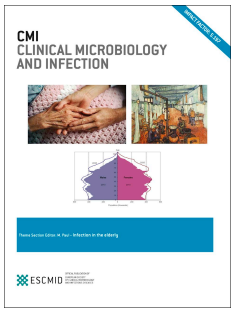
Accepted Manuscript



Prospective assessment of rapid diagnostic tests for the detection of antibodies to hepatitis C virus, a tool for improving access to care

Stéphane Chevaliez, Lila Poiteau, Isabelle Rosa, Alexandre Soulier, Françoise Roudot-Thoraval, Syria Laperche, Christophe Hézode, Jean-Michel Pawlotsky

PII: S1198-743X(16)00025-2

DOI: 10.1016/j.cmi.2016.01.009

Reference: CMI 502

To appear in: *Clinical Microbiology and Infection*

Received Date: 8 October 2015

Revised Date: 23 December 2015

Accepted Date: 6 January 2016

Please cite this article as: Chevaliez S, Poiteau L, Rosa I, Soulier A, Roudot-Thoraval F, Laperche S, Hézode C, Pawlotsky J-M, Prospective assessment of rapid diagnostic tests for the detection of antibodies to hepatitis C virus, a tool for improving access to care, *Clinical Microbiology and Infection* (2016), doi: 10.1016/j.cmi.2016.01.009.

Prospective assessment of rapid diagnostic tests for the detection of antibodies to hepatitis C virus, a tool for improving access to care

Stéphane Chevaliez[1,2], Lila Poiteau[1,2], Isabelle Rosa[3], Alexandre Soulier[1,2], Françoise Roudot-Thoraval[2,4], Syria Laperche[5], Christophe Hézode,[2,6] and Jean-Michel Pawlotsky[1,2]

[1]National Reference Center for Viral Hepatitis B, C and delta, Department of Virology, Hôpital Henri Mondor, Université Paris-Est, Créteil, France; [2]INSERM U955, Créteil, France, [3]Department of Hepatology and Gastroenterology, Centre Inter-communal de Créteil, Créteil, France; [4]Department of Public Health, Hôpital Henri Mondor, Université Paris-Est, Créteil, France; [5]National Reference Center for Viral Hepatitis B, C and delta in Blood Transfusion, Institut National de la Transfusion Sanguine, Paris, France; [6]Department of Hepatology and Gastroenterology, Hôpital Henri Mondor, Université Paris-Est, Créteil, France

Keywords: Hepatitis C, Rapid diagnostic test, screening, anti-HCV antibodies, capillary whole blood, crevicular fluid

Running title: Rapid diagnostic tests for hepatitis C

Correspondent author: Stéphane Chevaliez, Department of Virology, Hôpital Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France. Tel: +33-1-4981-2828; Fax: +33-1-4981-2839. E-mail: stephane.chevaliez@aphp.fr

ABSTRACT

Large-scale hepatitis C screening is required to prevent further spread of the infection, improve access to care in the context of new HCV drug regimens without interferon alpha and subsequently reduce the risk of long-term complications of chronic liver disease. Rapid diagnostic tests (RDTs) represent an attractive alternative to enzyme immunoassay using blood from venipuncture. The aim of the present study was to prospectively assess the clinical performance of CE-marked RDTs detecting anti-HCV antibodies in fingerstick capillary whole blood and/or oral fluid.

A total of 513 individuals, including 318 patients with chronic HCV infection, 25 patients with resolved HCV infection and 170 HCV-seronegative individuals, were prospectively enrolled.

The specificity of RDTs with fingerstick whole blood varied from 98.8% to 100%. The clinical sensitivity was high for the OraQuick® and Toyo® tests (99.4% and 95.8%, respectively), but low for the Labmen® test (63.1%). The specificity and clinical sensitivity in crevicular fluid were both satisfactory for the OraQuick® test (100% and 97.6%, respectively).

HCV antibody RDTs were easy and rapid to perform in the context of patient care. They were highly specific. Both the OraQuick® and Toyo® tests reached the expected level of performance for broad-scale use, with a performance advantage for the OraQuick® HCV test. RDTs appears as a promising new tool for broad-scale screening of HCV infection in high- to medium-risk populations. Thus, careful assessment of the performance of HCV RDTs must be recommended before they can be implemented in clinical practice.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma, and the main indication for liver transplantation in industrialized countries [1]. HCV infection is also highly prevalent, and responsible for an important burden of disease, in low- and middle-income areas. The complications of HCV infection represent the 10th most frequent cause of death of infectious origin, with approximately 350,000 deaths per year worldwide [2].

Because chronic hepatitis C is often asymptomatic until advanced stages of liver disease develop, up to approximately 60% of infected patients are unaware of their infection and related liver disease in industrialized areas [3, 4]. In low- to middle-income areas, the vast majority of infected patients have not been diagnosed. Because chronic HCV infection is curable and the virological results of therapy have dramatically improved recently due to the arrival of new direct-acting antiviral drugs [5, 6], large-scale screening is mandatory to identify infected patients and provide them with efficient therapies.

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). Rapid diagnostic tests (RDTs) represent an attractive alternative for HCV screening and diagnosis because they use various matrices, including serum, plasma, but also fingerstick capillary whole blood or oral fluid. RDTs offer the advantage of simplicity, limited need for instrumentation, minimal training required, and rapid performance at room temperature.

The aim of the present study was to prospectively assess the clinical performance of different CE-marked RDTs detecting anti-HCV antibodies in fingerstick capillary whole blood and/or oral fluid in a large cohort of HCV-infected and uninfected individuals.

MATERIELS AND METHODS

Patients

A total of 531 consecutive subjects were prospectively recruited between September 2012 and November 2013 in the Departments of Hepatology and Gastroenterology of Henri Mondor university hospital and "Centre Hospitalier Intercommunal de Créteil". The inclusion criteria were the following: 18 years or older, seronegative or seropositive for HCV infection by EIA, no hepatitis B virus (HBV) or human immunodeficiency virus (HIV) coinfection.

Group A comprised patients with chronic HCV infection, all of whom had detectable anti-HCV antibodies and quantifiable HCV RNA. Group B comprised 25 patients with resolved infection, including 12 patients who spontaneously cleared HCV infection after acute infection and 13 patients who cleared infection after antiviral therapy. Group C comprised 170 HCV-seronegative individuals. The study followed the principles of Good Clinical Practice and was approved by the local ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale) in accordance with the Helsinki Declaration. All patients gave their written informed consent.

Performance of the study

During their medical visit, each patient from group A, B or C had the RDTs performed on different matrix specimens, including fingerstick whole blood for all RDTs and crevicular fluid for one of them, by a dedicated nurse in the outpatient department. The RDT was initially performed with crevicular fluid and then additional crevicular fluid was collected 10-15 minutes later for one assay. Fingerstick whole blood was subsequently tested with this RDT. In parallel, serum was collected from venous puncture to determine

the patient's HCV status by means of standardized methods (EIA for anti-HCV antibodies and real-time PCR for HCV RNA). The RDT readout and interpretation were performed by the nurse, at the site of patient care. Another RDT readout was performed by an independent reader in the central laboratory.

Laboratory measurements

Total anti-HCV antibodies were sought in blood by means of an automated third-generation EIA assay (aHCV VITROS ECi™, Ortho-Clinical diagnostics, Raritan, New Jersey). Crevicular fluids were collected by means of the Intercept® Oral Specimen Collection Device (OraSure Technologies, Inc, Bethlehem, Pennsylvania) and anti-HCV antibodies were sought by means of the Ortho® HCV 3.0 ELISA Test System with enhanced SAVe (Ortho-Clinical Diagnostics), according to the manufacturer's instructions. When discrepant results were observed, additional serological tests were performed, including the HCV Ab PLUS Access EIA test (Bio-Rad, Marnes-la-Coquette, France) and a third-generation recombinant immunoblot assay (INNO-LIA™ HCV Score, Innogenetics, Ghent, Belgium), when needed.

Serum HCV RNA levels were measured by means of a real-time PCR assay (*m*2000, Abbott Diagnostics, Chicago, Illinois).

The HCV genotype was determined in all patients from group A by directly sequencing a portion of the NS5B gene encoding the RNA-dependent RNA polymerase, as previously described [7].

The HBV and HIV status were determined by means of commercial EIAs using the VITROS ECi/ECiQ immunodiagnostic system (Ortho-Clinical Diagnostics) and the Architect automated device (HIV Ag/Ab Combo, Abbott Diagnostic, Chicago, Illinois).

RDTs for anti-HCV antibody detection

Three CE-marked RDTs were evaluated for anti-HCV antibody detection in capillary whole blood (all tests) and in crevicular fluid (one test); they included OraQuick® HCV Rapid Antibody Test (OraSure Technologies, Bethlehem, Pennsylvania), Toyo® anti-HCV test (Türklab Medical Devices, Izmir, Turkey), and Labmen® HCV test (Türklab Medical Devices). Capillary whole blood was collected from a finger using a sterile, disposable Haemolance® Plus High Flow lancet (HaeMedic, Frankfurt-am-Main, Germany). Crevicular fluid was collected on the OraQuick® HCV Rapid Antibody Test. All RDTs were performed according to the manufacturer's instructions.

Statistical analysis

Performance of the RDTs was assessed using the EIA result in serum as the reference. Receiver operating characteristics (ROC) curve analysis was used to estimate the threshold value of the EIA assay for detection of anti-HCV antibodies in crevicular fluid. Confidence intervals were calculated by the Wilson score method. Statistical analysis was performed with Stata® 10.0 (StataCorp LP, College Station, Texas). p values <0.05 were considered as statistically significant.

RESULTS

Characteristics of the study population

Eighteen of the 531 initially screened individuals were excluded because they did not meet the inclusion criteria; they included 16 HBsAg-positive patients, 2 HIV-seropositive patients. As a result, 513 subjects were prospectively included in the analysis.

Table 1 shows the baseline characteristics of the analyzed population. Group A comprised 318 patients with chronic HCV infection (63.5% were males and the median age was 56 years). All of them had detectable anti-HCV antibodies (mean signal-to-cutoff value: 27.0±4.3; range 8.8-36.0) and HCV RNA (mean HCV RNA level: 5.8±0.8 Log IU/mL; range: 1.8-7.5). Most patients (94.3%) had never received anti-HCV therapy. Among the patients from group A, 60.4% were infected with HCV genotype 1.

Group B comprised 25 patients with resolved HCV infection. All of them had detectable anti-HCV antibodies (mean signal-to-cutoff value: 27.8±5.3; range: 11.5-34) and HCV RNA below the lower limit of detection. Their mean age was 62 years and 56% were male.

Group C comprised 170 HCV-seronegative individuals. Their median age was 41 years and 40.6% were males. Gender was the only baseline characteristic that statistically differed across the 3 groups ($p<0.001$). Age was the only baseline characteristic that statistically differed across HCV-seronegative and –seropositive subjects ($p<0.001$).

All of the 513 included patients' whole blood and crevicular fluid specimens were tested with the OraQuick® HCV Rapid Antibody test, whereas capillary whole blood was tested in 502 of them with the Toyo® anti-HCV test. In contrast, only 243 capillary whole blood specimens could be tested with Labmen® HCV test (177 from Group A, 2 from group B and 64 from Group C), because this test was made available only 6 months after the start of this prospective study.

Clinical performance of anti-HCV RDTs in fingerstick whole blood

Sensitivity and specificity

As shown in Table 2, the 3 RDTs had high specificity (98.8%-100%). Only 2 HCV-seronegative subjects from group C tested anti-HCV antibody-positive with the Toyo® anti-

HCV Test. Frozen plasma from these two individuals was then retested with the same RDT and the result was negative in both cases.

The clinical sensitivity, as compared to EIA in serum, was high for OraQuick® anti-HCV Rapid Test (99.4%; 95%CI: 97.9%-99.8%) and Toyo® anti-HCV Test (95.8%: 95%CI: 93.0%-97.5%). In contrast, the clinical sensitivity of Labmen® HCV test was low (63.1%: 95%CI: 55.8%-69.8%).

Supplementary Table S1 shows the virological characteristics of the samples that tested negative in RDTs in spite of being anti-HCV antibody-positive in serum by EIA. One patient infected with genotype 4f was anti-HCV antibody-negative in the three RDTs in spite of a high HCV RNA level (6.1 Log IU/mL) and a high signal-to-cutoff value in serum with two EIAs (VITROS ECi and Access®). Another patient with detectable anti-HCV antibodies in EIA was found negative with the OraQuick® test. Frozen plasma from both patients were retested and found positive in the corresponding RDTs in all instances. The INNO-LIA Score test was positive in both cases, with strong reactivity against core and NS3 proteins (Table S2).

Twelve patients with chronic hepatitis C from group A and 2 patients with resolved infection from group B had a negative result with the Toyo® anti-HCV Test (Supplementary Table S1). Twelve of them had a high signal-to-cutoff value in both EIAs in serum. Among patients with chronic hepatitis C from group A, 11 had a high HCV RNA level. Frozen plasma from all of them was retested with Toyo® anti-HCV Test. Twelve tested positive on retesting and all of them were positive in INNO-LIA (Supplementary Table S2). In the remaining two patients (Pt-5 and Pt-7 from group A), the Toyo® test tested negative again; they included a genotype 2l-infected patient with a high signal-to-cutoff value in VITROS ECi but a low one in Access® and a high HCV RNA level (6.4 Log IU/mL), and one patient with low signal-to-cutoff values in both EIAs and a low HCV RNA level (2.6 Log IU/mL), in

whom the HCV genotype could not be determined (Supplementary Table S1). The INNO-LIA Score test was positive for core only in the first patient and for NS3 and NS5A in the second one (Supplementary Table S2).

Finally, among the 179 HCV-positive patients tested with Labmen® HCV Test, anti-HCV antibodies were not detected by the RDT in 66 cases (36.9%). All except one had a high signal-to-cutoff value for anti-HCV antibody detection in serum by EIA (on average 25.6 and 8.3 with VITROS Labmen® HCV Test ECi and Access, respectively) and quantifiable HCV RNA (mean HCV RNA level: 6.0±0.6 Log IU/mL; range: 4.5-7.5). Supplementary Table S1 shows their individual characteristics. Frozen plasma from all of them was retested with Labmen® HCV Test and was found positive in only 30 cases (45.4%).

Overall, no demographic or virological parameter, such as the age, gender, HCV genotype or HCV RNA level, were found to be predictive of a falsely negative or positive result with any of the RDTs tested.

Indeterminate results

The rate of success of the 3 RDTs was high (OraQuick®: 99.8%; Toyo®: 98.2%; Labmen®: 99.2%). Only one specimen from an HCV-positive patient gave an indeterminate result with the OraQuick® rapid test. This specimen was from a genotype 1a-infected patient with an HCV RNA level of 5.3 Log IU/mL and a high signal-to-cutoff value for anti-HCV antibody detection in serum by EIA (23.0 and 8.6 with VITROS ECi and Access, respectively). In this patient, the INNO-LIA Score test was positive with strong reactivity against all HCV proteins except NS5A (Supplementary Table S2).

Nine specimens from 2 HCV-negative patients and 7 HCV-positive patients gave an indeterminate result with Toyo® anti-HCV Test. The 7 HCV-infected patients, including 5 from group A and 2 from group B, had high signal-to-cutoff values for anti-HCV antibody

detection in serum by EIA (ranges: 24.5-33.8 and 9.3-11.2 with VITROS ECi and Access, respectively), whereas the 5 patients from group A had high HCV RNA levels ranging from 5.0 to 7.5 Log IU/mL. The INNO-LIA Score test was positive in all cases, with at least a strong reactivity against core and NS3 proteins (Supplementary Table S2).

Only one specimen from an HCV-seronegative patient gave an indeterminate result with the Labmen® rapid test.

Frozen plasma from all patients with an indeterminate RDT result were retested with the corresponding RDTs and the result confirmed EIA in serum in all cases.

Clinical performance of an anti-HCV RDT in oral fluid

Specificity, sensitivity

The specificity of the OraQuick® HCV Rapid Antibody Test on this matrix was 100% (95%CI: 97.9%-100%). Its clinical sensitivity was 97.6% (95%CI: 95.4%-98.8%) (Table 2). A negative result with OraQuick® in crevicular fluid was observed in only 6 patients with chronic hepatitis C and 2 with resolved infection. No demographic or virological parameter predicted the false-negative result. All 8 patients had a high signal-to-cutoff value with both EIA assays in serum and 5 of 6 patients with chronic infection had an HCV RNA level higher than 5.2 Log IU/mL (Supplementary Table S3). The INNO-LIA Score test was positive in all cases, with a strong reactivity against at least the core and NS3 proteins.

In order to assess whether the false negative results could be explained by the absence of anti-HCV antibodies in crevicular fluid, crevicular fluid was tested with the EIA-based Ortho HCV 3.0 Test System with Enhanced SAVe in all HCV-infected subjects from groups A and B and in HCV-seronegative subjects from group C, and the results were expressed as optical densities (Figure 1). Among the 511 crevicular fluid specimens tested, the mean±standard error to the mean (SEM) optical densities were 1.2470±0.0513 and

0.0073±0.0021 in anti-HCV positive (groups A and B) and -negative (group C) patients, respectively (p<0.0001). Based on ROC curve analysis, the optimal optical density cutoff value splitting HCV-positive from -negative patients was 0.0195 (Figure 1). This cutoff value was associated with a sensitivity of 97.1% (95%CI: 94.7%-98.6%) and a specificity of 97.1% (95%CI: 93.3%-99.0%). Ten crevicular fluid specimens yielded a falsely negative EIA result with a mean optical density of 0.0074±0.0057 (Figure 1), including nine collected from patients with chronic hepatitis C and one from a patient with resolved infection. Among the 7 patients with available oral specimens and a falsely positive result as compared with serum, 5 had anti-HCV antibodies in crevicular fluid (Supplementary Table S3).

Indeterminate results

Only five HCV-positive patients from group A had an indeterminate result of the Oraquick® test in crevicular fluid. Their HCV RNA levels ranged from 1.8 to 6.9 Log IU/mL and the signal-to-cutoff EIA values in serum were high in all instances (range: 17.0-29.9 and 7.1-11.6 in VITROS ECi and Access, respectively). All of them had a positive detection of anti-HCV antibodies with the RDTs in capillary whole blood. The INNO-LIA Score test was positive in all cases, with strong reactivity against core and NS3 proteins (Supplementary Table S2).

DISCUSSION

Large-scale HCV screening is now required to prevent further spread of the infection, improve access to care in the context of new, potent and well-tolerated HCV drug regimens without interferon alpha [5, 6, 8], and subsequently reduce the risk of long-term complications of chronic liver disease. In this context, rapid diagnostic tests performed

from capillary whole blood or crevicular fluid, that can be performed at the site of patient care without the need for a virology laboratory, represent an interesting alternative to classical virological tests performed from serum or plasma obtained by venipuncture.

A recently published meta-analysis suggested that anti-HCV antibodies can be reliably sought by means of RDTs [9]. The results of this prospective study show that the OraQuick® and Toyo® RDTs display satisfactory clinical performance for the detection of anti-HCV antibodies in fingerstick capillary whole blood and, in the case of the OraQuick® test, in crevicular fluid. In contrast, the performance of the Labmen® RDT did not reach the level required by the Medical Devices and in Vitro Diagnostic Directive of the European Union, nor complied with the WHO performance acceptance criteria. These findings emphasize the need for proper evaluation of RDT performance prior to their broad use for screening and diagnosis of HCV infection.

In our experience, the OraQuick® HCV Rapid Antibody Test had the best performance for anti-HCV antibody detection in both capillary whole blood and crevicular fluid. The high positive and low negative likelihood ratios found with the OraQuick® and Toyo® rapid tests indicate that they can both meaningfully inform the probability of infection. Given the convenience of RDTs and their rapid turnaround time, these results illustrate their potential for expanded first-line screening of HCV infection and demonstrate the utility of capillary whole blood or crevicular fluid for large-scale screening of at-risk populations.

False-negative results are a particular concern in large-scale screening, because they can lead to a substantial number of missed cases. Surprisingly, the number of false-negative results was higher with the Toyo® RDT in fingerstick whole blood than with the OraQuick® test in oral fluid, although a slightly higher false-negative result rate was observed in crevicular fluid than in fingerstick whole blood with this test. These results are explained

by the high quality of oral fluid collection, and by the fact that oral fluid contains at least 5 times lower concentrations of antibodies than blood [10]. They are in keeping with the reported high sensitivity of anti-HCV antibody detection in oral fluid in HCV RNA-positive patients [11]. No specific anti-HCV antibody profile, as assessed by the INNO-LIA Score test, explained the false-negative results, whatever the matrix and rapid test used. Indeed, antibodies against two major epitopes in the core and NS3 proteins were found by this immunoblot-based assay in the vast majority of patients with a false-negative result of an RDT. Interestingly, the isolated presence of anti-core or anti-NS3 antibodies appeared to be sufficient for the OraQuick® test to be positive in fingerstick whole blood.

Our study has some limitations. First, it was performed in a population of patients with diagnosed chronic HCV infection. Whether similar performance can be obtained when testing low-risk populations remains to be determined. Secondly, no acute HCV infection cases were included. Thirdly, the number of clinical specimen tested with the Labmen test was lower. Finally, the performance of RDTs in HIV-coinfected patients was not tested. RDTs may indeed be less sensitive in such patients, as already suggested [12-14], due to a lower amount of immunoglobulins in oral fluid, particularly in patients with residual HIV replication [15].

In conclusion, our prospective study, based on a large number of well-characterized non-infected individuals and patients with chronic HCV infection related to different genotypes, showed that anti-HCV antibodies can be easily and reliably detected by RDTs. These tests are easy and rapid to perform in the context of patient care, and they are highly specific. Both OraQuick® HCV Rapid Antibody Test and Toyo® anti-HCV test reached the expected level of performance for broad-scale use, with a performance advantage for the OraQuick® HCV test. RDT detection of anti-HCV antibodies in fingerstick whole blood or crevicular fluid thus appears as a promising new tool for broad-scale screening of HCV

infection in high- to medium-risk populations, provided that the tests used have been properly evaluated and have demonstrated appropriate performance. Thus, careful assessment of the performance of HCV RDTs must be recommended before they can be implemented in clinical practice.

ACKNOWLEDGEMENTS

The authors are grateful to Elena Braychenko for technical assistance.

TRANSPARENCY DECLARATION

None to disclose in relation to this study

EXTERNAL FUNDING

This work was supported by the National Agency for Research on AIDS and Viral Hepatitis (ANRS), the French Ministry of Health and Janssen Pharmaceuticals, Inc. Virological tests and/or reagents have been kindly provided by Abbott Molecular, Roche Diagnostics, OraSure and Turklab.

SUPPORTING INFORMATION

Supplementary Table S1. Virological characteristics of the patients with a false-negative result in the 3 RDTs in fingerstick whole blood.

Supplementary Table S2. Results of INNO-LIA™ HCV Score test. The number of (+) indicates the intensity of the bands, according to the manufacturer's reading instructions.

Supplementary Table S3: Virological characteristics of the patients with a false-negative result with the OraQuick® RDT in oral fluid.

REFERENCES

1 Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis b virus and hepatitis c virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol*. 2006; **45**: 529-538.

2 Hatzakis A, Wait S, Bruix J, et al. The state of hepatitis b and c in europe: Report from the hepatitis b and c summit conference*. *J Viral Hepat*. 2011; **18 Suppl 1**: 1-16.

3 Denniston MM, Klevens RM, McQuillan GM, Jiles RB. Awareness of infection, knowledge of hepatitis c, and medical follow-up among individuals testing positive for hepatitis c: National health and nutrition examination survey 2001-2008. *Hepatology*. 2012; **55**: 1652-1661.

4 Meffre C, Le Strat Y, Delarocque-Astagneau E, et al. Prevalence of hepatitis b and hepatitis c virus infections in france in 2004: Social factors are important predictors after adjusting for known risk factors. *J Med Virol*. 2010; **82**: 546-555.

5 Pawlotsky JM. New hepatitis c therapies: The toolbox, strategies, and challenges. *Gastroenterology*. 2014; **146**: 1176-1192.

6 Pawlotsky JM. Hepatitis c treatment: The data flood goes on: An update from the liver meeting 2014. *Gastroenterology*. 2015.

7 Bronowicki JP, Ouzan D, Asselah T, et al. Effect of ribavirin in genotype 1 patients with hepatitis c responding to pegylated interferon alfa-2a plus ribavirin. *Gastroenterology*. 2006; **131**: 1040-1048.

8 Easl clinical practice guidelines: Management of hepatitis c virus infection. *J Hepatol*. 2014; **60**: 392-420.

9 Shivkumar S, Peeling R, Jafari Y, Joseph L, Pant Pai N. Accuracy of rapid and point-of-care screening tests for hepatitis c: A systematic review and meta-analysis. *Ann Intern Med*. 2012; **157**: 558-566.

10 McKie A, Vyse A, Maple C. Novel methods for the detection of microbial antibodies in oral fluid. *Lancet Infect Dis*. 2002; **2**: 18-24.

11 Cock De L, Hutse V, Vranckx R. Correlation between detection of antibodies against hepatitis c virus in oral fluid and hepatitis c virus rna in serum. *Eur J Clin Microbiol Infect Dis*. 2005; **24**: 566-568.

12 Jewett A, Smith BD, Garfein RS, Cuevas-Mota J, Teshale EH, Weinbaum CM. Field-based performance of three pre-market rapid hepatitis c virus antibody assays in stahr (study to assess hepatitis c risk) among young adults who inject drugs in san diego, ca. *J Clin Virol*. 2012; **54**: 213-217.

13 Larrat S, Bourdon C, Baccard M, et al. Performance of an antigen-antibody combined assay for hepatitis c virus testing without venipuncture. *J Clin Virol*. 2012; **55**: 220-225.

14 Smith BD, Teshale E, Jewett A, et al. Performance of premarket rapid hepatitis c virus antibody assays in 4 national human immunodeficiency virus behavioral surveillance system sites. *Clin Infect Dis*. 2011; **53**: 780-786.

15 Visseaux B, Larrouy L, Calin R, et al. Anti-hepatitis c virus antibody detection in oral fluid: Influence of human immunodeficiency virus co-infection. *J Clin Virol*. 2013; **58**: 385-390.

FIGURE LEGENDS

Figure 1. Dot-plot of optical density values assessed with the third-generation EIA assay (Ortho® HCV 3.0 ELISA Test System with enhanced SAVe, Ortho-Clinical Diagnostics) in crevicular fluid according to the HCV status: HCV-seropositive patients (groups A and B) and HCV-seronegative individuals (group C).

Table 1. Demographic and virological characteristics of the study population, including subjects with chronic HCV infection (Group A), subjects seropositive for HCV with resolved infection (Group B) and subjects seronegative for HCV (Group C).

	Group A (n=318)	Group B (n=25)	Group C (n=170)
Age, median (year, range)	56 (27-87)	62 (28-73)	41 (18-80)
Male gender, n (%)	202 (63.5)	14 (56.0)	69 (40.6)
Treatment-naïve, n (%)	300 (94.3)	12 (48.0)	NA
Anti-HCV antibodies signal/cutoff (mean±SD)	27.0±4.3	27.8±5.3	0.1±0.1
HCV RNA level, mean±SD [Log IU/mL]	5.80±0.80	<1.10	<1.10
HCV RNA > 800,000 IU/mL, n (%)	156 (49.1)	0	0
HCV genotype, n (%)			
1	189 (60.4)	NA	NA
2	16 (5.1)	NA	NA
3a	40 (12.8)	NA	NA
4	61 (19.5)	NA	NA
5a	3 (0.9)	NA	NA
6	4 (1.3)	NA	NA

NA: not applicable

Table 2. Performance of anti-HCV antibody RDTs with fingerstick whole blood or oral fluid, using the EIA result in serum as the reference

	Specificity (95%CI)	Sensitivity (95%CI)	LR+	LR-
Fingerstick whole blood				
OraQuick® HCV Rapid Antibody Test	100% (97.9%-100%)	99.4% (97.9%-99.8%)	∞	0.006
Toyo® anti-HCV test	98.8% (95.8%-99.7%)	95.8% (93.0%-97.5%)	78.50	0.043
Labmen® HCV test	100% (94.4%-100%)	63.1% (55.8%-69.8%)	∞	0.369
Oral fluid				
OraQuick® HCV Rapid Antibody Test	100% (97.9%-100%)	97.6 (95.4%-98.8%)	∞	0.024

EIA: enzyme immunosorbent assay; LR+: positive likelihood ratio; LR-: negative likelihood ratio.