

Results of a Multi-Center Evaluation of a New Rapid Test for Detection of HCV Infection Using Whole Blood, Serum, Plasma and Oral Fluid

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■ Background

The availability of a highly accurate, rapid, point-of-care (POC) test for hepatitis C virus (HCV) may be useful in addressing the problem of under-diagnosis of HCV, by increasing opportunities for testing outside of traditional laboratory settings. We report here, the development of a rapid, non-instrumented POC test for the detection of antibodies to HCV, which can be used with oral fluid or fingerstick blood specimens in addition to serum, plasma and venous blood sample types. Performance of this test was evaluated in a multi-center study involving prospective testing of subjects at-risk for HCV infection. Performance was compared to CE-approved laboratory EIA.

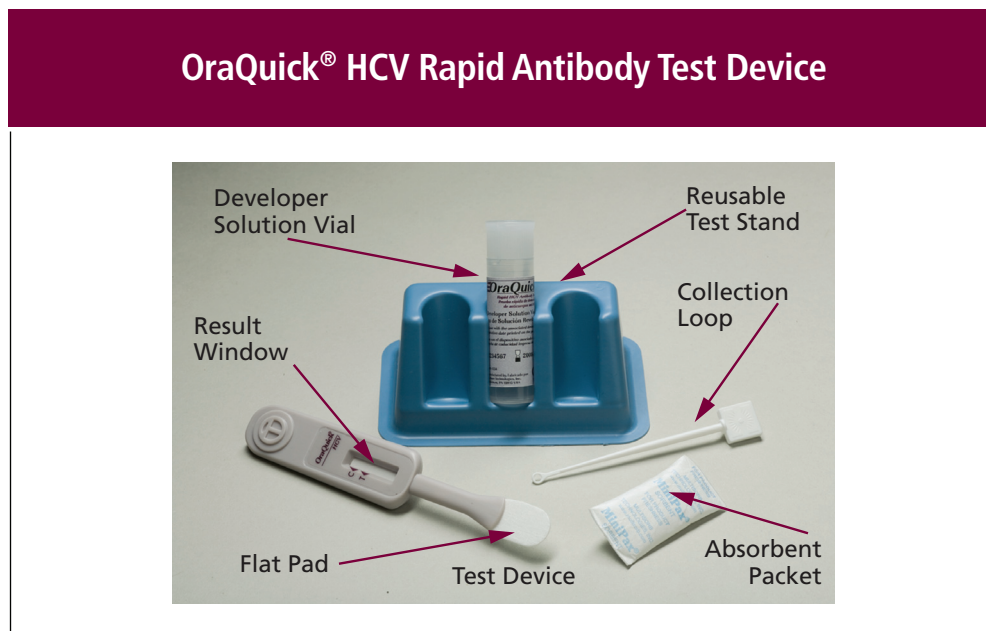


Figure 1

■ Methods

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 (Figure 1), 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, fingerstick 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 patient sample has been collected and has migrated beyond the test zone.

Performance was evaluated by prospective testing of 2206 subjects with signs and/or symptoms of hepatitis, or who were at risk for hepatitis C infection. Each subject was tested by the OraQuick® HCV Rapid Antibody Test using all 5 specimen types. Clinical performance was assessed compared to HCV serostatus established by CE-approved laboratory methods (EIA, RIBA® and PCR). Sensitivity for antibody in early infection was also compared to EIA in 27 seroconversion panels.

■ Results

In total, 2206 subjects at risk for HCV infection or with signs and/or symptoms of hepatitis were enrolled in the trial and classified as to HCV serostatus by CE approved tests. The distribution of HCV risk factors reported for the subject population is shown in Figure 2. The most prevalent risk factors were a history of incarceration, use of intravenous drugs and high risk sexual activity. Of

Hepatitis C Virus Risk Factors of Subjects with HCV EIA and OraQuick® HCV Rapid Test Results		
	Total (n-2206)	
	N*	(%)
HCV Risk Factors		
Had injected intravenous (IV) drugs	823	(37.3)
Born to a HCV positive mother	45	(2.0)
Had sex with a known Hepatitis C (HCV) positive partner	481	(21.8)
Had sex with more than 2 different sexual partners within the preceding 6 months	725	(32.9)
Had sex with an intravenous drug user	948	(43.0)
Currently have or have ever had a sexually transmitted disease	1065	(48.3)
Have been on long-term hemodialysis	41	(1.9)
HIV positive	837	(37.9)
Received a blood transfusion, blood product or organ transplant prior to 1992	224	(10.2)
Have been incarcerated (in jail)	1178	(53.4)

*Most subjects presented multiple risk factors

Figure 2

the 2206 total subjects, 123 (5.6%) had symptoms of hepatitis and 1930 (87.5%) were asymptomatic. There were 153 pregnant women in the test population who were not classified as symptomatic/asymptomatic. Of the 2206 subjects, 2183 were classified as either HCV positive (757, 34.3%) or HCV negative (1426, 64.6%) as a result of laboratory-based testing. A further 23 (1.04%) could not be classified as to HCV status due to an indeterminate RIBA® result and being negative for HCV RNA. These subjects were therefore excluded from the subsequent sensitivity and specificity analyses. Not all subjects had test data for all 5 specimen types with the OraQuick® HCV Rapid Antibody Test, resulting in slight differences in sample size for each specimen type (Figure 3).

Populations for Sensitivity and Specificity Analyses of the OraQuick® HCV Rapid Test

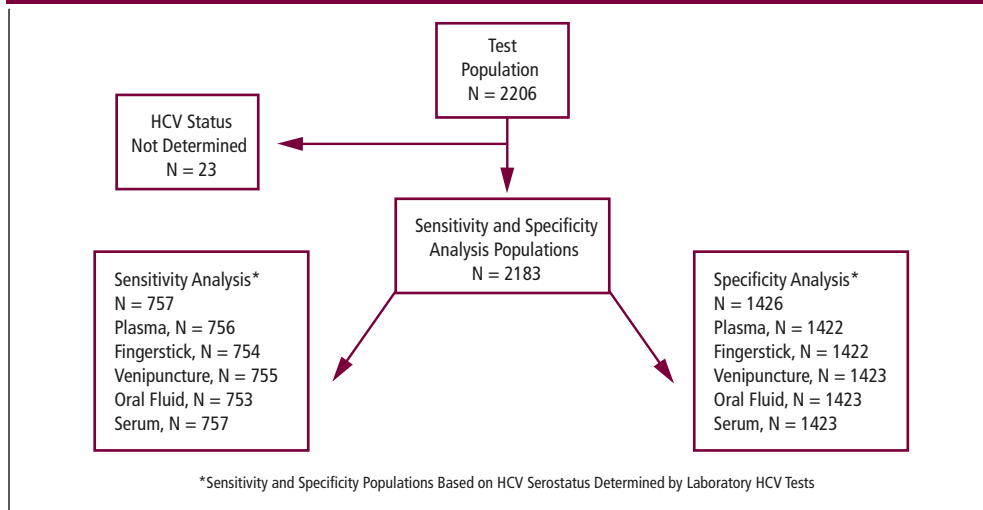


Figure 3

Clinical performance of the OraQuick® HCV Rapid Antibody Test in each specimen type is summarized in Figure 4. Specificities were virtually identical (99.6-99.9%) for all 5 specimen types and the 95% CIs substantially overlapped. Overall sensitivities were virtually identical for venous blood, fingerstick blood, serum and plasma (99.7-99.9%). Observed sensitivity was lower for oral fluid at 98.1% though the upper CI (99.0%) was equal to the lower CIs for venous and fingerstick blood. Significantly, of 12 HCV positive subjects (1.6%) which gave nonreactive results in oral

Sensitivities and Specificities in Each Specimen Type for the OraQuick® HCV Rapid Test

Matrix	Sensitivity ^a		Specificity ^a	
	TP	Proportion (95% CI ^b)	TN	Proportion (95% CI ^b)
Serum	756/757	99.9% (99.3%, 100.0%)	1422/1423	99.9% (99.6%, 100.0%)
Plasma	755/756	99.9% (99.3%, 100.0%)	1420/1422	99.9% (99.5%, 100.0%)
Venipuncture	753/755	99.7% (99.9%, 100.0%)	1421/1423	99.9% (99.5%, 100.0%)
Fingerstick	752/754	99.7% (99.0%, 100.0%)	1421/1422	99.9% (99.6%, 100.0%)
Oral Fluid	739/753	98.1% (96.9%, 99.0%)	1418/1423	99.6% (99.2%, 99.9%)
Abbreviations: TP = true positive; TN = true negative; and CI = confidence interval				
a	Sensitivity and specificity are calculated based on the HCV-infected or not HCV-infected samples with valid OraQuick® Rapid HCV Antibody Test result.			
b	The two-sided 95% exact CI of sensitivity is calculated using the exact method (Clopper-Pearson) by PROC FREQ with options BINOMIAL, EXACT, and ALPHA=0.05.			

Figure 4

fluid alone, only 4 were HCV RNA positive when tested by PCR. Sensitivity for anti-HCV in early seroconversion was essentially the same between the rapid test and EIA (Figure 5).

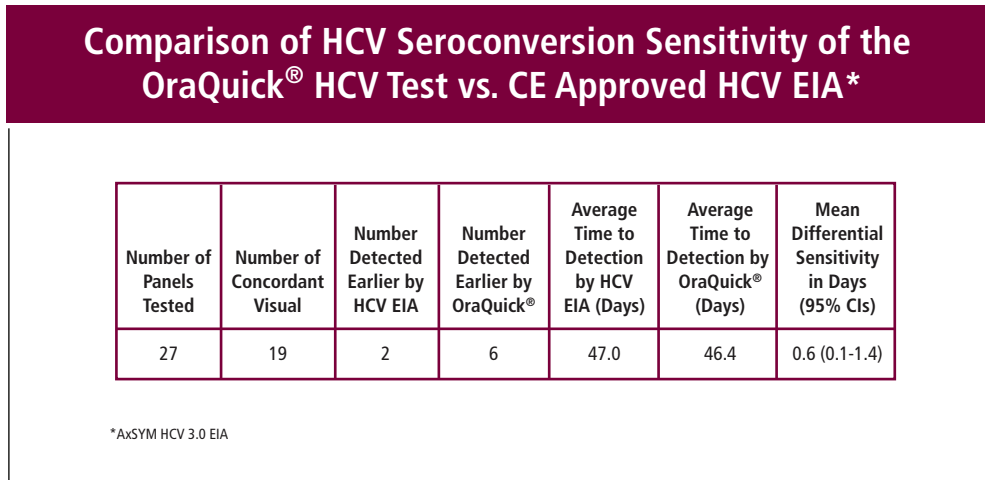


Figure 5

■ Conclusions

- The OraQuick® HCV Rapid Antibody Test demonstrated clinical performance using multiple specimen types that was equivalent to current CE-approved laboratory-based EIA for HCV antibodies.
- Sensitivity was slightly lower with oral fluid than with other specimen types, although the majority of oral fluid negative subjects that were reactive by EIA appeared to have resolved, remote infection.
- Sensitivity for anti-HCV seroconversion with this rapid test was equal to CE-approved laboratory EIA.
- This new, rapid test appears highly suitable as an aid in the diagnosis of HCV infection and may increase testing opportunities due to its portability and ability to use multiple specimen types, including fingerstick blood and oral fluid.



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