1 [0-0-1-0]**	**45 [5-1-19-20]**	**572 [0-0-0-572]**

Note: *T cruzi* RIPA results are in the bracket: [POS-IND-NEG-ND]

POS = Positive, IND = Indeterminate, NEG = Negative, ND = *T cruzi* RIPA Not Done

[a] These specimens included the following categories: cutaneous (6) and visceral (52).

[b] These specimens included the following categories: *P falciparum* (16) and *P vivax* (16)

[c] These 512 specimens included the following categories: anti-HBV positive (16), anti-HCV positive (16), HBsAg positive (16), anti-HTLV-I/HTLV-II positive (16), anti-HAV positive (16), anti-HIV-1/HIV-2 positive (16), anti-CMV positive (16), anti-EBV positive (16), anti-HSV positive (16), rubella antibody positive (16), West Nile virus antibody positive (16), varicella-zoster virus antibody positive (16), anti-nuclear antibody positive (16), human anti-mouse antibody positive (16), elevated IgG (16), elevated IgM (16), rheumatoid factor positive (16), toxoplasma antibody positive (16), yeast infection (16), tuberculosis positive (16), multiple myeloma (16), monoclonal gammopathy (16), multiparous females (16), elevated triglycerides (16), elevated bilirubin (16), elevated hemoglobin (16), influenza vaccine recipients (50), Dengue virus antibody positive (16), *E coli* infection (16), and Lyme disease positive (14).

[d] The 17 ABBOTT ESA Chagas indeterminate specimens from persons with other medical conditions were from the following categories: anti-HTLV-I/HTLV-II positive (2), anti-EBV positive (1), West Nile virus antibody positive (1), varicella-zoster virus antibody positive (2), anti-nuclear antibody positive (1), elevated IgG (1), yeast infection (1), tuberculosis positive (1), elevated triglycerides (1), elevated hemoglobin (1), influenza vaccine recipients (1), Dengue virus antibody positive (2), *E coli* infection (1), and Lyme disease positive (1) .

[e] The 3 *T cruzi* RIPA positive specimens from persons with other medical conditions were from the following categories: anti-HTLV-I/HTLV-II positive (1), *E coli* infection (1), and influenza vaccine recipients (1). The one *T cruzi* RIPA indeterminate specimen was from the anti-nuclear antibody positive category

Of the 58 Leishmaniasis specimens tested with ABBOTT ESA Chagas, 33 (56.9%) were negative, 24 (41.4%) were indeterminate, and 1 (1.7%) was positive, indicating cross-reactivity of some Leishmaniasis specimens with ABBOTT ESA Chagas (Table III). The 1 ABBOTT ESA Chagas positive specimen was negative on *T cruzi* RIPA. None of the 25 specimens (24 indeterminate and 1 positive) were repeatedly reactive with ABBOTT PRISM Chagas and therefore would not have been tested on ABBOTT

ESA Chagas. These specimens were not tested with a *T cruzi* antibody licensed ELISA.

Of the 32 Malaria Positive specimens tested with ABBOTT ESA Chagas, 29 (90.6%) were negative, and 3 (9.4%) were indeterminate and negative on *T cruzi* RIPA, indicating limited cross-reactivity of some Malaria Positive specimens with ABBOTT ESA Chagas (Table III). None of the 32 specimens were positive on ABBOTT ESA Chagas.

Of the remaining 528 specimens tested with ABBOTT ESA Chagas, 510 (96.6%) were negative, 18 (3.4%) were indeterminate and none were positive, indicating acceptable specificity of ABBOTT ESA Chagas with specimens from individuals with medical conditions unrelated to *T cruzi* infection (Table III).

Supplemental Testing of US Blood Donor Specimens Repeatedly Reactive by ABBOTT PRISM Chagas and/or the *T cruzi* Antibody Licensed ELISA

A total of 329 US blood donor specimens were tested with ABBOTT ESA Chagas and *T cruzi* RIPA. Of these specimens, 202 were preselected donor specimens repeatedly reactive by *T cruzi* antibody licensed ELISA, 64 repeatedly reactive by *T cruzi* antibody licensed ELISA and/or ABBOTT PRISM Chagas were prospectively identified by testing 41 760 fresh donor specimens and 63 were PRISM Chagas repeatedly reactive identified during the ABBOTT ESA Chagas post-marketing study.

A comparison of ABBOTT ESA Chagas results and *T cruzi* RIPA results for the 329 US blood donors that were repeatedly reactive on a *T cruzi* antibody licensed ELISA and/or ABBOTT PRISM Chagas is shown in Table IV. Of the 329 repeatedly reactive specimens, 151 were positive on both ABBOTT ESA Chagas and *T cruzi* RIPA, 5 were positive on ABBOTT ESA Chagas and indeterminate on *T cruzi* RIPA and 24 were positive on ABBOTT ESA Chagas and negative on *T cruzi* RIPA.

This study shows a high level of concordance of positives (151/153 or 98.7%) on ABBOTT ESA Chagas with specimens positive by *T cruzi* RIPA, demonstrating consistency with *T cruzi* RIPA for samples repeatedly reactive on ABBOTT PRISM Chagas and/or the *T cruzi* antibody licensed ELISA.

The most probable *T cruzi* antibody status for specimens with discordant results between ABBOTT ESA Chagas and *T cruzi* RIPA in these studies was interpreted based on the available evidence for those specimens, recognizing that the laboratory-developed assay, *T cruzi* RIPA, is not an absolute standard for determination of *T cruzi* antibody status. Specimens that were repeatedly reactive on the two licensed screening assays and positive with 3 or more antigen bands with ABBOTT ESA Chagas were interpreted as true positive. Specimens that were repeatedly reactive on one screening test and positive with 3 or less antigen bands with ABBOTT ESA Chagas were interpreted as an inconclusive status. Risk factors for *T cruzi* infection such as immigration from a Chagas-endemic country and intensity of the bands on ABBOTT ESA Chagas were also considered in the interpretation of the status as true positive and as inconclusive. Specimens that were negative on T cruzi RIPA and indeterminate with ABBOTT ESA Chagas with 2 or fewer reactive bands were interpreted as true negative. A negative result on a follow-up sample for both ABBOTT ESA Chagas and *T cruzi* RIPA were used to reinterpret the status of the donor as true negative.

Table IV
Supplemental Testing of Specimens Repeatedly Reactive by a Licensed Screening Test for Antibodies to *T cruzi*

ABBOTT ESA Chagas	*T cruzi* RIPA				Total *T cruzi* Antibody Status
	Total	POS	IND	NEG	
POS	180	151 TP	4 TP[c]	19 TP[d]	174 TP
			1 INC[c]	4 INC[e]	5 INC
				1 TN[f]	1 TN
IND	14	1 INC[a]	0	3 INC[g]	4 INC
				10 TN[g]	10 TN
NEG	135	1 TN[b]	0	134 TN	135 TN
Total	**329**	**153**	**5**	**171**	**329**

POS = Positive, IND = Indeterminate, NEG = Negative, INC = Inconclusive, TP= True Positive, TN = True Negative

[a] One specimen was ABBOTT PRISM Chagas repeatedly reactive, and nonreactive with a *T cruzi* antibody licensed ELISA. There was no follow-up specimen provided.

[b] One specimen was ABBOTT ESA Chagas negative and *T cruzi* RIPA positive. A follow-up sample from the donor was negative on both ABBOTT ESA Chagas and *T cruzi* RIPA, indicating that the original specimen was false positive with *T cruzi* RIPA.

[c] All 5 of these specimens were repeatedly reactive with both ABBOTT PRISM Chagas and a *T cruzi* antibody licensed ELISA, 4 specimens were positive with 3 or more antigen bands with ABBOTT ESA Chagas and were from donors from Chagas endemic countries, and 1 specimen was positive with 2 antigen bands with ABBOTT ESA Chagas.

[d] 19 specimens were repeatedly reactive with both ABBOTT PRISM Chagas and a *T cruzi* antibody licensed ELISA and positive for 3 or more antigen bands with ABBOTT ESA Chagas.

[e] One specimen was repeatedly reactive with both licensed screening assays and positive with 2 antigen bands with ABBOTT ESA Chagas, 1 specimen was repeatedly reactive with *T cruzi* antibody licensed ELISA and positive with 2 antigen bands with ABBOTT ESA Chagas, 1 specimen was repeatedly reactive with *T cruzi* antibody licensed ELISA and positive with 3 antigen bands with ABBOTT ESA Chagas, and 1 specimen was repeatedly reactive with ABBOTT PRISM Chagas and nonreactive with *T cruzi* antibody licensed ELISA and positive with 2 antigen bands with ABBOTT ESA Chagas.

[f] One specimen was nonreactive with one of the licensed screening assays and a follow-up specimen was nonreactive with both licensed screening assays and indeterminate with ABBOTT ESA Chagas.

[g] All 13 specimens were repeatedly reactive with ABBOTT PRISM Chagas or a *T cruzi* antibody licensed ELISA. Three specimens had 3 or more low-intensity reactive antigen bands with ABBOTT ESA Chagas, 2 specimens had only one antigen band with ABBOTT ESA Chagas, 1 specimen had 2 low-intensity reactive antigen bands with ABBOTT ESA Chagas and one had an identified risk factor (travel to rural Mexico).

Based on this analysis, out of the 174 true positives there were 174 (100%, with a 95% confidence interval of 97.9% to 100.0%) that were positive on ABBOTT ESA Chagas. These data demonstrate high sensitivity of ABBOTT ESA Chagas on repeatedly reactive samples, with sensitivity greater than *T cruzi* RIPA.

In addition, ABBOTT ESA Chagas was negative for 135 out of 146 specimens with a status interpreted as true negative (92.5%, with a 95% confidence interval of 86.9% to 96.2%), 10 (6.9%) were indeterminate, and 1 (0.7%) was positive. These data demonstrate specificity comparable to *T cruzi* RIPA.

Supplemental Testing of US Blood Donor Specimens Repeatedly Reactive by ABBOTT PRISM Chagas

A total of 284 US blood donor specimens that were repeatedly reactive by ABBOTT PRISM Chagas were tested with ABBOTT ESA Chagas and with *T cruzi* RIPA. Of the 284 blood donor specimens, 58 were prospectively identified by testing 41 760 fresh donor specimens,163 were identified by testing 202 preselected donor specimens that were repeatedly reactive by a *T cruzi* antibody licensed ELISA and 63 were PRISM Chagas repeatedly reactive identified during the ABBOTT ESA Chagas post-marketing study.

A comparison of ABBOTT ESA Chagas results and *T cruzi* RIPA results for the 284 ABBOTT PRISM Chagas repeatedly reactive US blood donor specimens is shown in Table V. Of the 284 repeatedly reactive specimens, 153 were positive by *T cruzi* RIPA, of which 151 were ABBOTT ESA Chagas positive, one specimen was ABBOTT ESA Chagas negative and one specimen was ABBOTT ESA Chagas indeterminate. Follow-up testing on a new specimen from the same donor that was ABBOTT ESA Chagas negative was nonreactive by both screening assays, and negative by both ABBOTT ESA Chagas and *T cruzi* RIPA, indicating that this donor was most likely not infected with *T cruzi*, and the negative result with ABBOTT ESA Chagas for the initial specimen was correct. There was no follow-up for the ABBOTT ESA Chagas indeterminate specimen.

The combined studies showed a high level of concordance of positive results (151/153 or 98.7%) from ABBOTT ESA Chagas with *T cruzi* RIPA positive results, demonstrating consistency with *T cruzi* RIPA for samples repeatedly reactive with ABBOTT PRISM Chagas.

Table V
Supplemental Testing of US Blood Donor Specimens Repeatedly Reactive by ABBOTT PRISM Chagas

ABBOTT ESA Chagas	*T cruzi* RIPA			
	POS	IND	NEG	Total
POS	151	5	22	178
IND	1[b]	0	10	11
NEG	1[a]	0	94	95
Total	**153**	**5**	**126**	**284**

POS = Positive, IND = Indeterminate, NEG = Negative

[a] The follow-up testing results for a new specimen from the same donor were nonreactive by ABBOTT PRISM Chagas (signal to cutoff, S/CO = 0.85, 0.91, 0.82), nonreactive by *T cruzi* antibody licensed ELISA (S/CO = 0.094), negative by ABBOTT ESA Chagas (FP10 -, FP6 +/-, FP3 - and TcF -), and negative by *T cruzi* RIPA.

[b] One specimen was nonreactive with *T cruzi* antibody licensed ELISA. There was no follow-up specimen provided.

Of the 41 760 blood donors screened with ABBOTT PRISM Chagas, 58 were repeatedly reactive of which 9 were positive with ABBOTT ESA Chagas (Table VI). Of these 9 specimens, 6 were positive with both ABBOTT ESA Chagas and *T cruzi* RIPA and 3 were positive on ABBOTT ESA Chagas only. Of the 3 specimens that were only positive with ABBOTT ESA Chagas, 2 specimens were repeatedly reactive with both screening assays and positive with 3 antigen bands with ABBOTT ESA Chagas. The

follow-up specimen for the first donor was positive with 3 antigen bands with ABBOTT ESA Chagas, *T cruzi* RIPA indeterminate, ABBOTT PRISM Chagas nonreactive (0.97 S/CO), nonreactive on ELISA (0.924 S/CO), and indicated several risk factors for *T cruzi* infection. No follow-up specimen was available for the second donor. The third specimen was repeatedly reactive with ABBOTT PRISM Chagas and positive with 2 antigen bands with ABBOTT ESA Chagas. A follow-up specimen tested indeterminate with ABBOTT ESA Chagas, negative by *T cruzi* RIPA, and nonreactive by the two licensed screening assays.

This study shows concordance (6 out of 6) for ABBOTT ESA Chagas positive results with *T cruzi* RIPA positive results for prospectively acquired US blood donor specimens found repeatedly reactive by ABBOTT PRISM Chagas.

Table VI
Supplemental Testing of ABBOTT PRISM Chagas
Repeatedly Reactive Specimens

Category	Number of Specimens Tested	ABBOTT PRISM Chagas Repeatedly Reactive/Number of Specimens Tested	ABBOTT ESA Chagas Positive/ Number of Specimens Tested
US Blood Donors	41 760	58/41 760 (0.14%)	9/58 (15.52%)

Of the 63 specimens that were identified during the ABBOTT ESA Chagas post-marketing study as PRISM Chagas repeatedly reactive, 7 were positive with ABBOTT ESA Chagas. Of these 7 specimens, 6 were positive with both ABBOTT ESA Chagas and *T cruzi* RIPA and 1 was ABBOTT ESA Chagas positive with 2 antigen bands and *T cruzi* RIPA negative. The specimen that was positive with ABBOTT ESA Chagas and *T cruzi* RIPA negative was repeatedly reactive with ABBOTT PRISM Chagas (1.26, 1.29, and 1.19 S/CO) and nonreactive on ELISA (0.361 S/CO). A follow-up specimen tested positive with 2 antigen bands on ABBOTT ESA Chagas, negative by *T cruzi* RIPA, and nonreactive by the two licensed screening assays.

The post-marketing study shows concordance (6 out of 6) for ABBOTT ESA Chagas positive results with *T cruzi* RIPA positive results for prospectively acquired US blood donor specimens found repeatedly reactive by ABBOTT PRISM Chagas.

The combined studies show concordance of (12 out of 12) for ABBOTT ESA Chagas positive results with *T cruzi* RIPA positive results for prospectively acquired US blood donor specimens found repeatedly reactive by ABBOTT PRISM Chagas.

Supplemental Testing of US Blood Donor Specimens Repeatedly Reactive by a *T cruzi* Antibody Licensed ELISA

A total of 221 US blood donor specimens that were repeatedly reactive by a *T cruzi* antibody licensed ELISA were tested with ABBOTT ESA Chagas and with *T cruzi* RIPA. Of the 221 blood donor specimens, 13 were from testing 16 292 fresh donor specimens, 202 were from testing preselected donor specimens repeatedly reactive by a *T cruzi* antibody licensed ELISA and 6 were identified during the ABBOTT ESA Chagas post-marketing study.

A comparison of ABBOTT ESA Chagas results with *T cruzi* RIPA results for the 221 *T cruzi* antibody licensed ELISA repeatedly reactive US blood donors is shown in Table VII. Of the 221 specimens, 149 were positive by both *T cruzi* RIPA and ABBOTT ESA Chagas. This study shows a high level of concordance of positive results (149/149 or 100.0%) on ABBOTT ESA Chagas with specimens positive by *T cruzi* RIPA, demonstrating consistency with *T cruzi* RIPA for samples repeatedly reactive on the *T cruzi* antibody licensed ELISA.

Table VII
Supplemental Testing of US Blood Donor Specimens
Repeatedly Reactive by a *T cruzi* Antibody Licensed ELISA

ABBOTT ESA Chagas	*T cruzi* RIPA			
	POS	IND	NEG	Total
POS	149	5	22	176
IND	0	0	3	3
NEG	0	0	42	42
Total	149	5	67	221

POS = Positive, IND = Indeterminate, NEG = Negative

Of the 16 292 blood donors screened with the *T cruzi* antibody licensed ELISA, 13 were repeatedly reactive with *T cruzi* antibody licensed ELISA and 7 were positive with ABBOTT ESA Chagas (Table VIII). Of these 7 specimens, 5 were positive on both ABBOTT ESA Chagas and *T cruzi* RIPA and 2 were positive with only ABBOTT ESA Chagas. These 2 specimens were repeatedly reactive with both screening assays and positive with 3 antigen bands with ABBOTT ESA Chagas. The follow-up specimen for the first donor was positive with 3 antigen bands with ABBOTT ESA Chagas, *T cruzi* RIPA indeterminate, ABBOTT PRISM Chagas nonreactive (0.97 S/CO), nonreactive on ELISA (0.924 S/CO), and indicated several risk factors for *T cruzi* infection. No follow-up specimen was available for the second donor.

This study showed concordance (5 out of 5) for ABBOTT ESA Chagas positive results with *T cruzi* RIPA positive results for prospectively acquired US blood donor specimens found repeatedly reactive by *T cruzi* antibody licensed ELISA.

Table VIII
Supplemental Testing of *T cruzi* Antibody Licensed ELISA
Repeatedly Reactive Specimens

Category	Number of Specimens Tested	*T cruzi* Antibody Licensed ELISA Repeatedly Reactive/Number of Specimens Tested	ABBOTT ESA Chagas Positive/ Number of Specimens Tested
US Blood Donors	16 292	13/16 292 (0.08%)	7/13 (53.85%)

Of the 6 specimens that were identified during the ABBOTT ESA Chagas post-marketing study as *T cruzi* antibody licensed ELISA repeatedly reactive, 5 were positive with both ABBOTT ESA Chagas and *T cruzi* RIPA and 1 was negative with both ABBOTT ESA Chagas and *T cruzi* RIPA. A follow-up specimen tested negative with both ABBOTT ESA Chagas and *T cruzi* RIPA, and repeatedly reactive by the two licensed screening assays.

The post-marketing study shows concordance (5 out of 5) for ABBOTT ESA Chagas positive results with *T cruzi* RIPA positive results for prospectively acquired US blood donor specimens found repeatedly reactive by *T cruzi* antibody licensed ELISA.

The combined studies show concordance (10 out of 10) for ABBOTT ESA Chagas positive results with *T cruzi* RIPA positive results for prospectively acquired US blood donor specimens found repeatedly reactive by *T cruzi* antibody licensed ELISA.

Clinical Sensitivity in Parasitologically Positive Non-US Specimens

A total of 110 serum specimens from individuals known to be positive for the *T cruzi* parasite were tested with ABBOTT PRISM Chagas and ABBOTT ESA Chagas (Table IX). Of the 110 specimens, 65 were from individuals that tested positive by identification of the parasite with xenodiagnosis. The remaining 45 specimens were from individuals known to be positive for the *T cruzi* parasite by historical identification of the parasite with xenodiagnosis or hemoculture. The specimens were obtained from the Chagas-endemic countries of Argentina, Bolivia, Brazil, and Peru. All 110 specimens were repeatedly reactive on ABBOTT PRISM Chagas and were positive for *T cruzi* antibodies with ABBOTT ESA Chagas demonstrating 100% sensitivity (Table IX).

Table IX
Sensitivity of ABBOTT ESA Chagas
with Specimens Parasite Positive for *T cruzi*

Category	Number Tested	ABBOTT ESA Chagas		
		POS	IND	NEG
Preselected *T cruzi* Parasite Positive	110	110	0	0

POS = Positive, IND = Indeterminate, NEG = Negative

Clinical Sensitivity in Serologically Positive Non-US Specimens

A total of 85 serum specimens from individuals reactive for *T cruzi* antibodies based on 2 different serologic tests for antibodies to *T cruzi* (ie, ELISA, immunofluorescence assay [IFA], or indirect hemagglutination assay [IHA]), were obtained from Argentina and were tested with ABBOTT PRISM Chagas and with ABBOTT ESA Chagas. All 85 specimens were repeatedly reactive on ABBOTT PRISM Chagas and were positive for *T cruzi* antibodies with *T cruzi* RIPA and with ABBOTT ESA Chagas demonstrating 100% sensitivity (Table X).

Table X
Sensitivity of ABBOTT ESA Chagas
with Preselected *T cruzi* Serologic Positive Specimens

Category	Number Tested	ABBOTT ESA Chagas		
		POS	IND	NEG
Preselected *T cruzi* Serologic Positive, South America	85	85	0	0

POS = Positive, IND = Indeterminate, NEG = Negative

Prospective Studies in High Risk Populations

A total of 524 serum specimens from individuals residing in Chagas-endemic areas were tested with ABBOTT ESA Chagas, ABBOTT PRISM Chagas, and a *T cruzi* antibody licensed ELISA. Specimens were obtained from

Argentina, Brazil, Guatemala, Panama, and Peru. Specimens that were positive and indeterminate with ABBOTT ESA Chagas and/or repeatedly reactive with either ABBOTT PRISM Chagas or a *T cruzi* antibody licensed ELISA, if available, were tested further with *T cruzi* RIPA.

A comparison of ABBOTT ESA Chagas results and *T cruzi* RIPA results for the 524 high risk specimens is shown in Table XI. This study shows a high level of concordance of positives (130/132 or 98.5%) on ABBOTT ESA Chagas with specimens positive by *T cruzi* RIPA, demonstrating consistency with *T cruzi* RIPA for samples repeatedly reactive on ABBOTT PRISM Chagas and/or the *T cruzi* antibody licensed ELISA.

The most probable *T cruzi* antibody status for specimens in these studies was interpreted based on the available evidence for those specimens, recognizing that the laboratory-developed assay, *T cruzi* RIPA, is not an absolute standard for determination of *T cruzi* antibody status. Specimens that were repeatedly reactive on the two licensed screening assays and positive with 3 or more antigen bands with ABBOTT ESA Chagas were interpreted as true positive. Specimens that were repeatedly reactive on one screening test and positive with 3 or less antigen bands with ABBOTT ESA Chagas were interpreted as an inconclusive status. Risk factors for *T cruzi* infection such as from a Chagas-endemic country and intensity of the bands on ABBOTT ESA Chagas were also considered in the interpretation of the status as true positive and as inconclusive.

Table XI
Supplemental Testing of High Risk Chagas Endemic Specimens Repeatedly Reactive by a Licensed Screening Test for Antibodies to *T cruzi*

ABBOTT ESA Chagas	*T cruzi* RIPA					Total *T cruzi* Antibody Status
	Total	POS	IND	NEG	Not Tested	
POS	147	130 TP	3 TP[b]	4 TP[c] 4 INC[d] 6 TN[e]	0	137 TP 4 INC 6 TN
IND	21	1 INC 1 TN[a]	0	12 TN[f]	7 TN[f]	1 INC 20 TN
NEG	356	0	0	2 TN	354 TN	356 TN[g]
Total	**524**	**132**	**3**	**28**	**361**	**524**

POS = Positive, IND = Indeterminate, NEG = Negative, INC = Inconclusive, TP= True Positive, TN = True Negative

[a] One specimen was repeatedly reactive with one screening assay and indeterminate with 2 antigen bands with ABBOTT ESA Chagas and 1 specimen was nonreactive on both screening assays and indeterminate with 2 antigen bands with ABBOTT ESA Chagas.

[b] All 3 specimens were repeatedly reactive with both ABBOTT PRISM Chagas and the *T cruzi* licensed antibody ELISA and positive with 3 or more antigen bands with ABBOTT ESA Chagas.

[c] 4 specimens were repeatedly reactive with both ABBOTT PRISM Chagas and *T cruzi* antibody licensed ELISA and were positive with 3 or more antigen bands with ABBOTT ESA Chagas

[d] 4 specimens were repeatedly reactive with one of the 2 licensed screening assays and positive with 3 or more antigen bands with ABBOTT ESA Chagas

[e] 6 specimens were nonreactive with both of the licensed screening assays

[f] All were nonreactive with both screening assays, and only one specimen had 3 antigen bands with ABBOTT ESA Chagas.

[g] Of the 356 specimens negative by ABBOTT ESA Chagas, 354 specimens were nonreactive by both ABBOTT PRISM Chagas and *T cruzi* antibody licensed ELISA and not tested by *T cruzi* RIPA. Two specimens were nonreactive by ABBOTT PRISM Chagas and repeatedly reactive by *T cruzi* antibody licensed ELISA and were *T cruzi* RIPA negative.

Based on this analysis, out of 137 true positives there were 137 (100%, with a 95% confidence interval of 97.3% to 100.0%) that were positive on ABBOTT ESA Chagas. These data demonstrate high sensitivity of ABBOTT ESA Chagas on repeatedly reactive samples, with sensitivity comparable to *T cruzi* RIPA

In addition, ABBOTT ESA Chagas was negative for 356 out of 382 specimens with a status interpreted as true negative (93.2%, with a 95% confidence interval of 90.2% to 95.5%), 20 (5.2%) were indeterminate, and 6 (1.6%) were positive. These data demonstrate specificity comparable to *T cruzi* RIPA.

ABBOTT ESA Chagas results and *T cruzi* RIPA results (shown in brackets) for the 524 High Risk specimens screened using ABBOTT PRISM Chagas are shown in Table XII. One (1) specimen that was nonreactive using ABBOTT PRISM Chagas was positive by both ABBOTT ESA Chagas and *T cruzi* RIPA.

Table XII
High Risk Chagas Endemic Specimens Tested with ABBOTT PRISM Chagas and ABBOTT ESA Chagas

ABBOTT PRISM Chagas	ABBOTT ESA Chagas			Total
	POS	IND	NEG	
Repeatedly Reactive	137 [129-3-5]	0	0	137
Nonreactive	10 [1-0-9]	21[a] [2-0-12]	356[b] [0-0-2]	387
Total	**147**	**21**	**356**	**524**

Note: *T cruzi* RIPA results are in the bracket: [POS-IND-NEG]

POS = Positive, IND = Indeterminate, NEG = Negative

[a] Out of 21 specimens that tested indeterminate with ABBOTT ESA Chagas, 7 specimens were not available for testing by *T cruzi* RIPA.

[b] *T cruzi* RIPA testing was not performed for 354 out of 356 nonreactive specimens.

ABBOTT ESA Chagas results and *T cruzi* RIPA results (shown in brackets) for the 524 High Risk specimens screened using the *T cruzi* antibody licensed ELISA is shown in Table XIII. Two (2) specimens that were nonreactive using the *T cruzi* antibody licensed ELISA were positive by both ABBOTT ESA Chagas and *T cruzi* RIPA.

Table XIII
High Risk Chagas Endemic Specimens Tested with a *T cruzi* Antibody Licensed ELISA and ABBOTT ESA Chagas

T cruzi Antibody Licensed ELISA	ABBOTT ESA Chagas			Total
	POS	IND	NEG	
Repeatedly Reactive	139 [128-3-8]	1 [1-0-0]	2 [0-0-2]	142
Nonreactive	8 [2-0-6]	20[a] [1-0-12]	354[b]	382
Total	**147**	**21**	**356**	**524**

Note: *T cruzi* RIPA results are in the bracket: [POS-IND-NEG]

POS = Positive, IND = Indeterminate, NEG = Negative

[a] Out of 20 specimens that tested indeterminate with ABBOTT ESA Chagas, 7 specimens were not available for testing by *T cruzi* RIPA

[b] *T cruzi* RIPA testing was not performed for 354 nonreactive specimens.

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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%).

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