Results of a US Multi-Center Evaluation of a New Rapid Test to Aid in the Diagnosis of HCV Infection

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Background

To date, there has been no FDA approved, rapid, point-of-care (POC) test for hepatitis C (HCV) infection. The availability of a highly accurate, rapid POC test for 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 the results of a multi-center evaluation of a new, rapid HCV test which provides results in 20 minutes and which requires no instrumentation.

Methods

The OraQuick® HCV test utilizes an indirect immunoassay method in a lateral flow device to detect antibodies to HCV. 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. Venous whole blood samples were 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 in prospective testing of 1207 subjects with signs and/or symptoms of hepatitis, or who were at risk for hepatitis C. Clinical performance was assessed compared to HCV serostatus established by FDA approved EIA, RIBA® and PCR (Figure 2).
The limit of detection (LoD) of the OraQuick® HCV test was determined by testing diluted HCV positive specimens in both EIA and the rapid HCV test. Reproducibility was assessed using HCV positive specimens (including the LoD specimen) over multiple sites, operators and product lots. Potential interference was investigated using 405 specimens from 16 unrelated disease states and from whole blood containing elevated levels of hemoglobin, lipid, bilirubin and protein.

## Results

In total, 1207 subjects at risk for HCV or with signs and/or symptoms of hepatitis were enrolled in the trial and classified as to HCV serostatus by FDA approved laboratory-based tests. The distribution of HCV risk factors reported for the asymptomatic subject population is shown in Figure 3.

Of the 1207 subjects, 142 (11.8%) had symptoms of hepatitis and 1064 (88.2%) were asymptomatic. There was 1 pregnant woman in the test population who was not classified as symptomatic or asymptomatic. Of the 1207 subjects, 436 were classified as either HCV positive (436, 36.1%) or HCV negative (762, 63.1%) as a result of laboratory-based testing. An additional 9 (0.7%) could not be classified as to HCV status due to an indeterminate RIBA® result and being negative for HCV RNA. Clinical performance of the OraQuick® HCV Rapid Antibody Test is summarized in Figure 4.

Sensitivity and specificity were 99.8% and 100% respectively, when subjects who could not be classified as to HCV status were excluded from the calculations. Assuming a worst case scenario, where “unable to determine” subjects were categorized as false negative or false positive depending on their reactivity in OraQuick®, positive and negative agreement vs. HCV status were 99.5% and 99.0% respectively.

Of the 443 subjects reactive in the OraQuick® HCV Rapid Antibody Test, 94.4% (418/443) were positive by RIBA® and 25 were RIBA indeterminate. Seventeen (17) of the RIBA® indeterminate results were positive for HCV RNA when tested by PCR; therefore, 98.2% (435/443) of the OraQuick® HCV reactives were positive by either RIBA® or PCR (Figure 5).
Analytical sensitivity of the rapid test compared favorably to EIA when diluted HCV positive specimens were tested (Figure 6). The LoD representing the lowest level of antibody detected 95% of the time by the rapid test, corresponded to a 0.75 s/c in EIA (below cutoff).

In a reproducibility study conducted over 5 days, 3 sites, 9 operators and 3 product lots, the LoD specimen was detected 98.7% of the time (Figure 7).

No significant interference was observed in 405 plasma specimens from 16 unrelated disease states or in whole blood containing elevated levels of hemoglobin (500 mg/dL), lipid (3500 mg/dl), bilirubin (10 mg/dL) or protein (12 g/dL).

**Conclusions**

- The OraQuick® HCV Rapid Antibody Test demonstrated clinical performance using venous whole blood that was equivalent to current FDA approved, automated EIA for HCV antibodies.

- Analytical sensitivity was slightly better than the automated EIA.

- The positive predictive value of the rapid test was 98% in this high risk population.

- This new, rapid test appears highly suitable as an aid in the diagnosis of HCV infection and may increase testing opportunities due to its simplicity and portability.

- Full utilization of this technology will require the approval for use with other specimen types, including fingerstick blood and oral fluid.