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Point-of-care testing for HCV infection: recent advances and implications for alternative screening

Maria Rita Parisi[1], Laura Soldini[2], Gianmarino Vidoni[3], Chiara Mabellini[4], Teresa Belloni[4], Livia Brignolo[3], Silvia Negri[5], Karin Schlusnus[6], Fernanda Dorigatti[2], Adriano Lazzarin[1]

[1]Division of Immunology, Transplantation and Infectious Diseases, Vita-Salute University, San Raffaele Scientific Institute, Milan, Italy;
[2]Laboraf of Diagnostic and Research OSR S.p.A., San Raffaele Scientific Institute, Milan, Italy;
[3]Prevention Department, Reference Centre for HIV and STDs, Local Public Health Unit, Milan, Italy;
[4]Vita-Salute University, Dentistry Department, San Raffaele Scientific Institute, Milan, Italy;
[5]ANLAIDS Lombardia Onlus, Milan, Italy;
[6]AIRON Communication s.r.l., Milan, Italy

SUMMARY

Over the last few years, hepatitis C virus (HCV) infection has emerged as one of the most significant causes of chronic liver disease worldwide, with an estimated prevalence ranging from 2.2 to 3.0%. In Italy, approximately 2% of subjects are infected with HCV. Considering that acute HCV infection is usually asymptomatic, early diagnosis is rare. Those people who develop chronic infection, even though undiagnosed, may suffer serious liver damage, making chronic HCV infection a major health problem. New initiatives are needed to identify a submerged portion of patients with chronic viral hepatitis and to propose controls and antiviral treatments to avoid the progression to liver cirrhosis or hepatocellular carcinoma (HCC). Since January 2011, the Infectious Diseases Department of San Raffaele Scientific Institute in Milan has been carrying out a prevention program called "EASY test project", using a new oral test, the OraQuick® HCV rapid antibody test (OraSure technologies, Inc.). The main objective of the project is to evaluate the acceptability of an alternative, free and anonymous HCV test offer, available in different settings (Points of Care, STDs Prevention clinics and General Practitioner clinics). From January 2011 to April 2014, 29,600 subjects were approached to inform them about HCV infection and other sexually transmitted diseases; 4,507 (15.2% of the contacted subjects) of them, total eligible volunteers, performed HCV tests on saliva and completed the interview in the alternative POCTs.
Twenty-seven subjects (0.6% of the total) turned HCV oral test reactive (27/4.507) during the evaluation period; all of them were confirmed by conventional test. All 27 patients were asymptomatic and without a history of HCV-related symptoms. The results from this analysis suggest that the promotion of alternative HCV test screening has not yet been fully developed as a strategy to increase levels of HCV testing among people at risk for HCV infection. Increasing awareness of these alternative tests among individuals at risk and providers may be an appropriate strategy to increase the number of people who know their serological status. The recent introduction of rapid oral HCV antibody test could completely change the HCV diagnosis approach by facilitating the possibility of testing millions of people worldwide (in particular in the developing countries).

KEY WORDS: HCV screening test, Saliva/oral fluid test, HCV positive people, HCV prevention.

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Corresponding author
Maria Rita Parisi
Division of Immunology
Transplantation and Infectious Diseases
San Raffaele Scientific Institute
Via Stamira d'Ancona, 20 - 20127 Milan (Italy)
E-mail: parisi.mariarita@hsr.it

INTRODUCTION

Over the last few years, hepatitis C virus (HCV) infection has emerged as one of the most significant causes of chronic liver disease worldwide, with an estimated prevalence ranging from 2.2 to 3.0% (Lavanchy, 2009).

The epidemiology of hepatitis C in Italy has also changed in recent decades, with a progressive decrease in incidence due to the same factors that have contributed to the downward trend of hepatitis B in the pre-vaccine era: improved hygienic and socio-economic conditions, reduced family size and therefore the intra-family circulation of viruses, increased knowledge about modes of transmission and better prevention with the introduction of measures such as blood screening, adoption of universal precautions, namely the discontinued use of non-disposable syringes, educational campaigns on HIV infection whose transmission modes are common to the HCV virus (EpaC).

In 2010, the incidence was 0.2 per 100,000, the decrease in incidence has primarily involved the age group 15-24 years (probably due to behavioural changes on the part of drug users). Today, individuals who develop hepatitis C are mainly males. The major risk factors are surgery, percutaneous exposure in the course of cosmetic treatments, promiscuous sexual activity and the use of intravenous drugs.

In Italy, the proportion of subjects infected with HCV is approximately 2% of the general population with a gradient that increases from the North to the South and the Islands and with age (60% of patients with hepatitis C are >65 years). It is estimated that about 1 million people in Italy are ill with hepatitis C (ISS).

Seroepidemiological surveys carried out in the 80s and early 90s in probability samples of the Italian population between 3 and 26 years, shown a very low prevalence of anti-HCV: 0.2% between 3 and 14 years, 0.6% between 14 and 19 years and 0.5% between 19and 26 years. These data show a very limited circulation of the hepatitis C virus in Italy among young people.

Several surveys have been conducted in various regions of the country to assess the prevalence of hepatitis C virus in all age groups of the general population. All studies have been conducted with similar methods, using a possible sample of the resident population. All investigations were characterized by a high rate of cooperation of the population tested. The results were surprising, showing values of fluctuating global prevalence from 3.9% to 16.2%. The high levels of HCV prevalence observed, however, cannot be considered representative of the entire regional area these municipalities belong to, as it is not urban centres whose limited size may have facilitated the extent of virus spread (D'Amelio *et al.*, 1995; Bellentani *et al.* 1999). Two aspects, however, can be considered valid also for Italy as a whole: a strong North-South gradient, which reflects many socio-economic differences between these areas, and a dramatic increase in the levels of prevalence with age, from extremely low values in adolescents and young adults to higher levels, up to 40%, in subjects older than 60 years, thereby outlining the new features of the target population.

Considering that acute HCV infection is usually asymptomatic, early diagnosis is rare. Those people who are developing chronic infection, even though undiagnosed, may suffer serious liver damage. In fact, a significant proportion of HCV-infected subjects will ultimately evolve to liver cirrhosis and/or hepatocellular carcinoma, making chronic HCV infection a major health problem (Hoofnagle 1997; Hutin *et al.* 2004).

Despite the excellent accuracy of the currently available tests for the detection of anti-HCV antibodies, the delay in reporting the results, the need for specialized equipment for processing the samples and interpreting the results, as well as the need to transfer individuals to sample collection and processing centers, limit their use as screening tools. Serologic points of care tests (POCTs) have several advantages, namely that they require little specialized apparatus, can be brought to individuals to be tested and allow diagnosis in as little as a few minutes in different clinical settings (Ferreira-Gonzales *et al.* 2004). These advantages might be translated into increased testing opportunities and, ultimately, identification of more patients who could benefit from antiviral treatment (Tucker *et al.* 2013). Over the last few years, several tests for rapid detection of anti-HCV have been developed and are currently in use in various countries. However, only recently, was the first POCT approved by the U.S. Food and Drug Administration (Food and drug administration). The investigation of the diagnostic accuracy of POCTs and rapid tests for the detection of anti-HCV is a highly relevant topic. Besides the great importance of the issue in terms of public

health, studies evaluating the performance of several of the currently used tests are lacking.
In London, during the last international congress on the liver, the scientific association of European specialists in the field (EASL), nine key recommendations for the treatment of HCV were made including: increasing the number of people to be controlled (screening) to mitigate the liver damage for those affected, and using the appropriate treatments for chronic infection. The real news, no longer experimental, is that today science has in hand a successful treatment: new drugs with fewer side-effects than oral treatments currently used and they seem to be able to eradicate the virus (EASL).
To achieve results similar to those obtained for the HIV infection prevention, new initiatives are needed to identify patients with chronic viral hepatitis and propose controls and eventually antiviral treatments.
Since January 2011, the Infectious Diseases Department of San Raffaele Scientific Institute in Milan has carried out a Prevention Program called "EASY test project", using two new oral tests: the OraQuick® HCV Rapid Antibody Test and the OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test (OraSure technologies, Inc.) to diagnose the HCV and HIV infection, respectively. The HCV rapid test received FDA approval for use with oral fluid on 28 June 2010.
The main objective of the project is no longer to test the reliability of the oral fluid rapid tests (sensitivity and specificity), but to evaluate the acceptability of an alternative, free and anonymous HCV test offer, available in different settings (in points-of-care, STD prevention clinics and general practitioner surgeries) (Parisi *et al.* 2009; Parisi *et al.* 2013). Furthermore, reaching the submerged people with this anonymous and free test offer, it could reduce or stop this public health problem.
From 2011 to date, the EASY test project took place regularly in three different locations in Milan: on each first Friday of month in two San Raffaele points-of-care and one STD (Sexually Transmitted Diseases) prevention clinic ("*FreeDay Easy-test*"); and now extended to six general practitioner surgeries.
Individuals are counselled by an infectious diseases specialist or by a psychologist and sign an informed consent form before taking the test. In addition, for the individuals who do not wish to participate in the study, the test was offered free and anonymous with an identification code for a subsequent evaluation of the results. This report summarizes EASY test results, collected from 2011 to date for the individuals who participated to EASY test study (73% of all tests offered).

METHODS

This programme has been running since 2011 with the objective of promoting HCV early diagnosis using an oral fluid antibody test, rapid and not invasive. This is a cross-sectional community study, implemented under the patronage of the Infectious Diseases Department of San Raffaele Hospital, in collaboration with the Department of Prevention-Reference Centre for HIV and STDs (Local Public Health Unit in Milan) and supported by the ANLAIDS-Lombardia association (National Association for the Fight against AIDS).
The programme has been conducted in Milan since January 2011 in three different appointments per year:
1) Two points of care at San Raffaele Hospital;
2) One HIV-STDs public prevention outpatient clinic ("FreeDay Easy");
3) Six general practitioner surgeries.

During these appointments, a biologist, an infectious diseases specialist and many volunteers of ANLAIDS-Lombardia association carried out the prevention day.
In each location there were different spaces for the privacy of the subjects performing the test: a waiting room with STDs prevention information and pre-test counselling, testing and giving back results (eventually blood sampling) with post-test counselling.
This HCV test offered was free and anonymous for the study participants. A skilled specialist explained the study objectives and procedures to all participants and each of them signed an informed consent form. Each subject received an identification code number with the possibility to choose to undergo an HCV test, an HIV test (also offered in the same project) or both. Subjects who underwent the test were asked to complete an anonymous questionnaire, which

allowed us to collect a series of data on risk behaviours of the population tested. The questionnaire was designed to collect demographic and risk behaviour data, as well as previous HCV/HIV testing experience, questions on sex, drug use, educational level, nationality, general behaviours, use of HIV/HCV prevention services, previous surgical practices, invasive diagnostic practises, dental care, tattoos or sexually transmitted diseases.
Subjects who did not accept the test were given personal counselling on the prevention of HCV infection and other sexually transmitted infections.
People eligible for inclusion in the project were aged >18 years, unaware of their HCV serological status and able to complete the questionnaire in Italian or English. Subjects were informed that they could refuse testing at any time. Post-test counselling was provided to all HCV reactive and non-reactive subjects, by the Infectious Diseases Department physicians involved in the study. All those with HCV reactive tests were offered a regular screening confirming test free of charge, and the results and their first specialist visit were guaranteed within a few days.

Performing the test
The testing step was carried out by a biologist or a practitioner, following the manufacturer's procedures.
Our prevention programme was performed using the new OraQuick ADVANCE® Rapid HCV Antibody Test (OraSure Technologies, Bethlehem, PA, USA).
The test is a single-use, immunoassay for the qualitative detection of antibodies to hepatitis C virus (anti-HCV) in oral fluid, fingerstick whole blood, venipuncture whole blood and plasma specimens. The clinical sensitivity and specificity of the OraQuick HCV test using oral fluid were 97.8% (95% confidence interval [CI]), and 100% (95% CI, 98.4-100%), respectively (OraQuick).
It is very important to follow the manufacturer's sampling procedure (saliva collection), because this step can affect the proper performance of the test, resulting in false positive test results. If the HCV oral test is reactive, a venipuncture is performed immediately, supported by post-test counselling to interview the subject about possible previous risk behaviours and to prepare them for an HCV positive diagnosis.
The blood sample is sent to the serology laboratory of San Raffaele Hospital for standard confirming test. The results are received in two working days. At this point, the HCV-positive patient is contacted directly by the infectious diseases specialist for the visit and the diagnostic procedures to define the liver disease status and eventually to start treatment, according to the guidelines when HCV viral load and genotype are identified.

Ethical committee and data collection
The project protocol was approved by the Institutional Review Board of the Ethical Committee of San Raffaele Hospital, Milan. An informed consent form was prepared explaining the details of the study, the HCV testing procedure and the interventions available for each individual.
HCV test results were entered immediately into a personal electronic file by the person responsible. Study data, including the questionnaires, were conveyed to the Department of Infectious Diseases of San Raffaele Hospital, used anonymously under the control of the Head of the project and the Department.

RESULTS

From January 2011 to April 2014, a total of 29,600 subjects were approached to inform them about HCV infection and other sexually transmitted diseases; 4,507 (15.2% of contacted subjects) of them, total eligible volunteers, performed HCV tests on saliva and completed the interview in the alternative "lab street" (Table 1). As both the rapid test for HIV and for HCV is offered during the Easy test days, of the total number of tests offered, 73% of the population performed both tests, and none was only tested for HCV.
The total number of subjects tested during the last three years increased gradually, due to the spread of initiatives among the general population. The subjects undergoing monthly HCV salivary tests appears to be represented, on the one hand, by those who have a high and

TABLE 1 - *Self-reported demographic characteristics of eligible respondents among individuals interviewed from January 2011 to April 2014, per year.*

	2011	*2012*	*2013*	*2014*	*Total*
N. Contacted and informed subjects (Test offer days)	14.000 (26 days)	10.000 (25 days)	5.000 (10 days)	600 (3 days)	29.600 (64 days)
N. Tested subjects (percentage of informed)	1.278 (9,1%)	1.407 (14%)	1.689 (16.9%)	133 (22%)	4.507 (15.2%)
N. HCV pos saliva test (percentage of tested)	7 (0.5%)	7 (0.5%)	7 (0.4%)	6 (4.5%)	27 (0,6%)
Gender: Male Female (percentage of tested)	933 (73%) 345 (27%)	1.125 (80%) 282 (20%)	1.064 (63%) 625 (37%)	84 (63%) 49 (37%)	3.206 (71%) 1.301 (29%)
Education: High level school (percentage of tested)	1.022 (80%)	985 (70%)	1.013 (60%)	84 (63%)	3.104 (69%)
Employment: Workers (percentage of tested)	1.086 (85%)	1.238 (88%)	1.351 (80%)	106 (80%)	3.781 (84%)
Median age (Range)	35 (20-50)	35 (20-50)	35 (20-50)	35 (20-50)	35 (20-50)
Race: Italian (percentage of tested)	383 (30%)	844 (60%)	1.182 (70%)	52 (70%)	2.461 (55%)
Previous tested: HCV negative (percentage of tested)	383 (30%)	281 (20%)	506 (30%)	0	1.170 (26%)

altered perception of risk and who need to test themselves repeatedly while not having risk behaviours, and on the other, by those who were unaware but underwent the test because it was there.

The portrait of the population responding to the EASY test initiative in these 39 months was quite different, dominated by Italian (55%) men (71%), aged between 20 and 50 years, with a medium-high level of education (graduates, managers, 69%), and 84% of them employed; 74% of subjects had never performed a test for HCV because they did not consider themselves at risk (Table 1).

During the first year of the initiative, 2011, a total of 1.278 rapid oral tests were performed and 7 (0.5%) reactive tests were found in all, confirmed positive by the blood test. Many people who underwent the test were employed males (73%) with a high level of education (85%), aged between 20 and 50 years; 30% of the people previously tested performed the test due to family events or following a transfusion.

Also during the second year 2012, the San Raffaele initiative of *FreeDay EASY test* started in February and finished in December. A total of 1,407 saliva tests were performed and 7 (0.5%) new HCV infections diagnosed; 80% of total tested subjects were males with a high level of education (70%) in employment (88%) and aged between 20 and 50 years.

TABLE 2 - *Self-reported demographic characteristics of HCV-positive saliva test subjects per year, from January 2011 to April 2014.*

	2011	2012	2013	2014	TOTAL
N. HCV pos saliva test	7	7	7	6	27
Gender:					
Male	5 (71%)	4 (57%)	3 (43%)	5 (84%)	17 (63%)
Female	2 (29%)	3 (43%)	4 (57%)	1 (16%)	10 (37%)
(percentage of tested)					
Education:					
High level school	5 (71%)	5 (71%)	4 (57%)	3 (50%)	17 (63%)
(percentage of tested)					
Employment:					
Workers	5 (71%)	5 (71%)	4 (57%)	3 (50%)	17 (63%)
(percentage of tested)					
Median age					
(Range)	30 (20-40)	30 (20-40)	30 (20-40)	37.5 (25-50)	32 median age
Race:					
Italian	5 (71%)	5 (71%)	4 (57%)	3 (50%)	17 (63%)
(percentage of tested)					
Previous tested:					
HCV negative	0	3 (43%)	0	4 (34%)	7 (27%)
(percentage of tested)					

2013 was the year with the highest number of tests offered, with 1,689 subjects tested and 7 new diagnoses of HCV (0.4% of tested subjects). The portrait of the population tested was similar to the previous years, with an increase in the percentage of women participating in the initiative (from 20% of the previous year to 37%).
During the first three days of the current year 133 tests were offered and 6 found HCV positive (4.5%).
Regarding the 27 (0.6% of the total tested subjects, 27/4.507) subjects who turned HCV oral test reactive during the 39 months, all of them were confirmed by conventional test; 63% were men and 37% women, aged between 20 and 50 years, coming from Italy (63%), with a medium-high education level (63%). Only 27% of them were previously tested for HCV screening, resulting HCV-negative (Table 2).
All 27 patients were asymptomatic and without a history of HCV-related symptoms or pathology. They were immediately directed to our clinical department for all medical needs related to HCV infection and most of them are still in care at our Clinic (data not shown).

DISCUSSION

In recent years, advances in detection technology have made available a range of POCTs for different infectious diseases. It is now possible to screen and diagnose those conditions in primary healthcare settings, using minimally invasive tests. In the present study, a new POCT for HCV infection was performed on oral fluid. The use of oral fluid is an attractive alternative based on the fact that collection of plasma or serum samples requires equipment and training, and is more time-consuming.
The FDA-approved OraQuick HCV Rapid Antibody Test (OraSure Technologies, Bethlehem, PA, USA) is one of the most widely studied rapid tests for the diagnosis of HCV infection. The development of rapid alternative tests for the diagnosis of HCV infection is to facilitate access to testing to reduce the individual risk of disease progression and social costs.
The US Centers for Disease Control and Prevention (CDC) recommended that a person be considered to have serologic evidence of HCV infection if a positive result of anti-HCV screen-

ing must be confirmed by positive results of a further test, using either a recombinant immunoblot antibody assay (RIBA) or NAT to detect HCV RNA. However, despite these recommendations, reflex supplemental testing has not been widely performed by many laboratories for various reasons, including the complexity of the assays, long turnaround time of test results, and high cost (OraSure).

Despite the excellent sensitivity and specificity of third-generation EIAs, the turnaround time for reporting test results is at least one day, thereby making it difficult to deliver the results to tested individuals at first visit. Rapid tests are formatted so that they do not require complicated instrumentation or testing by skilled technical staff. They potentially generate results within an hour and therefore may be used for point-of-care testing. The CDC recently completed evaluation of 3 rapid tests for detecting anti-HCV IgG (Orasure and Chembio finger-stick and oral fluid-rapid test; Medmira finger-stick rapid test) in laboratory and field settings. Regarding the oral fluid assays, which are based on recombinant antigens derived from core, NS3, NS4, and NS5 proteins in an immunochromatographic format were found to exhibit a specificity between 80.0% and 97.7% with sensitivity ranging from 88.8% to 92.2% (Chembio oral fluid rapid test) and a specificity and sensitivity ranging from 90.8% to 94.7% and 92.1% to 98.6% respectively for the OraSure oral assay.

Regarding the performances on blood assay, the sensitivity and specificity were respectively: 93.0%-94.0% and 97.1% for Chembio test; 78.9% and 83.3% for MedMira assay; and 95.9%-97.4% and 98.6%-100.0% for OraSure assay (Center for disease control and prevention; Smith, Thesale *et al.* 2011; Smith, Drobeniuc *et al.* 2011).

The OraQuick HCV rapid antibody test for the detection of anti-HCV IgG has been recently approved by the FDA also for use with fingerstick, whole blood, and venous blood specimens from individuals aged ≥15 years and at risk for infection with HCV and from persons with signs and symptoms of hepatitis. The test also received an FDA waiver under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) (Food and drug administration).

Since facilities that can perform CLIA-waived tests outnumber the laboratories performing screening EIAs, the test has the potential to be used far more widely, such as in physician surgeries, outreach clinics, and community-based organizations.

Rapid tests are obviously more expensive than conventional immunoassays and are not designed for testing large batches of specimens. However, in non-clinical settings and laboratories conducting low-volume testing, adoption of rapid oral testing can be cost-effective. CDC guidelines formulated for confirming screening anti-HCV results remain to be refined to accommodate rapid anti-HCV testing. It is important to emphasize that OraQuick HCV test has not been approved for general screening. A positive result of a rapid anti-HCV positive test is indicative of anti-HCV Ab and, again, does not indicate active infection (Center for disease control and prevention).

We successfully conducted this rapid HCV testing and counselling program with the goal of spreading the use of saliva test anonymously and free of charge. We aim to facilitate access to testing in alternative settings to verify whether the "submerged" population would access salivary rapid testing versus the conventional settings.

In the present study, non-responders commonly refer low accuracy as the reason for not using rapid tests. The rapid oral tests, whit a different testing method, have equally high specificity, and a lower value of sensitivity, not representing an obstacle, due to priority of saliva test to identify more or less recent new HCV infections.

Therefore, the concern that alternative tests may be less accurate is not supported by experience with the test and is likely due to lack of knowledge about test performance. This suggests a need for increased education among high-risk populations about the accuracy of these tests (Food and drug administration) (FDA, 2012).

Although a formative research process was conducted to select locations representative of communities at risk for HCV infection, sites were not randomly selected, and therefore our findings may not be generalizable to all individuals at risk for HCV infection in the areas

where the survey was conducted. In addition, our response rate of 15.2% (4.507 tested/29.600 informed) of interviewed individuals may introduce some bias if those refusing interviews were different from those accepting interviews. We did not collect the demographic information of those who declined to be interviewed and tested, and thus could not characterize this potential bias.

The analysis of our data showed that 15.2% of the general population informed, took the test because they wanted to take advantage of the opportunity that we were offering, few of them just because they had a family member infected or because they were actually at risk. Only in the HIV-STDs public prevention centre (Viale Jenner Local Health Authority) was the population not general but the a selected population at risk, being a reference centre for sexually transmitted diseases.

During the EASY test days of the first two years the focus was mainly on HIV testing, offered at the same time together with the HCV test. In the last year, instead, we found a growing interest also for the diagnosis of HCV infection, due to the information and prevention programme carried out recently. This interest was manifested by Italian subjects aged between 20 and 40 years of age and with a high level of education. Foreigners, on the other hand, did not know about the infection caused by the HCV virus and often only asked about it. Despite that, almost half of the subjects HCV positive were foreigners, who did not report a family history of blood transfusions or infection and did not know how to explain how they had contracted the infection. We believe that this submerged people at risk should be identified.

The percentage of patients who underwent the saliva test during our programme increased gradually over the years from 9.1% to 22% of contacted subjects to spread information, meaning there is certainly an increased attention to the diagnosis. The high percentage of HCV-positive subjects resulted in these first 3 days of the new year (4.5%). This may not mean an increase in prevalence, but is probably a self-selection of the at-risk population and others. The high prevalence rate achieved in these first days has certainly placed the focus on the importance of early diagnosis but especially on new therapies, having regard to the availability of more effective and safe oral drugs (EpaC).

The results of this analysis suggest that the promotion of alternative HCV test screening has not yet been fully developed as a strategy to increase levels of HCV testing among people at risk for HCV infection. Increasing awareness of these alternative tests among individuals at risk and providers may be an appropriate strategy to increase the number of people who know their serological status. However, our analysis does not make clear the extent to which the availability of alternative HCV tests would increase testing among those high- risk individuals previously untested for HCV.

The recent introduction of rapid oral HCV antibody test could completely change the HCV diagnosis approach by facilitating the possibility of testing millions of people worldwide (in particular in the developing countries).

For these reasons, we hope the oral-based rapid HCV tests could become the gold standard to facilitate the HCV screening access and become the standard of care and the basis for the national HCV testing algorithm in many countries with widespread HCV epidemics, to increase the proportion of persons aware of their HCV serological status.

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