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Protocols

Evaluation of a new, rapid test for detecting HCV infection, suitable for use with blood or oral fluid

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A B S T R A C T

The availability of a highly accurate, rapid, point-of-care 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 clinical settings. A new HCV rapid test device (OraQuick® HCV Rapid Antibody Test), approved recently in Europe for use with venous blood, fingerstick blood, serum, plasma, or oral fluid was evaluated in a multi-center study and performance compared to established laboratory-based tests for detection of HCV.

The HCV rapid test was evaluated in prospective testing of subjects with signs and/or symptoms of hepatitis, or who were at risk for hepatitis C using all 5 specimen types. Performance was assessed relative to HCV serostatus established by laboratory methods (EIA, RIBA and PCR) approved in Europe for diagnosis of hepatitis C infection. Sensitivity to antibody in early infection was also compared to EIA in 27 seroconversion panels. In addition, the reliability of the oral fluid sample for accurate detection of anti-HCV was assessed by studying the impact of various potentially interfering conditions of oral health, use of oral care products and consumption of food and drink.

In this large study of at-risk and symptomatic persons, the overall specificities of the OraQuick® HCV Rapid Antibody Test were equivalent (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 slightly lower for oral fluid at 98.1% though the upper CI (99.0%) was equal to the lower CI for venous blood and fingerstick blood. Most of the HCV positive subjects which gave nonreactive results in oral fluid had serological and virological results consistent with resolved infection. Sensitivity for anti-HCV in early seroconversion was virtually identical between the HCV rapid test and EIA. Detection of anti-HCV in oral fluid appeared generally robust to conditions of oral health, consumption of food and drink and use of oral care products.

The OraQuick® HCV Rapid Antibody Test demonstrated clinical performance that was equivalent to current laboratory-based EIA. This new, HCV rapid test appears suitable as an aid in the diagnosis of HCV infection and may increase testing opportunities due to its simplicity and flexibility to use multiple specimen types, including fingerstick blood and oral fluid.

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Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; NS3, non structural Protein 3; NS4, non structural Protein 4; IgG, immunoglobulin G; EIA, enzyme immunoassay; RIBA, recombinant immunoblot assay; CI, confidence interval; PCR, polymerase chain reaction; RNA, ribonucleic acid.

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1. Introduction

Since the early 1990s, laboratory-based enzyme immunoassay (EIA) testing for antibodies to hepatitis C virus (HCV) has been the standard for identification of HCV infection in at-risk individuals. These tests have been shown to be highly sensitive for detection of antibodies (Kleinman et al., 1992; Lee et al., 1995) and more recently; EIAs have been developed that are capable of detecting both circulating antibodies and HCV core antigen (Alzahrani, 2008). The deployment of sensitive antibody tests and nucleic acid tests to screen donated blood has all but eliminated transfusion-transmitted infection (Dodd et al., 2002). However, despite this evolution in the performance of laboratory-based tests, there remains a substantial amount of undiagnosed HCV in the general population. Although HCV incidence has declined significantly in developed countries from its peak in the 1970s and 1980s (Wasley et al., 2008), the overall prevalence of undiagnosed infection remains high. It is estimated that approximately 10 million individuals are infected in Europe (World Health Organization, 1999) and over 4 million infected in the United States (Armstrong et al., 2006) with the majority of these infections being undiagnosed (Kwiatkowski et al., 2002). Individuals with chronic infection are at risk for progressive liver disease leading to cirrhosis and/or liver cancer (Davis et al., 2003). It is now recognized that this reservoir of undiagnosed infection represents a growing healthcare burden worldwide as the infected population ages, and HCV-related mortality and morbidity is expected to rise substantially over the coming decades (Shuhart et al., 1997; Kuehn, 2009). Recent developments in therapeutic drugs for treatment of HCV (Thompson and McHutchison, 2009) suggest that future therapies may be more effective and of shorter duration than the current state of the art pegylated interferon and ribavirin therapy (Fried et al., 2002). This may in turn, enable more patients to enter treatment with a greater prospect of achieving sustained viral clearance. The success by which the virus can be eradicated from the infected population will be highly dependent on increasing primary diagnoses, in order to identify individuals who may be suitable candidates for therapeutic intervention.

A simple, non-instrumented, rapid, point-of-care test for HCV may be a useful tool to address the under-diagnosis of HCV infection, by increasing testing opportunities outside of traditional laboratory settings such as clinics and physician offices. This may be particularly true if such tests are easy to use and allow for use of a variety of less invasive specimen types such as oral fluid (Johnston-Roberts et al., 2007). This approach has been shown to be beneficial in identification of previously undiagnosed HIV infection (Zelin et al., 2008) in populations at high-risk. Widespread adoption of rapid HCV tests in primary diagnosis may have been slowed due to concerns about clinical performance and test quality compared to laboratory-based testing (World Health Organization, 2001; Gray, 2004; Gonen and Perry, 2007). In this study, a new, rapid, non-instrumented, point-of-care test for the detection of antibodies to HCV was evaluated, 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 multicenter study involving prospective testing of subjects at-risk for HCV infection.

2. Material and methods

2.1. Test device

The OraQuick® HCV Rapid Antibody Test utilizes an indirect immunoassay method in a lateral flow device, to detect antibodies to HCV. Antigens from the core, NS3, and NS4 regions of the HCV genome are immobilized on a single test line on a nitrocellulose strip contained within the device. Antibodies reactive with these antigens are detected (visualized) using colloidal gold labeled with Protein-A which is dried down on the test strip. Reactive results generate a reddish-purple line at the test zone. Oral fluid samples are collected by swiping the gums with the collection pad protruding from the device. The device is then placed in a vial of pre-measured developer solution which transports the sample into the device and allows it to run. Alternatively, fingerstick, venous whole blood, serum, or plasma is collected using a specimen loop and mixed in the developer solution before inserting the device into the vial. There is also a second control line which detects human IgG and ensures that patient sample has been collected and has migrated beyond the test zone. Devices are interpreted after 20 min.

2.2. Subjects and specimens tested

Subjects at risk for hepatitis C, or who had signs and/or symptoms of hepatitis were tested prospectively at eight separate clinical testing sites using the OraQuick® HCV Rapid Antibody Test. Subjects were recruited from outpatient clinics or physician offices specializing in hepatology, gastroenterology or infectious disease. All subjects included in the study were either symptomatic for hepatitis, or asymptomatic, but with one or more risk factors for HCV infection. Factors considered as putting an individual at risk for HCV infection are defined in Table 1. Each subject was tested using all 5 specimen types; oral fluid, venous blood, fingerstick blood, serum, and plasma in an unblinded fashion. Additionally, the effect of potential interfering factors on test results obtained with oral fluid was also studied at three additional testing centers. Oral fluid samples were collected from approximately 50 HCV negative individuals under various potentially interfering conditions and tested as normal, or using developer solution inoculated with a specimen containing low levels of anti-HCV (the positive kit control). Conditions tested were the presence of gingivitis (bleeding gums), use of dentures, consumption of food or a beverage, use of tobacco products and use of oral care products (tooth brushing, mouthwash and tooth whitening). Subjects who had dentures were instructed to remove them before testing. Subjects enrolled in the gingivitis sub-study had a clinical diagnosis of gingivitis by a physician or dentist. Specimens were tested after 5, 15, or 30 min following exposure to the interfering condition and the wait time required to achieve 100% positive and 100% negative agreement with the expected results was recorded. All testing was carried out according to protocols approved by the appropriate Institutional Review Boards (IRBs), and after informed consent was given by subjects. In a separate laboratory study, sensitivity to anti-HCV seroconversion was assessed by testing 27 commercially available panels of plasma specimens sequentially collected from individuals undergoing anti-HCV seroconversion following HCV infection. Panels tested were from Sera Care Life Science, Milford, MA, USA (panels: 901, 904, 905, 909, 910, 911, 912, 914, 915, 916, 917, 920 and 921), Profile Diagnostics, Clarkson, GA, USA (panels: RP006 and RP038) and Zeptometrix Corporation, Buffalo, NY, USA (panels: 6212, 6213, 6214, 6215, 6222, 6227, 6229, 9041, 9044, 9045, 9046 and 9047).

2.3. Reference methods

Performance of the OraQuick® HCV Rapid Antibody Test was compared to laboratory-based tests approved in Europe for detection of HCV infection. Laboratory tests used were: EIA (Abbott AxSYM® HCV version 3.0, Abbott Laboratories, Abbott Park, IL, USA), Recombinant Strip Immunoblot Assay (RIBA® 3.0; Chiron

Table 1
Hepatitis C virus risk factors in the study population.

HCV risk factors[a]	Total (n = 2206)	
	n	(%)
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 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)

[a] Most individuals had multiple risk factors.

Corporation, Emeryville, CA, USA) and PCR (COBAS® AMPLICOR® Hepatitis C Virus Test v2.0, Roche Molecular Systems, Pleasanton, CA, USA). Sensitivity and specificity of the OraQuick® HCV rapid test was assessed relative to HCV serostatus assigned using these reference methods. Subjects were considered HCV positive if they were EIA reactive and either RIBA positive or indeterminate and PCR positive for HCV RNA. Subjects who were indeterminate in RIBA and PCR negative were considered indeterminate as to HCV status and excluded from sensitivity and specificity calculations.

3. Results

Of the total population of 2206 subjects at risk for HCV or with signs and/or symptoms of hepatitis, 1220 (55.3%) were male and 986 (44.7%) were female. The mean age was 42.7 ± 11.5 years, with ages ranging 10–80 years. African–American subjects comprised 51.3% of the population and Caucasians 34.3%. The distribution of HCV risk factors reported for the study population is shown in Table 1. The most prevalent risk factors were a history of incarceration, use of intravenous drugs and high risk sexual activity. Of the 2206 total subjects, 123 (5.6%) had symptoms of hepatitis and 1930 (87.5%) were asymptomatic. There were 153 pregnant women in the study population who were not classified as either symptomatic or 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 complete data for all five (5) specimen types tested with the OraQuick® HCV Rapid Antibody Test resulting in slight differences in sample size for each specimen type. Sensitivities and specificities obtained in the OraQuick® HCV Rapid Antibody Test along with 95% exact confidence intervals (CIs) for each specimen type are shown in Table 2. Overall specificities were equivalent (99.6–99.9%) for all 5 specimen types and the 95% CIs overlapped substantially. Overall sensitivities were virtually identical for venous blood, fingerstick blood, serum and plasma (99.7–99.9%). Observed sensitivity was slightly lower for oral fluid at 98.1% though the upper CI (99.0%) was equal to the lower CI for venous and fingerstick blood. Of 12 HCV positive subjects (1.6%) who gave nonreactive OraQuick® results in oral fluid alone, only four (4) were positive for HCV RNA when tested by PCR (0.5%). Sensitivities and specificities were not different in symptomatic vs. asymptomatic individuals for any specimen type (data not shown). In a population with high HCV prevalence (34.3%), positive and negative predictive values (PPV and NPV) were extremely high for all various specimen types. In blood derived specimens, PPV ranged from 99.7% (venous blood) to 99.9% (fingerstick blood) and NPV was 99.9% for all 4 specimen types. Positive and negative predictive values were only slightly reduced in oral fluid, with a PPV of 99.3% and NPV of 99.0%.

In the oral interference study (Table 3), no interference was observed with either positive or negative specimens in the presence of gingivitis (bleeding gums) or wearing of dentures (removed before taking the oral fluid sample). Consumption of tobacco and most types of food and drink did not result in interference at the minimum wait time (5 min) tested. A low rate of false positive results were observed at the shortest wait time tested (5 min) after use of mouthwash, tooth whitening, tooth brushing and consumption of acidic beverage (Coca Cola®), although these effects were not observed when the wait time was extended to 15 min (acidic beverage) or 30 min (oral care products).

In the study of sensitivity in early infection, HCV antibody was detected at the same time by both the OraQuick® HCV rapid test and EIA in 19/27 seroconversion series tested (Table 4). The OraQuick® HCV rapid test detected antibody earlier in six cases and EIA detected antibody earlier in two. Overall, the OraQuick® HCV rapid test detected antibody an average of 0.6 days before EIA (95% CIs: 0.1–1.4), and in no series was there a large difference in seroconversion sensitivity between the two tests (maximum 7 days).

Table 2
Sensitivities and specificities of the OraQuick® HCV Rapid Antibody Test in each specimen type.

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; 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 calculated using the exact method (Clopper–Pearson) by PROC FREQ with options BINOMIAL, EXACT, and ALPHA = 0.05.

Table 3
Effect of potentially interfering factors on detection of Anti-HCV in oral fluid.

Condition tested	Sub-category tested	*N*	Wait time (min) before oral fluid collection at which 100% positive and negative agreement was obtained
Gingivitis		20	5[a]
Dentures		45	5[a]
Tobacco	Smokeless tobacco	27	5[a]
	Tobacco (cigarettes)	0	5[a]
Food and drink	Cereal bar	50	5[a]
	High protein shake (8 oz)	50	5[a]
	Coca Cola® (12 oz)	103[b]	15
	Orange juice (4 oz)	51	5[a]
	Whole milk (4 oz)	50	5[a]
	Whiskey	50	5[a]
Oral care products	Tooth brushing (2 min)	103[c]	30
	Mouthwash (30 s)	103[c]	30
	Tooth whitening (30 min)	103[c]	30

[a] 5 min was minimum wait time tested.
[b] 50 subjects tested after 5 min wait period, 53 subjects tested after 15 min wait.
[c] 50 subjects tested after 5 min wait, 53 subjects tested after 30 min wait.

4. Discussion

HCV is prevalent worldwide and the majority of infected persons are, as yet, undiagnosed (World Health Organization, 1999). Deployment of rapid tests outside of traditional laboratory settings has increased identification of HIV infection by enabling greater access to at-risk populations (Zelin et al., 2008). Although this approach has not yet been applied widely for detection of HCV infection, a new point-of-care test for detection of anti-HCV was recently approved in Europe and was evaluated in this study. As with any point-of-care test, demonstration of performance and quality equivalent to laboratory-based testing is critical to support widespread adoption, and earlier evaluations have suggested that the previous state of the art rapid tests remained less sensitive compared to current laboratory EIAs (World Health Organization, 2001; Gonen and Perry, 2007). In this multi-center study of individuals at risk for HCV infection, the OraQuick® HCV Rapid Antibody Test demonstrated clinical performance equivalent to laboratory-based tests across all specimen types for which the test is approved for in Europe. Sensitivity was equal to EIA for venous and fingerstick whole blood as well as serum and plasma. Sensitivity was slightly lower for oral fluid, although the majority of those HCV positive subjects that were nonreactive in oral fluid alone, had undetectable levels of viremia according to HCV PCR. The PCR method used (COBAS® AMPLICOR® Hepatitis C Virus Test v2.0, Roche Molecular Systems, Pleasanton, CA, USA) has an analytical sensitivity claim (at 95% detection) of 20 IU/ml and in previous studies has demonstrated a high concordance with other HCV RNA tests among specimens positive for HCV antibodies (Ross et al., 2001). Undetectable HCV RNA in these oral fluid nonreactive specimens strongly suggest an absence of viremia consistent with resolved infection (Kondili et al., 2002), although extremely low levels of viremia below the limit of detection cannot be ruled out. Previous reports have indicated that specimens with this serological and virological phenotype may generate results that are discordant between HCV EIAs (Myrmel et al., 2005).

The sensitivity of the HCV rapid test was also supported by the laboratory study of seroconversion sensitivity, in which the HCV rapid test detected antibody consistently at the same time as Abbott AxSYM® HCV version 3.0 EIA. This EIA has been used widely in Europe for detection of HCV antibodies (Zachary et al., 2005; Vermeersh et al., 2008) and previously published studies have indicated that it is one of the most sensitive laboratory tests for detection of anti-HCV (Myrmel et al., 2005; Dean and Perry, 2006). The similarity in seroconversion sensitivity between the HCV rapid test and the laboratory EIA is in contrast to previous reports showing rapid tests were in general less sensitive than EIA in early seroconversion (World Health Organization, 2001; Gonen and Perry, 2007).

Specificity of the HCV rapid test with all specimen types compared favorably with that reported for anti-HCV EIA (Zachary et al., 2005). The results obtained in this large, multi-center prospective study, are consistent with previous reports indicating that the performance of this HCV rapid test device improved significantly over the previous state of the art for HCV rapid tests (O'Connel et al., 2008; Lee et al., 2010). In this population of high prevalence for HCV antibodies (34%), positive and negative predictive values (>99%) were extremely high for the HCV rapid test, reflecting the high sensitivity and specificity compared to established laboratory methods for identifying HCV infection. Additional studies would be required to assess performance in lower prevalence settings.

Use of the OraQuick® HCV Rapid Antibody Test may enable testing in a broader range of clinical and non-clinical settings, due to the lack of requirement for phlebotomy or instrumentation and the ability to utilize fingerstick blood or oral swab specimens. Previous studies have indicated that oral fluid may provide a suitable alternative to blood-based rapid testing for identification of HIV infection (Johnston-Roberts et al., 2007). Moreover, oral fluid has been used successfully for detection of anti-HCV using EIAs (Bello et al., 1998; De Cock et al., 2004). The clinical performance observed in this study support the use of rapid HCV testing by oral fluid swab, as a convenient non-invasive alternative to blood specimens. Abil-

Table 4
Comparison of HCV seroconversion sensitivity of the OraQuick® HCV Test vs. current laboratory EIA[a].

Number of panels tested	Number of concordant visual	Number detected earlier by HCV EIA	Number detected earlier by OraQuick®	Average time to detection by HCV EIA (Days)	Average time to detection by HCV OraQuick® (Days)	Mean differential sensitivity (Days)
27	19	2	6	47.0	46.4	0.6 (0.1–1.4)

[a] AxSYM® HCV 3.0 EIA.

ity to detect anti-HCV in oral fluid did not appear to be affected by conditions of oral health, and relatively rapid clearing time (15 min) after which no interference was observed after consumption of food and drink, appear compatible with routine use.

This HCV rapid test was approved recently in Europe for all five specimen types used in this study, to provide presumptive evidence of HCV infection in individuals symptomatic for hepatitis, or who may present with risk factors for the HCV infection. However, it should be noted that as is the case for laboratory-based tests for antibodies to HCV, follow-up testing, including use of RNA tests, will be required to determine the actual state of HCV infection in these individuals (Pirisia et al., 1998; Dufour et al., 2003).

Availability of easy to use, high quality, point-of care tests, may increase testing opportunities for physicians and therefore enable identification of more patients who could benefit from therapeutic intervention. This in turn, may help to reduce the future morbidity and associated healthcare burden caused by progression of liver disease in individuals with as yet, unidentified HCV infection.

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