

OnSite® HCV Ab Plus Combo Rapid Test

REF R0024C

Instructions for Use

INTENDED USE

The OnSite HCV Ab Plus Combo 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 an aid in the diagnosis of infection with hepatitis C virus.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be used to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C virus (HCV) is the causative agent of significant acute and chronic liver disease worldwide. According to the WHO, approximately 71 million people globally live with chronic HCV infection, with 1.75 million new cases in 2015¹. 15-25% of acute HCV infections are cleared, while 75-85% of cases result in chronic infections². Sequelae of chronic HCV infection include liver scarring (cirrhosis), liver failure, and liver cancer, causing 400,000 deaths in 2016 and 20% of all liver cancer cases in 2012^{1,3}. Although no HCV vaccines are available, oral antiviral medications can cure chronic HCV infections in 90% of individuals in 8-12 weeks⁴.

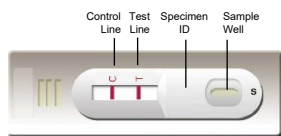
Hepatitis C viruses, which are positive-sense single-stranded RNA viruses, exhibit considerable genetic diversity that presents challenges for vaccine development. At least six major genotypes of HCV are recognized (1-6) and can differ as much as 30-35% genetically^{5,6}. Genotype 1 is the most prevalent genotype, accounting for 46.2% of HCV cases, followed by genotype 3 at 30.1%. Genotypes 2, 4, and 6 constitute 22.8% of remaining cases, with genotype 5 being the least prevalent, occurring in <1% of cases. These HCV genotypes also feature distinct global distributions (for example, genotype 1 is found worldwide, genotype 4 is more prevalent in Middle East and North Africa, and genotypes 5 and 6 are common to South Africa and Hong Kong) and differential responses to clinical treatment and therapies⁶.

Diagnosis for HCV infection can be accomplished through the detection of antibodies against HCV or through direct detection of the virus using nucleic acid amplification tests (NAT)⁷. HCV antibodies are generated primarily against the viral core, NS3, NS4, and NS5 proteins, usually around 6-8 weeks after infection⁸. As HCV antibodies can also be a marker of past infection, detection of HCV RNA via NAT remains the gold standard for detecting acute infections⁹. The CDC testing algorithm for HCV infection recommends testing for HCV antibody first, followed up by HCV NAT to confirm any antibody-positive samples². Multiple studies have demonstrated the utility for using point-of-care rapid tests for HCV antibody testing compared to conventional laboratory-based methods such as ELISA¹⁰⁻¹¹.

The OnSite HCV Ab Plus Combo Rapid Test detects anti-HCV antibodies (IgG, IgM, IgA) in human serum, plasma or whole blood. The test can be performed within 15 minutes by minimally skilled personnel without the use of cumbersome laboratory equipment.

TEST PRINCIPLE

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



When an adequate volume of specimen is dispensed into the sample well of the cassette, it migrates by capillary action across the cassette. Antibodies to HCV, if present in the specimen, will migrate through the conjugate pad and bind to the HCV Ag conjugates. The immunocomplex is then captured on the membrane by the pre-coated HCV fusion antigen forming a colored T line, indicating an HCV Ab positive or reactive test result. Lack of color development on the test line indicates a negative or non-reactive result for HCV Ab.

The test contains an internal control (C line) which should exhibit a colored line by capture of the control immunocomplex by the 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

- Individually sealed foil pouches containing:
 - One cassette device
 - One desiccant
- Plastic droppers
- Sample diluent (REF SB-R0024, 5 mL/bottle of Tris-buffered solution with preservatives containing 0.095% sodium azide)
- Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

- Clock or timer

WARNINGS AND PRECAUTIONS

For in Vitro diagnostic use only

- Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
- Do not open the sealed pouch unless ready to conduct the assay.
- Do not use expired devices or components.
- Use only one specimen per device. Do not combine specimens.
- Bring all reagents to room temperature (15-30°C) before use.
- Do not use components from any other test kit/lot as substitutes for components in this kit/lot.
- Do not use hemolyzed blood specimens for testing.
- Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
- Follow US CDC Universal Precautions for prevention of transmission of HIV, HBV and other bloodborne pathogens: <https://www.cdc.gov/niosh/topics/bbp/universal.html>
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Dispose of all specimens and materials used to perform the test as biohazardous waste.
- Handle external controls in the same manner as patient specimens.
- Read test results 15-20 minutes after a specimen is applied to the sample well of the device. Reading the test result after 20 minutes should be considered invalid and must be repeated.
- Do not perform the test in a room with strong air flow, i.e. electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

The OnSite HCV Ab Plus Combo Rapid Test is stable at 2-30°C for up to 24 months from the date of manufacture. All kit components are ready to use as supplied. Store unused test devices unopened at 2-30°C. 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 above 30°C.

SPECIMEN COLLECTION AND HANDLING

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

Step 1: Collect venous blood by venipuncture into collection tube containing EDTA, citrate or heparin for plasma or whole blood specimens, or collection tube containing no anticoagulants for serum specimens.

Step 2: **For whole blood specimens:** Test immediately or store refrigerated at 2-8°C for up to 24 hours after collection. Do not freeze specimens.

For plasma: Centrifuge collected specimen and carefully withdraw the plasma into a new pre-labeled tube.

For serum: Allow blood to clot, centrifuge collected specimen and carefully withdraw the serum into a new pre-labeled tube.

Step 3: **Plasma/Serum only:** Test specimens immediately after collection or store refrigerated at 2-8°C for up to 5 days. Specimens can 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.

Note: Do not test specimens demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

ASSAY PROCEDURE

Step 1: Ensure that specimen and test components are equilibrated to room temperature. If frozen, mix the specimen well after thawing, prior to performing the assay.

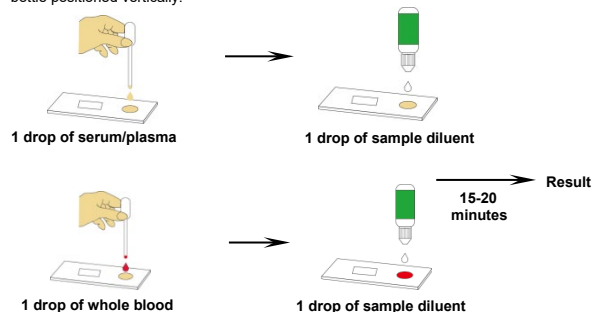
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

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

Step 4: Fill the plastic dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (approximately 45 µL) of serum/plasma or 1 drop of whole blood (approximately 55 µL) into the center of the sample well making sure that there are no air bubbles.

Immediately add 1 drop (approximately 55 µL) of Sample Diluent to the sample well with the bottle positioned vertically.



Step 5: Set up timer.

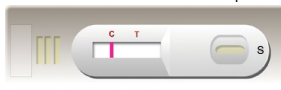
Step 6: Read results at 15-20 minutes. Positive results may be visible as soon as 1 minute. Negative results must be confirmed at the end of the 20 minutes. **Any results interpreted outside 15-20 minute window should be considered invalid and must be repeated. Discard used device after interpreting the results following local laws governing the disposal of device.**

QUALITY CONTROL

- Internal Control:** This test contains a built-in control feature, the C line that develops whether the specimen is positive or negative. If the C line does not develop, review the entire procedure and repeat the test with a new device.
- External Control:** Good Laboratory Practice recommends using external positive and negative controls to ensure the proper performance of the assay, particularly under the following circumstances:
 - A new operator uses the kit, prior to performing testing of specimens.
 - A new lot of test kits is used.
 - A new shipment of test kits is used.
 - The storage temperature of the kits falls outside of 2-30°C.
 - The temperature of the test area falls outside of 15-30°C.
 - To verify a higher than expected frequency of positive or negative results.
 - To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

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



- POSITIVE RESULT:** If both the C and T lines develop, the test indicates that the specimen contains detectable HCV antibodies. The result is HCV Ab positive or reactive.



Specimens producing very faint test lines (indeterminate) should be re-tested with another two devices or tested with alternative method. Samples with reactive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made.

- INVALID:** If no C line develops, 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. **Diagnostic Sensitivity**

1.1 Positive Specimens

Clinical evaluations at three different sites were performed with the OnSite HCV Ab Plus Combo Rapid Test and a commercial CE-marked rapid test. The data is summarized below.

Samples	OnSite HCV Ab Plus Combo		CE-marked Rapid Test		Relative Sensitivity
	Pos.	Neg.	Pos.	Neg.	
Anti-HCV Ab Positive	471	0	469	2*	100% (95% CI: 99.2-100%)

Overall Agreement: 100% (95% CI: 99.2-100%)

*: The discordant specimens were confirmed as true positives by CE-marked ELISA or PCR assay

1.2 HCV Genotypes

The following genotyped specimens were tested at a clinical site and detected as positive by the OnSite HCV Ab Plus Combo Rapid Test. The data is summarized below.

HCV Genotype	Samples Positive/ Samples Tested	HCV Genotype	Samples Positive/ Samples Tested
1	30/30	4	21/21
2	26/26	5	6/6
3	23/23		

1.3 Seroconversion Panels

30 commercially available seroconversion panels and 1 low titer panel were tested in-house with the OnSite HCV Ab Plus Combo Rapid Test and a commercial CE-marked rapid test. The data is summarized below.

Panels	No. of Samples	OnSite HCV Ab Plus Combo		CE-marked Rapid Test	
		Pos.	Neg.	Pos.	Neg.
Seroconversion	247	98	149	94	153
Low Titer	11	10	1	10	1

The OnSite HCV Ab Plus Combo Rapid Test detected 4 more Ab positive seroconversion panel members than CE-marked reference rapid test.

2. **Diagnostic Specificity**

Clinical evaluations at three different sites were performed with the OnSite HCV Ab Plus Combo Rapid Test and a commercial CE-marked rapid test. The data is summarized below.

Samples	OnSite HCV Ab Plus Combo		CE-marked Rapid Test		Relative Specificity
	Pos.	Neg.	Pos.	Neg.	
Blood donations	0	1007	0	1007	100% (95% CI: 99.6-100%)
Clinical Specimens	2*	693	0	695	99.7% (95% CI: 99.0-99.9%)
Pregnant Women**	0	214	0	214	100% (95% CI: 98.2-100%)
Potentially Interfering Specimens ^Δ	0	123	0	123	100% (95% CI: 97.0-100%)

Overall Agreement: 99.9% (95% CI: 99.6-100%)

*: The discordant specimens were confirmed as true negatives by CE-marked PCR assay

** Includes 20 multiparous pregnancy samples

^Δ: Naturally-occurring specimens high in glucose, triglycerides, bilirubin, creatinine, hemoglobin, or cholesterol

3. **Analytical Specificity – Cross-reactivity**

Specimens from the following disease states or conditions were tested with OnSite HCV Ab Plus Combo Rapid Test. No cross-reactivity was observed.

Disease/Condition	Samples Tested	Disease/Condition	Samples Tested
HBsAg	10	Influenza B	2
HIV	10	TBE	3
HBc	3	HTLV-1	5
HAV	3	HTLV-2	5
Syphilis	4	Malaria	5
Toxoplasma	4	Chagas	3
HSV1	3	Influenza vaccine (IgA)	3
HSV2	3	CRP	4
E. coli	4	dsDNA	4
CMV	3	Multiparous pregnancy	2
EBV	3	RF	12
VZV	3	ANA	4
Measles	5	HAMA	2
Rubella	5	SLE	2
Influenza A	4	Yellow Fever (vaccine)	3

4. **Specimen Equivalency**

Same day "fresh" samples (≤1 day after collection) were tested at a clinical site with the OnSite HCV Ab Plus Combo Rapid Test and a CE-marked rapid test. The data is summarized in the table below:

Sample	Matrix	No.	OnSite HCV Ab Plus Combo		CE-marked Rapid Test	
			Pos.	Neg.	Pos.	Neg.
Anti-HCV Ab Positive	Serum	52	52	0	52	0
	Plasma (EDTA)	52	52	0	52	0
	Plasma (heparin)	52	52	0	52	0
	Plasma (citrate)	52	52	0	52	0
	Whole Blood	52	52	0	52	0
Negative	Serum	52	0	52	0	52
	Plasma (EDTA)	52	0	52	0	52
	Plasma (heparin)	52	0	52	0	52
	Plasma (citrate)	52	0	52	0	52
	Whole Blood	52	0	52	0	52

5. **Interference**

The following common potentially interfering substances were spiked (at concentrations above physiological levels) into anti-HCV Ab positive and negative specimens and tested in-house with the OnSite HCV Ab Plus Combo Rapid Test. No interference was observed at all concentrations tested.

Potentially Interfering Substances	
Human IgG	EDTA
Acetaminophen	Sodium citrate
Aspirin	Albumin
Caffeine	Triglycerides
Ethanol	Glucose
Fluconazole (antifungal)	Quinine (antimalarial)
HIV medication (Tenofovir, Lamivudine, Efavirenz)	Ethambutol (antibiotic for tuberculosis)
Bilirubin	Creatinine
Heparin	Hemoglobin

6. **Precision**

Within-run precision was determined using 6 replicates of 3 serum or 3 whole blood specimens containing different concentrations of HCV antibodies.

Within-run precision of 100% was observed for both specimen matrices.

Between-day precision was determined over 5 different days, and observed as 100% for both serum and whole blood.

Between-lot precision was determined with 3 lots of devices. 100% precision was observed for both serum and whole blood.

Between-operator precision was determined by 3 operators. 100% precision was observed for both serum and whole blood.

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. Failure to follow the procedure may give inaccurate results.
- The OnSite HCV Ab Plus Combo 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 correlate with the antibody titer of the specimen.
- Specimens that produce very faint lines (indeterminate) should be re-tested with two new devices or an alternative method(s). However, a non-reactive or negative test result does not preclude the possibility of exposure to or infection with HCV.
- A negative or non-reactive result can occur if the concentration of HCV Ab present in the specimen is below the level detectable by the assay or HCV Ab was not present during the stage of disease in which a sample was collected.
- Infection may progress rapidly. If the symptoms persist, while the result from the OnSite HCV Ab Plus Combo Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
- Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
- The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

- World Health Organization. Fact Sheets – Hepatitis C. <https://www.who.int/news-room/fact-sheets/detail/hepatitis-c>
- Centers for Disease Control and Prevention. A Guide to Comprehensive Hepatitis C Counseling and Testing (2016). www.cdc.gov/hepatitis/resources/professionals/pdfs/CounselingandTestingPC.pdf
- Maucoit-Boulch D, de Martel C, Franceschi S, et al. Fraction and incidence of liver cancer attributable to hepatitis B and C viruses worldwide. *Int J Cancer* (2018); 142(12): 2471-2477.
- American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA). Recommendations for testing, management, and treating hepatitis C. HCV testing and linkage to care. <https://www.hcvguidelines.org>
- Messina JP, Humphreys I, Flaxman A. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* (2015); 61(1): 77-87.
- Zein NN. Clinical significance of hepatitis C virus genotypes. *Clin Microbiol Rev* (2000); 13(2): 223-35.
- Gupta E, Bajpai M, Choudhary A. Hepatitis C virus: Screening, diagnosis, and interpretation of laboratory assays. *Asian J Transfus Sci* (2014); 8(1): 19-25.
- Sillanpää M, Melén K, Porkka P, et al. Hepatitis C virus core, NS3, NS4B and NS5A are the major immunogenic proteins in humoral immunity in chronic HCV infection. *Virology* (2009); 6: 84.
- Kamili S, Drobeniuc J, Araujo AC. Laboratory diagnostics for hepatitis C virus infection. *Clin Infect Dis* (2012); 55 Suppl 1:S43-8.
- Shivkumar S, Peeling R, Jafari Y, et al. Accuracy of rapid and point-of-care screening tests for hepatitis C: a systematic review and meta-analysis. *Ann Intern Med* (2012) 16; 157(8): 558-66
- Tang W, Chen W, Amini A, et al. Diagnostic accuracy of tests to detect Hepatitis C antibody: a meta-analysis and review of the literature. *BMC Infect Dis* (2017); 17 (Suppl 1): 695.

Index of Symbols

	Consult instructions for use		For in vitro diagnostic use only		Use-by date
	Catalogue number		Batch code		Tests per kit
	Store between 2-30°C		Date of manufacture		
	Manufacturer		Do not reuse		

CTK Biotech, Inc.
 13855 Stowe Drive,
 Poway, CA 92064, USA
 Tel: 858-457-8698
 Fax: 858-535-1739
 E-mail: info@ctkbiotech.com

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