

INTENDED USE

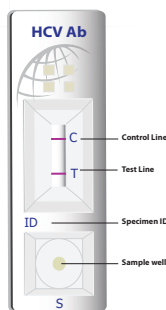
The **TRUSTline** HCV Ab Rapid Test is a double antigen lateral flow chromatographic immunoassay for the qualitative detection of anti-hepatitis C virus antibodies (IgG, IgM, IgA) in human serum, plasma or whole blood. It is intended to be used by healthcare professionals as a screening test and as an aid in the diagnosis of infection with HCV. The test kit is not automated and does not require any additional instrument. Any reactive specimen with the **TRUSTline** HCV Ab Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C virus (HCV), which was formerly described as the parentally transmitted form of non-A, non-B hepatitis (NANBH)¹, causes chronic disease in 50% of cases². HCV can also be transmitted through intravenous drug abuse and sexual contact³. Hepatitis C virus is a single-stranded RNA virus with structural similarities to the flavivirus family. Nucleic acid sequences of HCV cDNA clones provide the basis for the construction of recombinant peptides representing putative hepatitis C virus proteins^{4,5}. Anti-hepatitis C virus antibody screening of blood using synthetic or recombinant proteins helped to identify apparently healthy blood donors with anti-HCV antibodies who otherwise might have transmitted the virus⁶. Therefore, the **TRUSTline** HCV Ab Rapid Test is a useful tool for blood bank screening safety.

The **TRUSTline** HCV Ab Rapid Test was developed to detect anti-HCV antibodies (IgG, IgM, IgA) in human serum, plasma or whole blood. The test can be performed by minimally trained personnel and without cumbersome laboratory equipment.

TEST PRINCIPLE



The **TRUSTline** HCV Ab Rapid Test is a double antigen lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant HCV fusion antigen (core, NS3, NS4 and NS5) conjugated with colloidal gold (HCV Ag conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with recombinant HCV fusion antigen (core, NS3, NS4 and NS5), and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. Antibodies to HCV, if present in the specimen, will bind to the HCV Ag conjugates. The immunocomplex is then captured on the membrane by the pre-coated, non-conjugated HCV fusion antigen forming a burgundy colored T line, indicating a HCV Ab positive or reactive test result. Absence of the T line suggests a negative result.

The test contains an internal control (C line), which should exhibit a burgundy colored line of the immunocomplex of control antibodies regardless of the color development on the T line. If the C line does not develop, the test result is invalid, and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
 - a. One cassette device
 - b. One desiccant
2. Specimen transfer device
3. Sample diluent (5 mL/bottle)
4. One package insert (instruction for use)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer
2. Disposable gloves
3. Lancing device for whole blood test
4. Alcohol swab

WARNINGS AND PRECAUTIONS

For in Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert may lead to inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use the test device if pouch is not intact.
4. Do not use expired devices or components.
5. Bring all reagents to room temperature (15-30°C) before use.
6. Do not use the components of different lots and of any other type of test kit as a substitute for the components in this kit.
7. Do not use hemolyzed blood for testing.
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
10. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
11. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
12. Handle the negative and positive controls in the same manner as the patient specimens.
13. The test result should be read 15 minutes after a specimen is applied to the sample well of the device. Reading the test result after 20 minutes may give erroneous results.
14. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air conditioning.
15. Clean up spills thoroughly using appropriate disinfectant.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store test kit at 1-30°C. If stored at 2-8°C, ensure that all reagents are brought to room temperature before opening. The sample Diluent (Opened and unopened) and unopened test device is stable through the expiration date printed on the label, when stored at recommended temperature. Do not freeze the kit or expose the kit to temperatures above 30°C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the test device from the foil pouch.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

- Step 1: Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by venipuncture.
- Step 2: Separate the plasma by centrifugation.
- Step 3: Carefully withdraw the plasma into a new pre-labeled tube.

Serum

- Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
- Step 2: Allow the blood to clot.
- Step 3: Separate the serum by centrifugation.
- Step 4: Carefully withdraw the serum into a new pre-labeled tube.

Test serum and plasma specimens as soon as possible after collecting. Store serum and plasma specimens at 2-8°C if not tested immediately. Serum and plasma specimens can be stored at 2-8 °C for up to 5 days. The serum and plasma specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

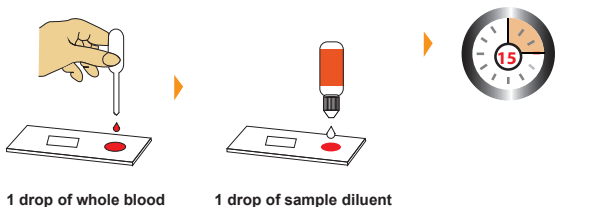
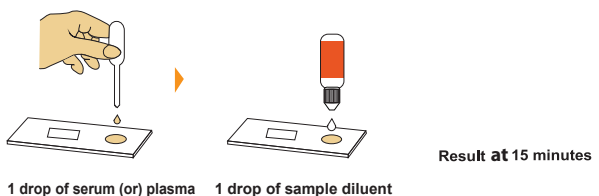
Whole Blood

Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, Citrate or heparin, respectively, in vacutainer®). Do not use hemolysed blood for testing. Capillary blood (fingertip puncture) can be used directly without anti-coagulant. Collect blood with specimen transfer device and transfer it to sample well of device.

Whole blood specimen should be stored in refrigeration (2-8°C), if not tested immediately. The specimens must be tested within 24 hours of collection.

ASSAY PROCEDURE

- Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.
- Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat and dry surface.
- Step 3: Label the device with the specimen's ID number.
- Step 4: Fill the specimen transfer device with the specimen.



- Step 5: Set up timer.
- Step 6: Read the result at 15 minutes. Positive results may be visible as soon as 1 minute. **Do not read the result after 20 minutes. To avoid confusion, discard the test device after interpreting the result.**

QUALITY CONTROL

Internal Control: This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the whole procedure and repeat the test with a new device.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT:** If only the C line is developed, the test indicates that no detectable antibodies to HCV are present in the specimen. The result is negative or non-reactive.



2. **POSITIVE RESULT:** If both the C and the T lines are developed, the test indicates the presence of antibodies to HCV in the specimen. The result is positive or reactive.



Samples with reactive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic decision is made.

3. **INVALID:** If no C line is developed, the assay is invalid regardless of development on the T line as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

A total of 1089 samples including serum, plasma and whole blood from susceptible subjects were tested with the TRUSTline HCV Ab Rapid Test and with a commercial HCV ELISA Kit. Comparison for all subjects is shown in the following table.

HCV ELISA	TRUSTline HCV Ab Rapid Test		Total
	Positive	Negative	
Positive	130	0	130
Negative	2	957	959
Total	132	957	1089

Relative Sensitivity: 100%, Relative Specificity: 99.79%, Overall Agreement: 99.82%

2. **Cross-Reactivity**

No cross reactivity was observed when tested the TRUSTline HCV Ab Rapid test with the following infectious diseases samples with the standard test procedure

Cross reactivity Specimen	Sample Size	HCV Ab Reactivity	
Dengue Positive	Serum	5	Negative
	Plasma	3	Negative
	Whole Blood	2	Negative
HAV Positive	Serum	5	Negative
	Plasma	2	Negative
	Whole Blood	3	Negative
HBsAg Positive	Serum	5	Negative
	Plasma	2	Negative
	Whole Blood	3	Negative
HIV Positive	Serum	5	Negative
	Plasma	3	Negative
	Whole Blood	2	Negative
Syphilis Positive	Serum	5	Negative
	Plasma	3	Negative
	Whole Blood	2	Negative
ANA Positive	Serum	3	Negative
	Plasma	1	Negative
	Whole Blood	1	Negative
RF Pos (≤2,500 IU/ml)	Serum	3	Negative
	Plasma	1	Negative
	Whole Blood	1	Negative

3. **Precision**

Within run and between run precisions have been determined by testing 20 replicates with four categories of Serum, Plasma and whole Blood specimens: negative, weak, medium and strong positive specimens. The negative, weak, medium and strong positive specimens were correctly identified in all of the tests performed in each run.

4. **Interference**

Common substances (such as pain and fever medication, blood components) may affect the performance of the TRUSTline HCV Ab Rapid Test. This was studied by spiking of these substances into three levels of HCV standard Serum, Plasma and whole Blood control. The results are presented in the following table and demonstrate that at the concentrations tested, the substances studied did not affect the performance of the TRUSTline HCV Ab Rapid Test.

Potential interfering substances spiked	HCV Reactivity		
	Negative	Weak Positive	Strong Positive
Control	-	+	+++
Bilirubin	1 mg/dL	-	+
	15 mg/dL	-	+
Creatinine	1.5 mg/dL	-	+
	5 mg/dL	-	+
Glucose	80 mg/dL	-	+
	120 mg/dL	-	+
Albumin	3.5 g/dL	-	+
	5 g/dL	-	+
CRP	1 mg/dL	-	+
	4 mg/dL	-	+
Urea	9 mg/dL	-	+
	40 mg/dL	-	+
Bicarbonate 0.23 g/dL	-	+	+++
EDTA 3.48 μmol/L	-	+	+++
Sodium citrate 3.8%	-	+	+++

EXTERNAL EVALUATION RESULTS

The TRUSTline HCV Ab Rapid test was externally evaluated by The National Institute of Biologicals and it complies with the CDSCO's specifications. The TRUSTline HCV Ab rapid test qualified the evaluation with a sensitivity of 99% and a specificity of 100%.

LIMITATIONS OF TEST

- The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to HCV in serum plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
- The TRUSTline HCV Ab Rapid Test is limited to the qualitative detection of antibodies to HCV in human serum, plasma or whole blood. The intensity of the test line does not have linear correlation with the antibody titer in the specimen.
- A non-reactive result for an individual subject indicates absence of detectable antibodies to HCV. However, a non-reactive test result does not preclude the possibility of exposure to or infection with HCV.
- A non-reactive result can occur if the quantity of the antibodies to HCV present in the specimen is below the detection limits of the assay or if the antibodies that are detected are not present during the stage of disease in which a sample is collected.
- False negative results may arise because of hook effect due to very high titer of antibodies in sample. Repeat the test by using different dilution of same sample.
- Hemolytic samples may give reddish background even after end of the reading time.
- Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
- Infection may progress rapidly. If the symptoms persist and the result from the TRUSTline HCV Ab Rapid Test is non-reactive, it is recommended to test with an alternative device or to re-sample the patient a few days later.
- The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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- Miyamura T, Saito I, Katayama T, et al. Detection of antibody against antigen expressed by molecularly cloned hepatitis C virus cDNA: application to diagnosis and blood screening for posttransfusion hepatitis. Proc Natl Acad Sci USA 1990. 87:983-7.
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- Houghton M, Weiner A, Han J, Kuo G, Choo Q-L. Molecular Biology of the Hepatitis C viruses: Implications for diagnosis, Development, and Control of Viral Disease. Hepatology 1991. 14:381-8.
- Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo Q-L, Kuo G. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med 1989. 321:1494-1500.
- ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

Index of Symbols

	Consult instructions for use	REF Catalogue number		Caution
IVD	In vitro diagnostic medical device	LOT Batch code		Non-sterile
	Temperature limit 1-30 °C			Use-by date
	Manufacturer			Keep dry
	Do not use if package is damaged			Keep away from sunlight

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