



# WL Check

## Chagas

For the detection of antibodies against *Trypanosoma cruzi* in serum, plasma and whole blood

### SUMMARY

Chagas disease (american trypanosomiasis) is a parasitic infection caused by *Trypanosoma cruzi*. Laboratory diagnosis depends on the stage of the disease. During the acute phase, diagnosis is done by identification of parasites in the blood or through immunological methods that detect IgM antibodies. During the chronic phase, immunological methods may be used, such as hemagglutination, immunofluorescence and enzyme immunoassay or western blot. Since none of these tests individually has optimum sensitivity and specificity, it is recommended the parallel use of at least two different serological tests to confirm infection by *T. cruzi*.

In recent years, immunochromatographic rapid tests based on recombinant antigens have been developed. For their simplicity and rapidity, these tests are a useful complementary tool for both epidemiological field trials and for clinical diagnosis, thereby facilitating early detection of infection.

**WL Check Chagas** is a rapid test to detect antibodies against *Trypanosoma cruzi* based on a special combination of recombinant antigens capable of detecting the presence of antibodies in serum, plasma and whole blood.

### PRINCIPLE

**WL Check Chagas** test is an "in vitro" immunochromatographic test for qualitative detection of antibodies against *Trypanosoma cruzi* in serum, plasma and whole blood. The test consists of a plastic cassette containing:

- A nitrocellulose membrane sensitized in the «T» test zone with specific recombinant antigens from epimastigote and trypomastigote stages of *T. cruzi*.

- A patch impregnated with *T. cruzi* specific recombinant antigens for conjugated to colloidal gold.

The sample and buffer are added into the «S» sample well, solubilizing and mixing with the conjugate of recombinant antigens. Then, this mixture migrates by capillary action through the nitrocellulose membrane. If the sample is reactive, the antibodies to *T. cruzi* that are present, form a complex with the antigens conjugated to colloidal gold. This complex will then bind to the immobilized antigens in the «T» test zone of the nitrocellulose membrane, forming pink-purple red a line. The absence of this line indicates a negative result. As a control procedure, the test includes a yellow colored «C» control zone that changes to pink-purple red after the addition of the sample. The absence of this line invalidates the results.

### PROVIDED MATERIAL

**A. Reagent A:** plastic cassette composed of a nitrocellulose membrane sensitized with *Trypanosoma cruzi* specific recombinant antigens and recombinant antigens conjugates.

**B. Reagent B:** 50 mM sodium phosphate buffer, 0.90 g/L sodium azide, surfactant, pH = 8.0.

### INSTRUCTIONS FOR USE

The provided material is ready to use.

### NON-PROVIDED REQUIRED MATERIAL

- Micropipette for measuring stated volumes.
- 40 uL disposable device for capillary whole blood sample collection (only provided in some package sizes).
- Disposable tips.
- Stopwatch or timer.
- Material for sample collection.
- Lancet for capillary whole blood collection
- Disposable gloves, lab coats, eye protection.
- Container for disposal of biological waste.
- Sodium hypochlorite.

### WARNINGS

- Carefully read the instruction manual before testing, and follow the instructions.
- Avoid using the test if the package is damaged.
- Avoid using reagents beyond the expiration date stated on the packaging.
- The reagents are for "in vitro" diagnostic use.
- This test provides a qualitative and visual outcome. A good light source is required to observe the results.
- Avoid mixing reagents from different lots.
- Avoid using reagents from other sources.
- Avoid touching the nitrocellulose membrane with the fingers.
- Follow safe practices when using biological samples and reagents:
  - . Handle all patient specimens as potentially infectious.
  - . S24/25: Avoid contact with skin and eyes.
  - . S37/39: Wear suitable gloves and eye/face protection.
  - . Do not pipette by mouth.
  - . Clean and disinfect spills of specimens or reagents using sodium hypochlorite (5% final concentration) or other suitable disinfectant. To inactivate the material used autoclave for 1 hour at 121°C.
- Avoid bubble formation in «S» sample well when adding the sample and the Reagent B.
- When performing the test, place the cassette on a clean, flat surface without vibrations.
- Avoid shaking the cassette during the test.
- The cassette is disposable, not reusable. Discard into containers for biohazard risk material.
- Reagent B has low concentrations of sodium azide as preservative.

- The reagents and samples must be discarded according to current regulations.

### STABILITY AND STORAGE INSTRUCTIONS

The kit is stable between 2 and 30°C until the expiration date stated on the box. Do not freeze. If stored refrigerated, ensure that the pouch reaches room temperature before use, otherwise content moistening will be favored.

The cassette should remain in its original sealed pouch. Do not open the package until use.

### SAMPLE

Serum, plasma and whole blood obtained by venipuncture or capillary puncture.

**a) Collection:** collect specimens aseptically and avoid hemolysis.

- Serum: obtain serum as usual. Remove serum from clot as soon as possible within two hours from collection. Serum collected in tubes with accelerator and separator gel may be used.

- Plasma: use plasma collected with EDTA, citrate or heparin.

- Whole blood (venipuncture): use blood collected with EDTA, citrate or heparin.

- Whole blood (capillary puncture): use blood collected with 40 uL disposable device (only provided in some package sizes). Perform the test immediately. If another device is used, the user is responsible for validating the device for capillary whole blood sample collection.

### Capillary whole blood collection

**1-** Collect the sample from the tip of the middle finger or ring finger. If necessary warm the hand of the patient to increase blood flow. To that effect use a warm, moist towel or hot water (< 42°C).

**2-** Clean the finger with aqueous 70% v/v isopropanol solution and let dry. Isopropanol residues may cause sample hemolysis.

**3-** Place the hand palm up and hold the patient's finger firmly.

**4-** Use a new lancet for each patient. Place the lancet perpendicular to the skin surface. Firmly puncture the fingertip on the distal phalanx.

**5-** Discard the lancet in a suitable container for sharp objects and biohazardous material.

**6-** Keep the finger below the elbow level and gently press the base of the finger at regular intervals.

**7-** Absorb the first drop of blood with sterile gauze and place in a suitable container. This ensures the removal of tissue fluid from the sample, thus avoiding erroneous results.

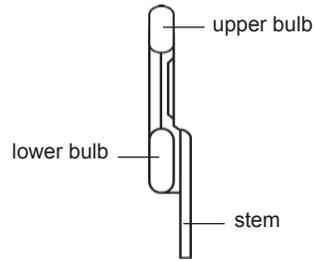
**8-** Collect the blood sample with the disposable device provided. To this effect:

**8a-** Hold the device upright with a slight angle to the skin surface. Press the upper bulb.

**8b-** Collect the blood sample to fill the stem, releasing the upper bulb gently avoiding bubble formation.

**8c-** Shake the device gently and carefully to avoid splashing. Excess sample should be retained in the bottom of the lower bulb. It is very important to take this precaution to avoid dispensing excess blood.

**8d-** Press the upper bulb and unload the 40 uL sample contained in the stem on the «S» sample well.



### b) Stability and storage:

- Serum and Plasma: store between 2 and 10°C. If the test is not performed within 3 days, store the sample at -20°C.

Avoid performing multiple freezing and thawing cycles. This can lead to erroneous results. If using frozen samples, they must be homogenized and centrifuged before use.

- Blood (venipuncture): can be stored up to 3 days between 2 and 10°C. Do not freeze.

- Blood (capillary): use immediately. Do not freeze.

**c) Interfering Substances:** no interference was observed by:

- . Hemolysis: up to 1.1 g/dL hemoglobin.

- . Lipemia: up to 1500 mg/dL triglycerides.

- . Bilirubin: up to 30 mg/dL.

- . Ascorbic acid: up to 50 mg/dL.

**d) Transportation:** if the samples must be transported, pack according to legal specifications regarding the shipment of infectious material.

### PROCEDURE

**1.** The reagents and samples must be at room temperature (18-30°C) before use.

**2.** Remove the cassette from its sealed pouch immediately before use.

**3.** Place the cassette on a clean, flat surface without vibrations.

**4.** Place the sample on the «S» sample well.

- **For Serum - Plasma - Whole Blood:**

- . Place 40 uL with automatic micropipette\*.

- . Wait for 10-15 seconds until the sample is absorbed.

- . Add 3 drops (100 uL) of Reagent B in the «S» sample well.

- . Start the timer.

NOTE: \* If capillary whole blood is used employ the 40 uL disposable device (only provided in some Package Sizes).

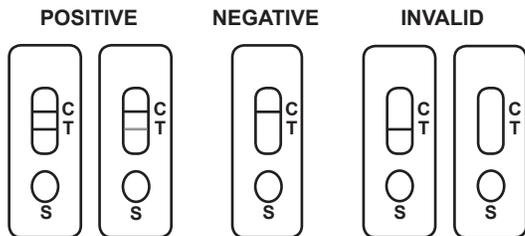
**5.** In either case, read the results from 25 to 35 minutes. Do not read after 35 minutes since erroneous results may be obtained. Some positive samples react immediately while others react more slowly within the specified reading time. Due to particular characteristics of some samples, the background color of the membrane may be slightly pink without affecting the interpretation of results.

### ASSAY VALIDATION CRITERIA

- The cassette has a yellow line in the «C» control area that identifies the **WL Check Chagas** determination.

- When the test is performed, the yellow line should change to pink-purple red color. This color change confirms that the appropriate volume of sample was added, migration was appropriate and that the completion of the procedure was successful.

## RESULT INTERPRETATION



**Positive Result (2 lines):** There are two pink-purple red colored lines, one in the «T» test zone and one in the «C» control area. The color intensity of the «T» line will depend on the sample under study. Any visible pink color, even very faint must be interpreted as positive. The result is positive although the intensity of the color of the «T» and «C» lines is different.

**Negative Result (1 line):** There is only one pink red-purple colored line in the «C» control area. No colored line appears in the «T» test zone.

**Invalid Result:** The absence of the pink-purple red colored line in the «C» control zone invalidates the result; whether or not the pink-purple red colored line is present in the «T» test zone. An invalid result usually indicates an error in performing the procedure or a problem with the sample. With certain samples difficulties like incomplete migration, very viscous sample or presence of fibrin may appear. In any case, review the procedure, centrifuge the sample again and repeat the test using a new cassette.

## PROCEDURE LIMITATIONS

- **WL Check Chagas** is a test to the diagnosis of infection by *T. cruzi*. Any result obtained by this test should be confirmed by other methods and checked against the patient's clinical data before making a definitive diagnosis.
- A reactive result by **WL Check Chagas** indicates the presence of anti-*T. cruzi* antibodies. This result must be verified by another technique. Take into account the criteria recommended by the Fatale Chabén Institute, whereby the immunodiagnosis of the infection must be confirmed by at least two of the following methods: indirect immunofluorescence, indirect hemagglutination and ELISA, duly validated by the National Reference Center.
- A negative result does not exclude the possibility of infection with *T. cruzi*. It can be obtained a false negative result in samples with low levels of anti-*T. cruzi* or in early stages of infection. For these reasons a negative result interpretation must be followed closely, especially in patients with clinical symptoms and risk factors.
- False positive results can occur in the following situations: autoimmune diseases, tuberculosis, lupus erythematosus,

pregnancy, vaccination against hepatitis B and other immunizations, hemodialysis, liver disease, other parasitic disease such as Leishmaniasis and other infectious diseases other than Chagas (HIV, HTLV, hepatitis C, hepatitis B, syphilis, etc).

- Erroneous results may be obtained with serum or plasma with turbidity due to bacterial contamination or that have been subjected to several freezing and thawing cycles.
- The use of heat-inactivated samples may yield erroneous results.
- Avoid using pools of samples or diluted samples.
- See Known Interfering Substances under SAMPLE.

## PERFORMANCE

### a) Sensitivity

#### Clinical Sensitivity of Performance Panels

The following results were obtained in a study performed on different international commercial panels:

- PMT 202 (Anti-*T. cruzi* Performance Panel, BBI, USA): 13 out of 14 reactive samples were detected.
- PP 0407 (Chagas Performance Panel, Q Panel, Brazil): 16 out of 16 reactive samples were detected.
- PP 0508 (Anti-*T. cruzi* Performance Panel, Q Panel, Brazil): 16 out of 16 reactive samples were detected.
- PP 0409 (Chagas Performance Panel, Q Panel, Brazil): 16 out of 16 reactive samples were detected.

#### Analytical Sensitivity with International Controls

- VIROTROL® Chagas - Bio-Rad: Reactive result was obtained.
- ACCURUN Positive Control® 190 anti-*Trypanosoma cruzi* (Chagas) - Sera Care Life Sciences: Reactive result was obtained.

#### Clinical Sensitivity in Panels of anti-*T. cruzi* reactive samples

In a study performed on a panel of 326 samples reactive by ELISA and indirect hemagglutination (IHA), the sensitivity obtained was 93.87% with  $CI_{95\%} = 91.11\% - 96.62\%$ .

In another study of 83 reactive samples of children from endemic regions for *T. cruzi* infection, 78 samples were found reactive. A study carried out in a Reference Center for Diagnosis of Chagas disease tested a panel of 72 reactive samples yielding a sensitivity of 98.61% with  $CI_{95\%} = 92.51\% - 99.96\%$ . Another study, tested in parallel, 106 reactive plasma and whole blood samples collected from the same patient. In the case of the plasmas, 106 samples were detected, whereas in the case of the whole blood, 97 samples were reactive.

### b) Specificity

In a study of 1419 samples of serum, plasma and whole blood from 3 different health centers, a specificity of 97.89% was obtained with  $CI_{95\%} = 97.10\% - 98.67\%$ . For 200 of these samples, serum and plasma samples from the same patient were tested in parallel and for 17 of these 200 samples, whole blood obtained by capillary puncture was also tested. With all types of samples negative results were obtained.

In another study of 313 blood bank samples, a specificity of 98.08% was obtained with  $CI_{95\%} = 96.40\% - 99.76\%$ .

The possible occurrence of cross-reactivity was also studied testing 257 samples from individuals with different clinical

conditions that may be causing nonspecific reactions with the **WL Check Chagas** test. These conditions include pregnant women, patients on hemodialysis, patients with autoimmune diseases or infectious diseases other than Chagas (HIV, HTLV, Hepatitis C, Hepatitis B, Syphilis, etc.). For this population, the specificity was 98.44% with  $CI_{95\%} = 96.73\%-100.0\%$ . For this population, the specificity was 98.44% with  $CI_{95\%} = 96.73\%-100.0\%$ . This sampling included 12 plasmas from patients with cutaneous leishmaniasis and 12 with mucocutaneous leishmaniasis. **WL Check Chagas** has not shown cross-reactions by Leishmaniasis in any of the samples tested.

### c) Accuracy

The test accuracy was evaluated following the protocol EP5-A recommended by the CLSI (formerly NCCLS). The tests were performed with four positive samples with different reactivity levels and a negative sample. Two daily trials were performed to evaluate each sample in duplicate during 20 days. The results were visually read after 25 minutes by 3 independent operators and instrumentally, using an immunochromatographic reader.

The positive and negative samples were correctly identified in 100% of the cases.

Instrumental interpretation	T Line					C Line				
	Mean (OD)	Intra-assay		Total		Mean (OD)	Intra-assay		Total	
		S	CV	S	CV		S	CV	S	CV
Positive sample 1	0,590	0,068	11,61%	0,067	11,38%	0,833	0,076	9,18%	0,079	9,45%
Positive sample 2	0,437	0,047	10,86%	0,050	11,36%	0,851	0,062	7,30%	0,059	6,96%
Positive sample 3	0,401	0,056	13,87%	0,054	13,57%	0,873	0,066	7,51%	0,066	7,54%
Positive sample 4	0,145	0,020	13,74%	0,021	14,45%	0,846	0,061	7,25%	0,071	8,34%
Negative sample	(-)	NA	NA	NA	NA	0,812	0,061	7,47%	0,078	9,64%

N = 80; (-) = non-reactive by visual interpretation; NA = Not Applicable

### WIENER LAB PROVIDES

- 25 tests (Cat. N° 1690011)

### REFERENCES

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- WHO. Control of Chagas disease. Ginebra: WHO Press, 2002.
- OMS. Reporte sobre la enfermedad de Chagas. Grupo de trabajo científico 17-20 de abril de 2005. Guhl F, Lazdins-Helds J, editores. Buenos Aires - Argentina. [http://www.who.int/tdr/publications/publications/pdf/swg\\_chagas.pdf](http://www.who.int/tdr/publications/publications/pdf/swg_chagas.pdf).
- Centers for Disease Control and Prevention - Chagas

Disease. <http://www.cdc.gov/chagas>.

- Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline. EP5-A, Vol. 19, N° 2, NCCLS.
- Interference testing in Clinical Chemistry. Approved Guideline. EP7-A, Vol. 22, N° 27, CLSI (ex NCCLS).
- User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline. EP12-A, Vol. 22, N° 14, NCCLS.
- Evaluation of Stability of "in vitro" Diagnostic Reagents. Approved Guideline. EP-25A, Vol. 29, N° 20, CLSI (ex NCCLS).
- Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard, 5ª Edición. H4-A5, Vol. 24, N° 21, CLSI (ex NCCLS).

# Symbols

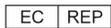
The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices



Manufactured by:



Authorized representative in the European Community



Harmful



"In vitro" diagnostic medical device



Corrosive / Caustic



Contains sufficient for <n> tests



Irritant



Use by



Consult instructions for use



Temperature limitation (store at)



Calibrator



Do not freeze



Control



Biological risks



Positive Control



Volume after reconstitution



Negative Control



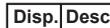
Contents



Catalog number



Batch code



Disposable devices

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