REF

Catalog Number R0023C

IVD

In vitro Diagnostic

INTENDED USE

The *OnSite* HCV Ab Plus 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 as a screening test and as an aid in the diagnosis of infection with HCV. Any reactive specimen with the *OnSite* HCV Ab Plus Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

SUMMARY AND EXPLANATION OF THE TEST

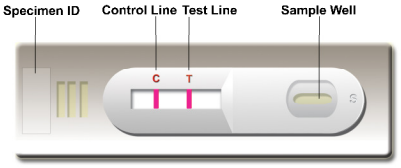
Hepatitis C virus (HCV), which was formerly described as the parentally transmitted form of non-A, non-B hepatitis (NANBH)[1], causes chronic disease in 50% of patients[2]. HCV can also be transmitted through intravenous drug abuse and sexual contact[3].

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 *OnSite* HCV Ab Plus Rapid Test is a useful tool for blood bank screening safety.

The *OnSite* HCV Ab Plus 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 *OnSite* HCV Ab Plus 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 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 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. Plastic droppers
3. Sample Diluent (REF SB-R0023, 5 mL/bottle)
4. One package insert (instruction for use)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer

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 expired devices.
4. Bring all test materials to room temperature (15-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood specimens for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The test result should be read 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the result after 20 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C. The positive and negative controls should be kept at 2-8°C or the temperature indicated. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures over 30°C.

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 with 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.

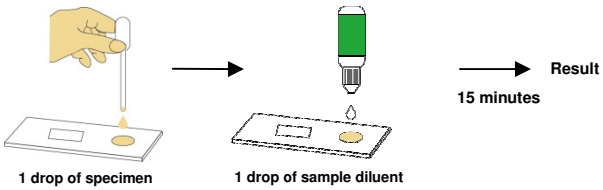
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with the specimen's ID number.

Step 4: Fill in the plastic dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (about 30-45 µL) of specimen into the center of 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.



Step 5: Set up a timer.

Step 6: Read the result in 15 minutes. Positive results may be visible in as soon as 1 minute.

Do not read result after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

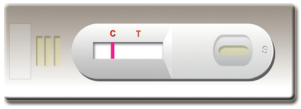
QUALITY CONTROL

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 entire procedure and repeat the test with a new device.

2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to ensure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing the testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of kits is used.
   d. The temperature used during storage of the kits fall outside of 2-30°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 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 positive determination 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 1050 samples from susceptible subjects were tested with the *OnSite* HCV Ab Plus Rapid Test and with a commercial HCV ELISA kit. Comparison of the results for all subjects is shown in the following table.

	OnSite HCV Ab Plus Rapid Test		
HCV ELISA	**Positive**	**Negative**	**Total**
Positive	312	4	316
Negative	3	731	734
Total	315	735	1050

Relative Sensitivity: 98.7%, Relative Specificity: 99.6%, Overall Agreement: 99.3%

2. **Worldwide Performance Panel**

BBI's (Boston Biomedica Inc.) worldwide performance panel (WWHV301) was tested with the *OnSite* HCV Ab Plus Rapid Test. The results are shown in the following table.

Member ID	Origin	Genotype	Abbott EIA	*OnSite* HCV Ab Plus Rapid Test
301-01	Argentina	1b	Positive	Positive
301-02	Argentina	1b	Positive	Positive
301-03	Argentina	3a/b	Positive	Positive
301-04	Argentina	2a/c	Positive	Positive
301-05	Argentina	Not tested	Negative	Negative
301-06	Uganda	4c/d	Positive	Positive
301-07	Uganda	Not tested	Positive	Positive
301-08	Ghana	Not tested	Negative	Negative
301-09	China	1b, 2a/c	Positive	Positive
301-10	China	2	Positive	Positive
301-11	China	1b	Positive	Positive
301-12	China	2	Positive	Positive
301-13	China	1a/b, 2a/c	Positive	Positive
301-14	Egypt	3a	Positive	Positive
301-15	Egypt	4	Positive	Positive
301-16	Egypt	4h	Positive	Positive
301-17	Egypt	Not tested	Positive	Positive
301-18	USA	1b	Positive	Positive
301-19	USA	1a	Positive	Positive
301-20	USA	1a	Positive	Positive

3. **Seroconversion Panel**

BBI's (Boston Biomedica Inc.) seroconversion panel (PHV910 – (M)) was tested with the *OnSite* HCV Ab Plus Rapid Test. The results are shown in the following table.

Member ID	Days Bleeding	Abbott HCV EIA 2.0 s/co*	*OnSite* HCV Ab Plus Rapid Test
910-01	0	0.2	Negative
910-02	4	0.3	Negative
910-03	8	1.3	Positive
910-04	11	2.9	Positive
910-05	15	2.4	Positive

* EIA results expressed as specimen absorbance to cut-off ratio (S/CO). Ratios ≥ 1.0 are considered reactive.

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 lead to inaccurate results.
2. The *OnSite* HCV Ab Plus Rapid Test is limited to the qualitative detection of antibodies anti-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 the 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 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. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
6. If symptoms persist when the result from *OnSite* HCV Ab Plus Rapid Test is non-reactive, it is recommended to re-sample the patient a few days later or test with an alternative test device.
7. Results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. Kuo,G, Choo Q-L, Alter, HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989. 244:362-4.
2. Esteban JI, Gonzalez A, Hernandez JM, et al. Evaluation of antibodies to hepatitis C virus in a study of transfusion-associated hepatitis. N Engl J Med 1990. 323:1107-12.
3. 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.
4. Estaban JI, Esteban R, Viladomiu L, et al. Hepatitis C virus antibodies among risk groups in Spain. Lancet 1989. 2:294-7.
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	For *in vitro* diagnostic use only	Use by
REF Catalog #	LOT Lot Number	Tests per kit N
Store between 2-30°C	Do not reuse	
Manufacturer	Date of manufacture	

CTK Biotech, Inc.
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English version

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