

# *Evaluation of the Sensitivity, Specificity and Accuracy of the HCV Rapid Test Cassette for Detecting HCV Antibodies in Serum or Plasma Specimens*

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**Abstract:** Hepatitis C virus (HCV) is a bloodborne pathogen that primarily targets the liver, leading to the development of chronic liver disease. Timely identification and effective management of HCV infection are essential to prevent complications and halt further transmission. The aim of this study was to evaluate the diagnostic efficacy of the HCV Rapid Test Cassette, an immunoassay that employs specific antibodies to qualitatively detect HCV antibodies directed against viral antigens. To assess the sensitivity, specificity, and accuracy of the HCV Rapid Test, we utilized a comprehensive collection of 936 seroconversion panels. The test demonstrated an accuracy of 99.0%, with a sensitivity of 98.7% and a specificity of 99.1%. The specificity of the test was confirmed through cross-reactivity testing, which yielded negative results for various viral strains. Developed by Hangzhou All Test Biotech Co., Ltd, the HCV Rapid Test offers high accuracy, reliability, and rapid results, making it a valuable adjunctive diagnostic tool in clinical settings, particularly in resource-limited environments. Early identification facilitated by this test enables timely intervention and effective management of Hepatitis C, leading to improved patient outcomes and reduced viral transmission.

## 1. Introduction

Hepatitis C is a viral infection caused by the hepatitis C virus (HCV) and primarily impacts the liver.<sup>[1]</sup> It poses a significant global health concern, with an estimated 50 million people worldwide living with chronic HCV infection, and around one million new infections occurring annually.<sup>[2]</sup> The profound impact of hepatitis C is evident from the estimated 242,000 deaths attributed to the disease in 2022, primarily resulting from cirrhosis and hepatocellular carcinoma, which is a form of primary liver cancer.<sup>[2]</sup> The prevalence of hepatitis C varies regionally, with certain geographic areas experiencing higher rates. This can be attributed to factors such as injection drug use, unsafe medical procedures, and blood transfusions.<sup>[3]</sup> Low- and middle-income countries bear the greatest burden of

Hepatitis C Virus (HCV), accounting for over 70% of the total cases worldwide.<sup>[4]</sup>

Hepatitis C transmission occurs through various routes, with blood-to-blood contact being the predominant mode. Key risk factors include sharing drug paraphernalia or needles contaminated with the virus, unsafe medical procedures and transfusion of blood that has not undergone proper screening. While sexual contact potentially contributes to transmission, its associated risk is relatively lower compared to other blood-borne viruses such as HIV.<sup>[5]</sup>

Hepatitis C infection can range from an acute, self-limiting illness to a chronic condition. Many individuals with acute infection may not exhibit noticeable symptoms<sup>[6]</sup>, while others may experience fatigue, jaundice, abdominal pain, loss of appetite, and joint pain. Chronic HCV infection results in liver damage, cirrhosis, and an elevated risk of liver cancer. Thus, conducting large-scale screening is essential to identify individuals infected with the hepatitis C virus (HCV) and provide them with effective therapies.

Screening for HCV is performed by detecting HCV-specific antibodies. The World Health Organization (WHO) recommends to use a single, quality-assured serological in vitro diagnostic test, such as a laboratory-based immunoassay or a rapid diagnostic test (RDT).<sup>[7]</sup> HCV screening is often performed using an enzyme-linked immunosorbent assay (ELISA) or chemiluminescence immunoassay (CLIA) for HCV antibody detection.<sup>[8]</sup> In regions with limited access to laboratory testing services due to equipment shortages, training requirements, and transportation challenges, Rapid Diagnostic Tests (RDTs) offer a feasible solution.<sup>[9]</sup> RDTs offer cost-effectiveness, ease of use, and versatility beyond traditional laboratory settings, accommodating various specimen types such as plasma, serum and capillary whole blood. As a result, RDTs are an appealing option for identifying and screening for HCV in resource-constrained regions.

## **2. Materials and Methods**

### **2.1 Study Design**

The objective of this study was to evaluate the performance and effectiveness of the HCV Rapid Test, developed by Hangzhou AllTest Biotech Co., Ltd, in diagnosing Hepatitis C Virus (HCV) infection. To ensure reliable results, a total of 936 seroconversion panels, consisting of positive and negative specimens collected at different time points, were included in the study. The specimen size was determined based on statistical considerations and the desired level of precision required to achieve adequate statistical power for the evaluation.

Reference testing was conducted at diagnostic reference laboratories using plasma samples. For the HCV Rapid Test kit, either serum or plasma samples can be used for detection. It is crucial to separate the serum or plasma from the blood as soon as possible to prevent hemolysis (rupture of red blood cells). Only clear and non-hemolyzed specimens should be used for testing. Immediate testing is recommended and specimens should not be kept at room temperature for an extended period. Serum and plasma specimens should be stored at 2-8 °C for up to 3 days. For long-term storage, specimens should be kept below -20 °C. Before testing, the specimens should be brought to room temperature. If frozen, the specimens must be completely thawed and thoroughly mixed before the test.

### **2.2 Screen Test**

#### **2.2.1 HCV Rapid Test**

To ensure precise outcomes, researchers diligently adhered to the manufacturer's instructions. The test kit, sample and/or controls were allowed to reach a state of equilibrium at room temperature (15-30 °C), as per the specified guidelines. The most favorable results were achieved when the test was

conducted within a one-hour timeframe.

The researchers placed the test cassette on a clean and level surface. For serum or plasma specimens, they assumed a vertical grip on the dropper and introduced 1 drop of serum or plasma (approximately 25  $\mu$ L) into the sample well. Following this, they added 2 drops of buffer solution (approximately 80  $\mu$ L) as directed and initiated the timer as indicated in the accompanying diagram. A patient wait ensued for the appearance of colored lines and the test results were subsequently examined after a duration of 10 minutes. It is worth noting that any interpretation of results beyond the 20-minute mark was deemed inappropriate.

The study employed the HCV rapid test kit (serum/plasma), a membrane-based immunodiagnostic method of qualitative nature, explicitly designed for the detection of HCV antibodies in serum or plasma specimens. Within the test cassette, the membrane's test line region was pre-coated with recombinant HCV antigen. During the test, the serum or plasma specimen interacted with the colloidal gold-labeled recombinant HCV antigen. Through capillary action, the resulting mixture ascended the membrane, ultimately encountering the recombinant HCV antigen and eliciting the emergence of a colored line. A positive outcome was indicated by the presence of colored test line, while a negative outcome was indicated by its absence. The presence of a line in the control line region acted as a procedural control, affirming the appropriate addition of the sample's volume and the membrane's wicking process.

### 2.2.2 Enzyme Immunoassay (EIA)

Large-scale screening of HCV infection is usually based on the detection of anti-HCV antibodies in whole blood collected by venous puncture by means of enzyme immunoassay (EIA). The detection of Hepatitis C Virus (HCV) commonly employs the Enzyme Immunoassay (EIA) technique. This widely utilized method utilizes enzymes labeled with specific antibodies to identify HCV antigens or antibodies within blood specimens. The procedure commences with the collection of a blood sample from the individual, followed by its incubation in a microplate coated with HCV-specific antigens. This allows for the binding of any HCV antigens or antibodies present in the specimen. Subsequently, unbound components are eliminated through a washing process and enzyme-labeled antibodies are introduced. These antibodies selectively bind to HCV antigens or antibodies. Another round of washing is conducted to eliminate any excess labeled antibodies. To produce a detectable signal, a substrate solution is added. If HCV antigens or antibodies are present, the enzyme catalyzes a reaction, resulting in the generation of a measurable signal. The intensity of this signal correlates with the quantity of HCV antigens or antibodies present. Interpretation of the results involves comparing the measured signal to predetermined cutoff values, thereby determining whether the results are positive or negative.

### 2.3 Quality Control

The HCV screening test incorporates internal procedural controls to ensure the reliability and accuracy of the results. These controls are essential for verifying that the test has been executed accurately and that all necessary steps have been followed. One of these controls is the presence of a colored line in the control region (C) of the test. This line serves as an internal procedural control, indicating that the test has been conducted properly. Its presence confirms that an adequate volume of the specimen was used, the membrane effectively absorbed the sample and the procedural technique was executed correctly.

In addition to internal controls, it is recommended to include both positive and negative external controls when utilizing the HCV screening kit to further ensure the accuracy and reliability of the test results. These external controls are provided as part of the kit itself. By incorporating these controls,

laboratories can verify the performance of the test and evaluate any potential deviations from expected outcomes.

### 3. Results and Discussion

#### 3.1 Sensitivity and Specificity

The HCV Rapid Test Cassette (Serum/Plasma) employs a recombinant antigen that is derived from genes encoding both structural (nucleocapsid) and non-structural proteins of the hepatitis C virus (HCV). The performance of the HCV Rapid Test Cassette (Serum/Plasma) has been rigorously validated, including testing against a seroconversion panel and comparison with a widely-used commercial HCV enzyme immunoassay (EIA) test using clinical specimens. As shown in the Table 1, the relative sensitivity of the HCV Rapid Test Cassette (Serum/Plasma) is 98.7%, the relative specificity is 99.1% and the overall accuracy is 99.0%

Table 1: Performance Characteristics of HCV Rapid Test.

Method	Results	EIA		Total Results
		Positive	Negative	
HCV Rapid Test Cassette (Serum/Plasma)	Positive	235	6	241
	Negative	3	692	695
Total Results		238	698	936

Relative sensitivity: 98.7% (95%CI:\*96.4%-99.7%);

Relative specificity: 99.1% (95%CI:\*98.1%-99.7%);

Accuracy: 99.0% (95%CI:\*98.2%-99.6%);

\*: Confidence Intervals.

#### 3.2 Precision

The precision of the HCV Rapid Test Cassette (Serum/Plasma) was evaluated both within-run and between-run to ensure its reliability and consistency.

For within-run precision assessment, 20 replicates of three specimens were employed: a negative specimen, a specimen with a low titer of HCV and a specimen with a high titer of HCV. The test cassette demonstrated exceptional accuracy, correctly identifying the negative specimen, the HCV low titer positive specimen and the HCV high titer positive specimen in 100% of the replicates. This outcome provides strong evidence of the test's precision and consistency within a single assay run.

To evaluate between-run precision, 20 independent assays were conducted using the HCV Rapid Test Cassette (Serum/Plasma) and the same three specimens: a negative specimen, a specimen with a low titer of HCV and a specimen with a high titer of HCV. These assays were performed using three different lots of the test cassette. Across all three lots, the test consistently and accurately identified the negative specimen, the HCV low titer positive specimen, and the HCV high titer positive specimen in 100% of the assays. This finding demonstrates the reproducibility and reliability of the test across multiple runs and various test cassette lots.

#### 3.3 Cross-Reactivity

Through subjecting the HCV Rapid Test Cassette (Serum/Plasma) to rigorous evaluation, its performance was thoroughly assessed using a diverse range of positive specimens. These specimens included HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, Syphilis, HIV, *H. Pylori*, MONO, CMV, Rubella and TOXO. The obtained results unveiled the exemplary specificity of the test, as it

accurately identified HCV antibodies without demonstrating any cross-reactivity with antibodies from other pathogens. This outcome underscores the test's capability to deliver dependable and precise HCV detection.

To appraise the potential impact of diverse substances on the test, various compounds were introduced to both HCV negative and positive specimens, each at specific concentrations. These substances encompassed Acetaminophen (20 mg/dL), Caffeine (20 mg/dL), Acetylsalicylic Acid (20 mg/dL), Gentisic Acid (20 mg/dL), Ascorbic Acid (2g/dL), Albumin (2 g/dL), Creatine (200 mg/dL), Hemoglobin (1000 mg/dL), Bilirubin (1g/dL) and Oxalic Acid (60 mg/dL).

The conclusive assay results indicated the absence of any interference with the accuracy and reliability of the HCV Rapid Test, even when the tested substances were present at their specified concentrations. This finding further confirms the test's robustness, ensuring reliable outcomes unaffected by commonly encountered interfering substances in clinical specimens.

### 3.4 Discussion

Accurate and timely diagnosis of Hepatitis C virus (HCV) infection is crucial in clinical practice to identify infected individuals, prevent disease progression and implement appropriate management strategies. Thus, the development of reliable diagnostic methods is therefore of paramount importance.

In this study, we evaluated the diagnostic efficacy of the HCV Rapid Test Cassette, an immunoassay designed for detecting HCV antibodies. This test utilizes specific antibodies targeting viral antigens, enabling qualitative detection of HCV antibodies in serum or plasma samples. Our findings highlight the potential of this test as a valuable diagnostic tool for Hepatitis C infection.

The diagnostic performance of the HCV Rapid Test was assessed using a comprehensive collection of 936 seroconversion panels, comprising both positive and negative specimens of HCV infection. The test exhibited high sensitivity, accurately identifying HCV antibodies in 98.7% of true positive cases, underscoring its effectiveness in detecting HCV infection. Compared to immunoassays, the rapid diagnostic test (RDT) offers the advantages of simplicity, minimal instrument requirements, minimal training, and rapid results obtainable in less than 20 minutes, with the ability to deliver results at the point of testing.

This study suggests that the RDT is a convenient and reliable method for detecting HCV antibodies in plasma or serum, with excellent performance in terms of sensitivity (98.7%), specificity (99.1%) and overall accuracy (99.0%). However, this study has limitations, including a limited sample size, indicating that the presence of HCV antibodies in the specimen can be detected but should not be considered the sole criterion for diagnosing HCV infection. It cannot differentiate between acute and chronic HCV infection. In cases where the test result is negative but clinical symptoms persist, further testing using alternative clinical methods is recommended. It is important to note that a negative result at any time does not exclude the possibility of HCV infection.

### 4. Conclusion

In conclusion, the HCV Rapid Test, developed by Hangzhou AllTest Biotech Co., Ltd, presents multiple benefits that enhance its appropriateness for clinical utilization, especially in resource-constrained environments. The test demonstrates remarkable performance, characterized by its exceptional precision and dependable outcomes. This enables healthcare practitioners to rapidly detect individuals afflicted with HCV infection, thereby expediting early intervention and the implementation of suitable management strategies. The timely diagnostic capabilities offered by the test contribute to enhanced patient outcomes and play a pivotal role in mitigating the risk of further transmission of the viral infection. These factors collectively emphasize the potential value of the HCV Rapid Test in the realm of clinical practice.

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