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# Evaluation of a rapid, point-of-care test device for the diagnosis of hepatitis C infection

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ABSTRACT

*Background:* Despite considerable evolution in the quality of laboratory-based testing for detection of HCV, the availability of rapid, point-of-care tests may increase diagnoses by increasing opportunities for testing outside of traditional laboratory settings.
*Objectives:* We evaluated the performance of a new, rapid HCV test that can be used with venous blood, finger stick blood, serum, plasma, or oral fluid and compared it to FDA-approved laboratory methods.
*Study design:* HCV positive subjects as well as subjects at low risk for HCV were tested with the rapid test using all 5 specimen types and results compared to FDA-approved laboratory methods. In addition, performance was assessed in commercially available seroconversion panels.
*Results:* Sensitivity and specificity of the rapid test was equivalent to laboratory EIA and performance was comparable across all 5 specimen types.
*Conclusions:* The OraQuick® HCV Rapid Antibody Test appears suitable as an aid in the diagnosis of HCV infection.



## 1. Background

Following the identification of the etiological agent of hepatitis C (HCV) infection in 1989,[1] numerous laboratory-based tests have been developed and commercialized to aid in the diagnosis of HCV infection and the screening of donated blood. These include both immunoassay-based tests for antibodies produced in response to infection,[2,3] as well as nucleic acid based tests for direct detection of virus.[4] However, despite significant evolution in the performance of these technologies, there remains substantial under-diagnosis of HCV in the general population. Although the incidence of new infections has declined substantially in many geographies over the past two decades due to greater risk awareness[5] and the implementation of highly sensitive screening of donated blood,[6] HCV remains highly prevalent worldwide. The World Health Organization (WHO) has estimated that about 170 million people or 3% of the world's population are infected, with approximately 10 million individuals infected in Europe[7] and over 4 million infected in the United States.[8] It is estimated that the majority of these infections remain undiagnosed.[9] Most HCV seropositive individuals are chronically infected[8] and these individuals are at-risk for progressive liver disease leading to cirrhosis and/or hepatocellular carcinoma. This silent epidemic represents a growing healthcare burden worldwide.[10] In the US, cirrhosis-related deaths due to chronic HCV infection have risen to more than 10,000 annually and HCV-related mortality is expected to rise substantially over the next 20 years.[11] HCV is the leading indication for liver transplants worldwide.[12]

Treatment of infected patients with the current standard of care regimen of pegylated interferon and ribavirin is effective in achieving sustained viral clearance in approximately 40–50% of patients infected with genotype 1,[13] the most common genotype in the US and Europe. More recent studies have suggested that newer drugs such as protease inhibitors offer the promise of higher rates of viral clearance and shorter treatment courses.[14] With the availability of more effective therapies, eradication of viral infection within the infected population will be highly dependent on increasing primary diagnosis in order to identify previously undiagnosed individuals who may be suitable candidates for therapeutic intervention.

Despite the success of the deployment of rapid tests in increasing opportunities for testing and diagnoses of HIV positive

*Abbreviations:* HCV, hepatitis C virus; WHO, World Health Organization; US, United States; POC, point-of-care; CLIA, clinical laboratory improvement amendments; FDA, food and drug administration; NS3, non-structural protein 3; NS4, non-structural protein 4; IgG, immunoglobulin G; EIA, enzyme immunoassay; RIBA, recombinant immunoblot assay; CI, confidence intervals; PCR, polymerase chain reaction; S/C, signal to cutoff; RNA, ribonucleic acid.

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individuals,[15] there has, as yet, been no FDA-approved rapid test to aid in the diagnosis of HCV infection. A rapid, point-of-care (POC) test for anti-HCV antibodies may have substantial benefit through increased testing convenience and the ability to obtain a result while the patient is still available to the physician. Moreover, a simple POC test that was CLIA-waived for use outside of traditional laboratory settings, such as clinics and physician's offices, may provide increased opportunities for testing.

## 2. Objectives

We report here the development of a rapid, POC test for the detection of antibodies to hepatitis C virus (HCV) using the OraQuick® rapid test platform, which does not require phlebotomy and can be used with any 1 of 5 different specimen types, including whole blood from finger stick and oral fluid. This test provides a result in 20 minutes and requires no instrumentation. We have evaluated the performance of this test compared to FDA-approved laboratory-based assays currently used for HCV diagnosis.

## 3. Study design

### 3.1. Test device

The OraQuick® HCV assay utilizes an indirect immunoassay method in a lateral flow device to detect antibodies to HCV in blood or oral fluid. In this device, antigens from the core, NS3 and NS4 regions of the HCV genome are immobilized on a single test line on a nitrocellulose strip and antibodies reactive with these antigens are visualized by colloidal gold labeled with protein-A. Oral fluid samples are collected directly on a collection pad protruding from the device, before placing the device in a vial of pre-measured developer solution which transports the sample into the device and allows it to run. Alternatively, finger stick or venous whole blood, serum or plasma are collected using a specimen loop and mixed in the developer solution before inserting the device in the vial. Reactive results generate a reddish-purple line at the test zone. A second control line which detects human IgG ensures that the patient sample has been collected and has migrated beyond the test zone. Devices are interpreted between 20 and 40 minutes.

### 3.2. Specimens and reference methods

Performance of the prototype OraQuick® HCV test was compared to currently approved, laboratory-based tests for HCV (EIA and strip immunoblot assay—RIBA®). Sensitivity and specificity in the OraQuick® HCV test was assessed using serum, plasma, venous blood, finger stick blood, and oral fluid specimens from 122 HCV positive subjects as well as 450 low risk subjects of unknown HCV status. Testing was carried out under informed consent according to protocols approved by the appropriate Institutional Review Board (IRB). Sensitivity to anti-HCV seroconversion was tested in 19 commercially available panels of plasma specimens sequentially collected from individuals undergoing HCV seroconversion following recent infection.

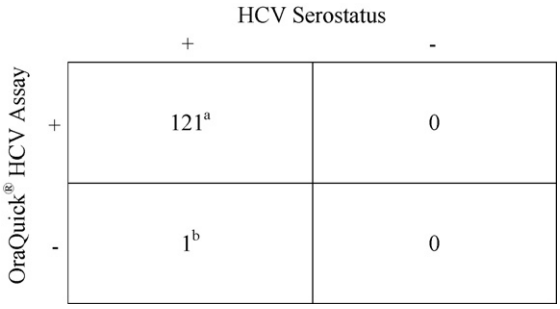


**Fig. 1.** Performance of the OraQuick® rapid HCV test in HCV positive subjects. [a]Subjects gave concordant positive results in all 5 specimen types. [b]One subject was false negative in oral fluid only and was negative when tested for HCV RNA.

## 4. Results

Of the 19 seroconversion series tested, HCV antibody was detected at the same time by both OraQuick® and EIA in 9 cases and earlier by OraQuick® HCV in 10 (Table 1). In no cases did EIA detect antibody before OraQuick®. Mean time to detection of seroconversion (relative to first available specimen in each series) was 61.3 days in the case of EIA and 56.4 days in the case of OraQuick® HCV. Overall, the OraQuick® HCV test detected antibody 4.9 days before EIA (95% CIs: 1.4–8.3).

Sensitivity in human subject testing was evaluated with matched sets of 5 specimen types (oral fluid, venous blood, finger stick blood, plasma, and serum) comparing the result obtained in the OraQuick® HCV test with the HCV serostatus established by FDA-approved laboratory-based HCV testing. Individuals were considered HCV positive if they were EIA and RIBA reactive or EIA and PCR positive. Of the 122 HCV positive subjects tested, 121/122 (99.2%) were detected by the OraQuick® rapid test in all matrices (Fig. 1). One subject was negative by oral fluid, but reactive for the other 4 matrices. Serum from this individual was RIBA (2-band) positive, weakly reactive by EIA (S/C = 2.3) and negative for HCV RNA.

Matched sets of all 5 specimen types prospectively obtained from 450 low risk subjects were also tested with the OraQuick® HCV test. Results obtained compared to laboratory EIA are shown in Fig. 2. In 449/450 cases (99.8%), concordant results were obtained between EIA and the OraQuick® test in all 5 specimen types. One subject gave a false positive result in serum and plasma (RIBA and PCR negative), but was non-reactive in the other matrices. One subject was prospectively identified as HCV positive by both EIA and the POC test and was confirmed positive by RIBA and PCR. Percent sensitivities (99.2–100%) and specificities (99.8–100%) were calculated for the OraQuick® HCV test for each of the 5 matrices with 95% confidence intervals (Fig. 3).

## 5. Discussion

A simple, non-instrumented rapid point-of-care test for HCV may be a useful tool to address under-diagnosis of HCV infection by increasing testing opportunities outside of traditional laboratory settings, such as clinics, community outreach centers and physician offices. This approach has been shown to be beneficial in the

**Table 1**
Comparison of seroconversion sensitivity of the OraQuick® rapid HCV test and EIA.

| Number of panels tested | Panels with concordant results | Panels detected earlier by HCV EIA | Panels detected earlier by OraQuick® | Average time to detection by HCV EIA (days)[a] | Average time to detection by HCV OraQuick® (days)[a] (95% C.I.) | Mean differential sensitivity (days)[a] (95% C.I) |
|---|---|---|---|---|---|---|
| 19 | 9 | 0 | 10 | 61.3 | 56.4 | 4.9 (1.4–8.3) |

[a] Time to detection from first available specimen in each panel.

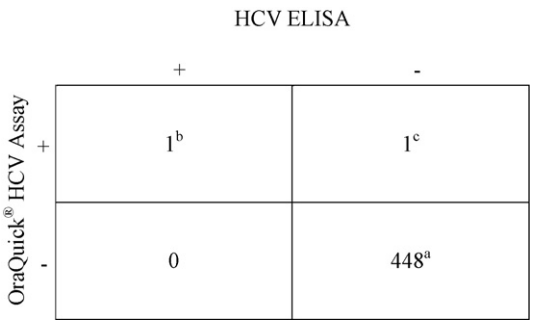


**Fig. 2.** Performance of the OraQuick® rapid HCV test in all 5 specimen types compared to HCV EIA. [a]Subjects concordantly non-reactive in all 5 specimen types. [b]One subject concordant positive in all 5 specimen types (RIBA and RNA positive). [c]One specimen false positive in serum and plasma only.

| Matrix | Specificity[a] | Sensitivity[a] |
|---|---|---|
| Oral Fluid | 100% (99.2 – 100%) | 99.2% (95.5 – 100%) |
| Venous Whole Blood | 100% (99.2 – 100%) | 100% (97.0 – 100%) |
| Fingerstick Blood | 100% (99.2 – 100%) | 100% (97.0 – 100%) |
| Plasma | 99.8% (98.8 – 100%) | 100% (97.0 – 100%) |
| Serum | 99.8% (98.8 – 100%) | 100% (97.0 – 100%) |

**Fig. 3.** Summary of performance of the OraQuick® rapid HCV test in each specimen type. [a]95% confidence intervals in parentheses.

identification of previously undiagnosed HIV infection.[15] However, one of the obstacles to more widespread adoption of rapid testing for infectious diseases has been concerns about clinical performance and test quality.[16] Based on the results presented here, the OraQuick® rapid HCV test appears suitable as an aid in the diagnosis of HCV infection, demonstrating a clinical performance comparable to currently approved laboratory-based tests. Sensitivity in HCV positive individuals was equal to EIA and comparable for all 5 specimen types. The identification of one subject who generated a false negative result in oral fluid may reflect reduced analytical sensitivity in this matrix, although the negative RNA status of this individual was consistent with low antibody titer as a result of previously resolved infection.[17] The occurrence of discordant results between EIA tests in specimens of this type has been reported previously.[18] Overall specificity across all specimen types was comparable to that reported for currently approved laboratory EIAs.[19] Detection of anti-HCV seroconversion in response to infection also compared favorably to performance of currently approved EIAs.[20] These findings are consistent with a previous report indicating that the performance of this test device was significantly improved over the current state of the art for rapid HCV tests and comparable to EIA.[21]

The OraQuick® HCV Rapid Antibody Test appears to provide sensitivity and specificity that is equal to laboratory-based tests, even when antibody levels are low, such as in the early acute phase of disease. Moreover, this platform provides substantial flexibility for rapid testing of subjects, due to its ability to utilize a finger stick blood specimen or an oral swab in addition to the serum and plasma specimens traditionally used in laboratory-based testing. Previous studies have indicated that oral fluid may provide a suitable alternative to blood-based testing for identification of HIV infection.[22] Validation of the suitability of this test device, which is not yet FDA-approved, as an alternative to laboratory EIA for diagnosis of HCV infection, will require prospective testing of at-risk populations in clinical studies.

Despite substantial progress in the development of laboratory-based tests for HCV infection, the majority of HCV cases remain undiagnosed.[9] With the future availability of new therapeutic agents of increased efficacy,[14] identification of chronically infected individuals will be critical to reducing morbidity and mortality associated with the disease.[11] Availability of rapid, point-of-care HCV tests that can be deployed in various clinical settings may increase testing opportunities and therefore enable identification of more patients who could benefit from therapeutic intervention.

## Conflict of interest

Financial relationship with OraSure Technologies, Inc.

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