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 or plasma. 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.

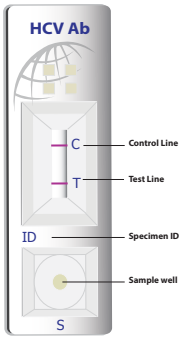
SUMMARY AND EXPLANATION OF THE TEST

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 cases[2]. HCV can also be transmitted through intravenous drug abuse and sexual contact[3].

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 virus[6]. 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 or plasma. 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

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 bio-safety 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 specimens as soon as possible after collecting. Store specimens at 2-8°C if not tested immediately. Specimens can be stored at 2-8 °C for up to 5 days.The 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.

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.

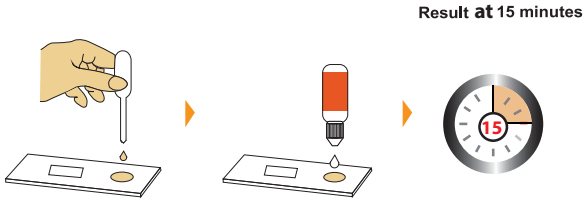
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

Step 3: Label the device with the specimen's ID number.

Step 4: Fill the specimen transfer device with the specimen.

Holding the specimen transfer device vertically, dispense 1 drop (about 30-45 µL) of serum/plasma into the sample well making sure that there are no air bubbles.

Immediately add 1 drop (about 35-50 µL) of Sample Diluent to the sample well with the bottle positioned vertically.



1 drop of specimen **1 drop of sample diluent**

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

1. **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.

2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. New operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature used during storage of the kits falls outside of 1 - 30 °C.
   e. The temperature of the test area falls outside of 15 - 30 °C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

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 color development on the T line as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

A total of 856 samples 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.

	TRUSTline HCV Ab Rapid Test		
HCV ELISA	**Positive**	**Negative**	**Total**
Positive	120	1	121
Negative	4	731	735
Total	124	732	856

Relative Sensitivity: 99.17%, Relative Specificity: 99.5%, Overall Agreement: 99.42%

2. **Cross-Reactivity**

The negative specimen was spiked with serum specimens of infectious diseases and then tested according to the standard procedure. The results showed that the TRUSTline HCV Ab Rapid Test had no cross-reaction with the following tested serum specimens of infectious disease.

Specimen	Sample Size	HCV Reactivity
Dengue Positive Serum	10	Negative
HAV Positive Serum	10	Negative
HBsAg Positive Serum	10	Negative
HIV Positive serum	10	Negative
Syphilis Positive Serum	10	Negative
ANA Positive Serum	5	Negative
RF positive Serum (≤2,500 IU/ml)	5	Negative

3. **Precision**

Within run and between run precisions have been determined by testing 20 replicates with four categories of the 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 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 20 mg/dL	-	+	+++
Creatinine 442 mol/L	-	+	+++
Glucose 55 mmol/L	-	+	+++
Albumin 60g/L	-	+	+++
Salicylic acid 4.34 mmol/L	-	+	+++
Heparin 3,000 U/L	-	+	+++
EDTA 3.48 µmol/L	-	+	+++
Human IgG 1000 mg/dL	-	+	+++
Sodium citrate 3.8%	-	+	+++

LIMITATIONS OF TEST

1. 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 or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTline HCV Ab Rapid Test is limited to the qualitative detection of antibodies to HCV in human serum or plasma. The intensity of the test line does not have linear correlation with the antibody titer in the specimen.
3. 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.
4. 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.
5. 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.
6. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
7. 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.
8. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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5. 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.
6. Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo Q-L, Kuo G. Detection of antibody to hepatitis C virus in prospectivity followed transfusion recipients with acute and chronic non-A,non-B hepatitis. N Engl J Med 1989. 321:1494-1500.

Index of Symbols

Consult instructions for use		REF	Catalogue number		Use-by date
IVD	For *in vitro* diagnostic use only	LOT	Batch code		Tests per kit
1°C 30°C	Temperature limit 1-30 °C		Do not re-use		Keep dry
	Manufacturer		Date of manufacture		

Do not use if package is damaged — Keep away from sunlight

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