

SHORT COMMUNICATION

Performance of rapid diagnostic tests for the detection of antibodies to hepatitis C virus in whole blood collected on dried blood spots

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SUMMARY. Rapid diagnostic tests (RDTs) represent an attractive alternative to enzyme immunoassays. A total of 207 individuals, including 68 HCV-seronegative subjects, 10 patients with resolved infection and 129 patients with chronic HCV infection, were studied. The specificity of RDT detection of anti-HCV antibodies in whole blood was 100% with the four RDTs tested: OraQuick[®] HCV Rapid Antibody Test, First Response HCV Card Test, ASSURE HCV Rapid Test and Multi-

Sure HCV Antibody Assay. Their diagnostic sensitivity varied between 98.6% and 100%. RDT detection of anti-HCV antibody in whole blood collected on dried blood spots appears to be a promising new tool for broadscale screening of HCV infection in high- to medium-risk populations.

Keywords: anti-HCV antibodies, hepatitis C, rapid diagnostic test, screening.

Chronic hepatitis C is a 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]. In low- to middle-income areas, the vast majority of infected patients have not been diagnosed. Chronic HCV infection is curable by antiviral therapy, and the rates of viral elimination have been dramatically improved with the arrival of new, interferon-free direct-acting antiviral drug combinations that are well tolerated [4]. Global control of HCV infection is now a realistic objective, as recently underlined by the World Health Organization [5]. Thus, large-scale screening of HCV infection is now needed to identify infected patients and provide them with efficacious therapies.

Screening of HCV infection is usually based on the detection of anti-HCV antibodies in whole blood collected by venous puncture by means of third-generation enzyme immunoassay (EIA). Rapid diagnostic tests (RDTs) represent an attractive alternative for HCV screening and diagnosis because they were validated using whole venous or capillary blood, some of them can accept a variety of matrices including oral fluid, serum or plasma. RDTs offer the advantage of simplicity, limited need for instrumentation, minimal training required and rapid performance at room temperature. RDTs have proven to have good specificity, but their analytical sensitivity varies [6,7]. These findings emphasize the need for careful assessment of the performance of RDTs before using them for the detection of antibodies to HCV in clinical practice. Collection on filter paper is the standard approach to store whole blood without alteration of viral markers, ensuring simplicity and accuracy of blood collection and transfer to the RDT.

The aim of the present study was to evaluate the diagnostic performance of CE-marked RDTs for the detection of anti-HCV antibodies in venous whole blood collected on dried blood spot (DBS).

The performance of 4 CE-marked RDTs (OraQuick[®] HCV Rapid Antibody Test from OraSure Technologies, Inc, Bethlehem, Pennsylvania; First Response[®] HCV Card Test from Premier Medical Corporation Ltd, Watchung, New Jersey; ASSURE HCV Rapid Test and MultiSure HCV Antibody

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Assay from MP Diagnostics, Santa Ana, California, USA) detecting anti-HCV antibodies in whole blood collected on DBS has been assessed in 207 subjects recruited in the Departments of Hepatology and Gastroenterology of Henri Mondor University Hospital and 'Centre Hospitalier Intercommunal de Créteil', including 68 HCV-negative individuals (group A: no detectable anti-HCV antibodies or HCV RNA), 10 subjects who spontaneously cleared infection (group B: presence of anti-HCV antibodies in the absence of detectable HCV RNA; mean signal-to-cut-off value: 26.0 ± 6.9 ; range: 11.5–33.5) and 129 HCV-seropositive patients with chronic HCV infection (group C), all of whom had detectable anti-HCV antibodies (mean signal-to-cut-off value: 26.8 ± 4.5 ; range: 8.8–35.5) and HCV RNA (mean HCV RNA levels: 5.70 ± 0.90 log IU/mL). Based on sequencing of a portion of the nonstructural 5B gene of HCV followed by phylogenetic analysis, 71 patients from group B were infected with HCV genotype 1 (29 with 1a, 36 with 1b and 6 with another genotype 1 subtype), 7 with genotype 2, 19 with genotype 3a, 26 with genotype 4 and 3 with genotype 6. The HCV genotype could not be determined in 3 patients due to a low HCV RNA level (range: 2.2–2.6 log IU/mL).

Anti-HCV antibodies were sought by means of a 3rd-generation EIA assay, aHCV VITROS ECITM (Ortho-Clinical Diagnostics, Raritan, New Jersey, USA), while HCV RNA was detected and quantified by means of the *m2000* assay and device (Abbott Diagnostics, Chicago, IL, USA) (Table S1). The DBS was stored for 12–24 months at -80 °C.

None of the HCV-seronegative individuals from group A were positive for anti-HCV antibody detection in the four RDTs tested. The specificity was thus 100% (95%CI: 94.6–100%) (Table 1). The clinical sensitivity, as compared to EIA in serum, was high for the four RDTs studied, including: OraQuick® Rapid Antibody Test (100%; 95%CI: 97.3–100%), First Response® HCV Card Test (99.3%; 95%CI: 96.0–99.9%), ASSURE HCV Rapid Test (98.6%; 95%CI: 94.9–99.6%) and MultiSure HCV Antibody Assay (98.6%; 95%CI: 94.9–99.6%). The positive and negative likelihood ratios of the four RDTs are shown in Table 1.

Receiver operating characteristic (ROC) curves were used to compare the performance of RDTs according to the Hanley and McNeil method; they showed no difference

($P = 0.384$). Table S2 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 1b was found negative with the First Response® HCV Card Test in spite of a high HCV RNA level (5.6 log IU/mL) and a high signal-to-cut-off value in serum with two EIAs (VITROS ECi and Access®) (Table S2). Frozen plasma was retested and found positive. Two patients had a negative result with the ASSURE HCV Rapid Test. One had a high signal-to-cut-off value in both EIAs in serum and a high HCV RNA level. The remaining patient had low signal-to-cut-off value and HCV RNA level. Both patients were positive on retesting. Finally, among the 129 HCV-positive patients tested with MultiSure HCV Antibody Assay, anti-HCV antibodies were not detected in two cases. Among them, one patient infected with genotype 4h had a high HCV RNA level (5.1 log IU/mL) and a high signal-to-cut-off value in serum with the two EIAs. The other one had an indeterminate genotype, a low signal-to-cut-off value in both EIAs and a low HCV RNA level (2.6 log IU/mL). Frozen plasma from all of them was retested with the MultiSure HCV Antibody Assay and was found invalid or negative.

Because of the need for increasing the worldwide rate of HCV screening in the context of new, highly effective interferon-free treatment strategies, new sensitive and easy-to-use virological tools are needed. In this respect, RDTs will be particularly useful. In some organizations, RDTs can be performed at the patient's care site from capillary whole blood taken from a fingerstick. This, however, requires that RDTs be available at the patient's care site and that the staff are able to use and interpret them correctly. Another option, particularly well suited for low- to middle-income areas, is the use of whole blood taken from fingerstick or venous puncture stored on DBS and sent for analysis to a central location. DBS is indeed an adequate solid device to store whole blood in the long term without degradation of virological markers [8]. Our study shows that anti-HCV antibodies can be easily and reliably detected by RDTs in whole blood collected on DBS. These tests are easy and rapid to perform. They are highly specific and highly sensitive for anti-HCV antibody detection. RDT detection of anti-HCV antibody in whole blood collected from DBS thus appears as a promising

Table 1 Performance of anti-HCV antibody RDTs in venous whole blood collected on DBS, using the EIA result in serum as the reference

Test	Specificity (95%CI)	Sensitivity (95%CI)	LR+	LR–
OraQuick® HCV Rapid Antibody Test	100% (94.6–100%)	100% (97.3–100%)	∞	∞
First Response® HCV Card Test	100% (94.6–100%)	99.3% (96.0–99.9%)	∞	0.007
Assure HCV Rapid Test	100% (94.6–100%)	98.6% (94.9–99.6%)	∞	0.014
MultiSure HCV	100% (94.6–100%)	98.6% (94.9–99.6%)	∞	0.014

EIA, enzyme immunosorbent assay; LR+, positive likelihood ratio; LR–, negative likelihood ratio; 95%CI, 95% confidence interval.

new tool for broadscale screening of HCV infection in high- to medium-risk populations. They will have a significant impact on diagnosing HCV infection in low- to middle-income areas where access to care must be improved in the framework of large-scale low-cost treatment programmes currently being implemented. Careful assessment of the performance of HCV RDTs must be recommended before they can be implemented in clinical practice. The good performance of the four RDTs tested here suggests that they can be confidently used in these settings.

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COMPETING INTERESTS

None.

ETHICAL APPROVAL

None.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1: Demographic and virological characteristics of the study

population, including HCV-seronegative subjects (group A), patients with resolved infection (group B) and patients with chronic hepatitis C (group C).

Table S2: Virological characteristics of the patients with a false-negative result in one of the 4 RDTs in venous whole blood collected on DBS.