Willi Fox

Troponin I Test

Bloodtest

Instructions for use

(IFU)

Rapid test for the detection of cardiac Troponin I

in whole blood, serum or plasma

1. Intended use

The *Willi Fox* – Troponin I test is a rapid visual immunoassay for the qualitative presumptive detection of cardiac Troponin I in human whole blood, serum or plasma specimens. The test is an aid in the diagnosis of myocardial infarction (MI) and is intended for professional use as for medical doctors and labarotories. The test gives an optically readable result within 10 minutes.

Troponin I (cTnI) Cardiac Troponin I (cTnI) is a protein found in cardiac muscle with a molecular weight of 22.5 kDa. Troponin I is part of a three subunit complex comprising of Troponin T and Troponin C. Along with tropomyosin, this structural complex forms the main component that regulates the calcium sensitive ATPase activity of actomyosin in striated skeletal and cardiac muscle. After cardiac injury occurs, Troponin I is released into the blood 4-6 hours after the onset of pain. The release pattern of cTnI is similar to CK-MB, but while CK-MB levels return to normal after 72 hours, Troponin I remains elevated for 6-10 days, thus providing for a longer window of detection for cardiac injury. The high specificity of cTnI measurements for the identification of myocardial damage has been demonstrated in conditions such as the perioperative period, after marathon runs, and blunt chest trauma. cTnI release has also been documented in cardiac conditions other than acute myocardial infarction (AMI) such as unstable angina, congestive heart failure, and ischemic damage due to coronary artery bypass surgery. Because of its high specificity and sensitivity in the myocardial tissue, Troponin I has recently become the most preferred biomarker for myocardial infarction.

The *Willi Fox* – Troponin I Test is a simple test that utilizes a combination of particle conjugated anti-cTnI antibodies and capture reagent to selectively detect cTnI in whole blood, serum or plasma. The minimum detection level is 0.5 ng/mL.

2. Test principle

The *Willi Fox* – Troponin I Test is a qualitative, membrane based immunoassay for the detection of cTnI in whole blood, serum or plasma. The membrane is pre-coated with capture reagent on the test line region of the test. During testing the Troponin I in the whole blood, serum or plasma specimen reacts with two specific anti-cTnI antibodies. One of the antibodies mediates binding to the capture reagent, the other antibody is colour labelled.

The mixture migrates upward on the membrane by capillary action. In the test line region the cTnI-antibody complex is captured by the immobilised capture reagent so that a red line is generated. The presence of a red line in the test line region indicates a positive result. If the sample does not contain cTnI no line will form in the test result line region indicating a negative result.

In addition a red line must form in the control line region (C) independent of the cTnI concentration in the sample. The control line serves as a procedural control and indicates that sufficient volume of specimen has been added and membrane wicking has occurred.

3. Material provided

• Individually packed test devices	*Kit with single-pouched test cassettes in foil pouches together with desiccant – The desiccant is not a test component, please give it to the waste!*
• Disposable pipettes	inside pouch
• 1 Buffer	
• Package insert	Information for user

4. Materials required but not provided

- Tubes for taking blood samples
- Lancets (only for whole blood from fingertip)
- Centrifuge (for plasma / serum)
- Heparinised capillaries and dispensary bulb (only for whole blood from fingertip)
- Timer

5. Storage and stability

- The *Willi Fox* – Troponin I rapid test should be stored at 2-30°C and is stable until the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use.
- Do not freeze!
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

6. Precaution

- The cTnI Rapid Test Device (Whole Blood/Serum/Plasma) is intended for use with human whole blood, serum, or plasma specimens only.
- Dispose the used test device according to the local regulations.
- Do not use after the expiration date.
- The test device should remain in the sealed pouch until ready to use.
- Do not use test of pouch has been damaged.
- Humidity and high temperature can adversely affect results.
- All specimens might be potentially infectious. Proper handling and disposal methods should be established. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.

7. Specimen collection and preparation

General comments:

- The *Willi Fox* –Troponin I rapid test should be only used with human blood, whole blood serum or plasma..
- Only clear, non-hemolyzed specimens are recommended for use with this test. Serum or plasma should be separated as soon as possible to avoid hemolysis.
- Perform testing immediately after specimen collection.
- Do not leave specimens at room temperature for prolonged periods.
- Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.

- Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens.
- Whole blood collected by fingertip should be tested immediately.
- Containers containing anticoagulants such as EDTA, citrate, or heparin should be used for whole blood storage.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.
- Icteric, lipemic, hemolysed, heat treated and contaminated specimens may cause erroneous results.
- There is a slight possibility that some whole blood specimens with very high viscosity or which have been stored for more than 2 days may not run properly on the test device. Repeat the test with a serum or plasma specimen from the same patient using a new test device.

Specimen collection:

Whole blood from fingertip:

- Wash the hand of the patient with soap and warm water or clean the puncture site thoroughly with alcohol.
- Massage the hand in direction of the fingertip of the middle finger or ring finger without touching the puncture.
- Prick the fingertip with a sterile lancet. Wipe the first drop of blood.
- Rub the hand from the wrist to the palm and to the finger to form a round drop at puncture.

Collection of whole blood from fingertip using a capillary:

- Take a blood sample filling the capillary with 120 µl of the sample. Avoid air pockets.
- Put the dispensary bulb at the top of the capillary and press it to dribble blood sample in the specimen well (S) of the test device.

Dispensary of whole blood from fingertip by hanging drops:

- Position the finger of the patient exactly above the specimen well (S) of the test device.
- Drop 2-(3) hanging drops of whole blood from the puncture of the finger into the specimen well (S). The finger of the patient can be moved over the specimen well so that the drop has contact with the well. A direct contact of the finger and the specimen well should be avoided.

8. Test procedure and results

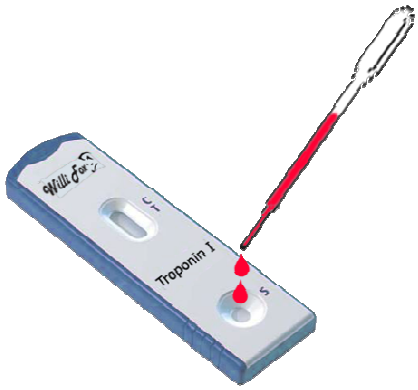
Bring tests, specimens, buffer, and/or controls to room temperature (15-30°C) before use.

1. Remove the pouch and label the test device

- Remove the test device from the sealed pouch and use it as soon as possible.
- Best results will be obtained if the assay is performed within one hour.
- Place the *Willi Fox* –Troponin I test device on a clean and level surface.

2.a Serum or plasma

- Hold the provided pipette vertically and transfer **2 drops of serum or plasma** (ca. 50 µl) into the round specimen well (S) of the *Willi Fox* –Troponin I test.



- Start the timer.

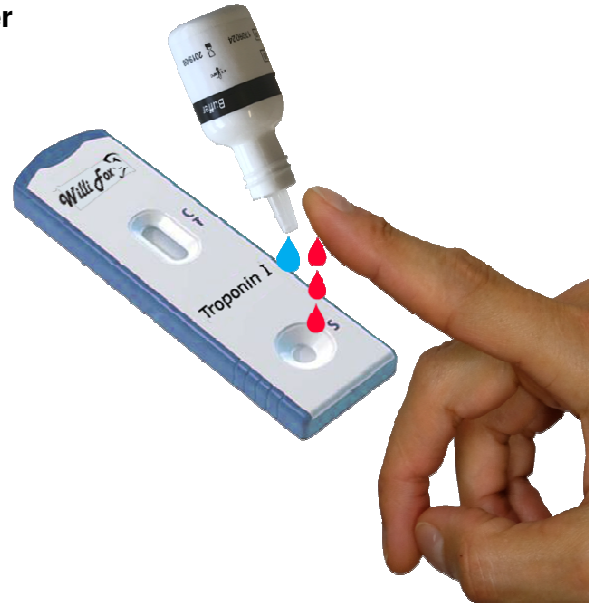
2.b Venipuncture blood

- Transfer **3 drops of whole blood specimen** (approximately 75 µL) to the specimen well of the device with the provided disposable pipette.
- Add **1 drop of buffer**

- Start the timer.

2.c Fingertip whole Blood

- Allow **3 hanging drops of fingertip whole blood** specimen to fall into the center of the specimen well (S) on the device.
- Add **1 drop of buffer**



- Start the timer.

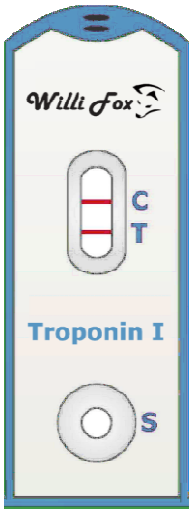
Attention: Avoid trapping air bubbles in the specimen well (S), and do not add any solution to the result area.

3. Wait for the red line (S) to appear.

- As the test begins to work, color will migrate across the membrane.
- The result should be read **at 10 minutes**.
- Do not read results after more than 20 minutes.

Do not read results after more than 20 minutes!

Interpretation of results



Positive:

Two red lines appear on the membrane.

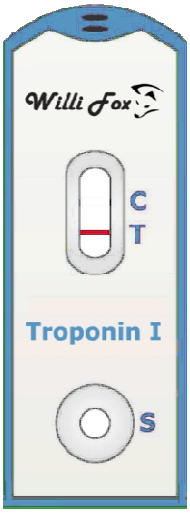
One line appears in the control region (C) and another line appears in the test region (T).

Attention: The intensity of the red colour in the test line region (T) will vary depending on the concentration of cTnI present in the specimen. Therefore, also faint reddish test result lines (T) should be considered positive.

Negative:

One red lines appears on the membrane in the control line region (C)..

No apparent red line appears in the test line region (T). Negative results should be confirmed after 20 minutes.



Invalid:

No red lines appears on the membrane in the control line region (C).

The test is not valid. Review the procedure and repeat with a new test.

- Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.
- If the problem persists, discontinue using the test kit immediately and contact your local distributor.

9. Quality control

Internal procedural control:

As internal procedural control the *Willi Fox* –Troponin I test includes the control line. It is only formed if sufficient specimen volume has been added and the chromatography has been finished successfully.

External procedural control:

Control standards are not supplied with this kit; yet, we recommend that positive and negative controls should be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

10. Limitations of the test

- The *Willi Fox* – Troponin I test is for professional in vitro diagnostic use, and should only be used for the qualitative detection of cardiac Troponin I.
- No meaning should be inferred from the colour intensity or width of any apparent lines.
- The *Willi Fox* – Troponin I test will only indicate the presence of cTnI in the specimen and should not be used as the sole criteria for the diagnosis of myocardial infarction.
- If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. The minimum detection limit of the assay is 0,5 ng/ml of cTnI in specimens. Thus, a negative result does not at anytime rule out the existence of Troponin I in blood, because the protein concentration may be below the minimum detection level of the test. Please keep in mind that the rise of Troponin I takes place several hours after the onset of pain. If the testing takes place too early, cTnI concentrations might still be too low to be detected by the assay. A negative test result does not exclude the possibility of myocardial infarction at any time.
- Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
- Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor (RF) may affect expected results. Even if the test results are positive, further clinical evaluation should be considered with other clinical information available to the physician.
- In rare cases it is possible that the antigen-antibody reaction of the test is inhibited by the presence of auto-antibodies in the patient's blood, which block the binding sites. False negative test results might be the consequence. Please note that this is a general problem with all detection methods based on an antigen-antibody reaction for the detection of proteins.

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12. Test performance

A. Analytical sensitivity and specifity

The *Willi Fox* – Troponin I test has been evaluated with a leading commercial Troponin I EIA test using clinical specimens.

Willi Fox – Troponin I rapid test compared with commercial Troponin I EIA:

Method		**EIA**		**Total results**
Willi Fox – Troponin I rapid test	**Results**	Positive	Negative	
	Positive	251	2	253
	Negative	4	648	652
Total results		255	650	905

Relative Sensitivity: 99,2% (97,2% - 99,9%)*

Relative Specificity: 99,4% (98,4% - 99.8%)*

Accuracy: 99,3% (98,6% - 99.8%)*

***95% Confidence Interval**

B. Precision

Intra-Assay

Specimens at the concentration of 0 ng/mL, 0.5 ng/mL, 2 ng/mL, 5ng/mL and 10 ng/mL were tested in 10 replicates with each of three lots to examine whether they are consistency in characteristics. Read the testing result at 10 minutes and 20 minutes according to color card.
The specimens were correctly identified >99% of the time.

Inter-Assay

Specimens at the concentration of 0 ng/mL, 0.5 ng/mL, 2 ng/mL, 5 ng/mL and 10ng/mL were tested in triplicate with 3 lots of the *Willi Fox* – Troponin I rapid test to compare each other in their performance. Read the testing result at 10 minutes and 20 minutes according to color card.
The specimens were correctly identified >99% of the time.

Cross-Reactivity

Sera containing known amounts of antibodies to Troponin I have been tested with 20`000 ng/mL Skeletal Troponin I and 2`000 ng/mL Troponin T. No cross-reactivity was observed, indicating that the *Willi Fox* – Troponin I rapid test has a high degree of specificity for Troponin I.

Interfering Substances

The below analytes were spiked into negative whole blood, plasma pools (EIA confirmed), 0.5 ng/mL, 2 ng/mL and 5 ng/mL specimens (EIA confirmed) at the concentrations listed. The specimens were tested in triplicate test devices. Visual interpretations were made at 10~20 minutes after specimen application.

Analytes/ Substances	**Concentration**
Human albumin	110 mg/mL
Acetaminophen	50 µg/mL
Acetylsslicylic acid	50 µg/mL
Ascorbic Acid	50 µg/mL
Atenolol	50 µg/mL
Atorvastatin Calcium	50 µg/mL
Anisodamine	50 µg/mL
Bilirubin	6 mg/mL
Chloramphanicol	50 µg/mL
Chlordiazepoxide	50 µg/mL
Cholesterol	5 mg/mL
Caffeine	50 µg/mL
Captopril	50 µg/mL
Cilazapril	50 µg/mL
Diclofenac	50 µg/mL
Digoxin	50 µg/mL
Erythromycin	50 µg/mL
Isosorbide Mononitrate	50 µg/mL
Furosemide	50 µg/mL
Hydrochlorothiazide	50 µg/mL
DL-Tyrosine	50 µg/mL
Labetalol	50 µg/mL
Oxazepam	50 µg/mL
Phenobarbital	50 µg/mL
Quinine	50 µg/mL
Triglycerides.	15 mg/mL
Trimethoprim	50 µg/mL
Verapamil	50 µg/mL
Felodipine	50 µg/mL
Nifedipine	50 µg/mL
Bisoprolol Fumarate	50 µg/mL
Ramipril	50 µg/mL
Metoprolol Tartrate	50 µg/mL
Moracizine Hydrochloride	50 µg/mL
Pentoxifyline	50 µg/mL
Flunarizine Hydrochloride	50 µg/mL
Hemoglobin	10 mg/mL

None of the substances at the concentration tested interfered in the assay.

13. Literature

1. Lee TH, Goldman L. *Serum enzyme assays in the diagnosis of acute myocardial infarction.* Ann Intern Med, 105:221-233, 1986.
2. Alpert JS, et al. Myocardial Infarction Redefined, Joint European Society of Cardiology American College of Cardiology: J. Am. Coll. Cardio., 36(3):959, 2000.
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5. Mehegan JP, Tobacman LS. *Cooperative interaction between troponin molecules bound to the cardiac thin filament.* J.Biol.Chem. 266:966, 1991.
6. Hossein-Nia M, et al. Cardiac troponin I release in heart transplantation. Ann. Thorac. Surg. 61: 227, 1996.
7. Wong SS. *Strategic utilization of cardiac markers for diagnosis of acute myocardial infarction.* Ann Clin Lab Sci, 26:301-12, 1996.

14. Symbols

REF	Article number		For single use only
LOT	Lot number		Expiry date
	Storage		Content
IVD	Only for in vitro diagnostics use		Instructions for use

CE

All *Willi Fox* –Troponin I rapid tests are manufactured and distributed by:

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