Inovações Tecnológicas no Rastreio e Diagnóstico da Hepatite C: Revisão Focada de Literatura

Technological Innovations in Hepatitis C Screening and Diagnosis: Focused Literature Review

Inês Coelho Rodrigues[1], Inês Botto[1], Sérgio Bronze[1], Diogo Medina[2], Rita Almeida Freire[2], Fátima Serejo[1]

Resumo:

A norma de avaliação diagnóstica da infeção pelo vírus da hepatite C em Portugal (2017) limita a confirmação de testes de anticorpo positivos a consultas especializadas em meio hospitalar, com testes moleculares. Outros profissionais, entidades prestadoras ou tecnologias não são contemplados, apesar das inovações que permitiriam um atendimento mais centrado na pessoa.

Realizámos uma revisão focada de literatura publicada entre 2011 e 2021 sobre o diagnóstico reflexo (em que a confirmação de anticorpo positivo é efetuada no espécimen inicial) ou num-só-passo, o teste de antigénio core e os testes moleculares *point-of-care*.

Incluímos um total de 44 artigos relevantes, na sua maioria originários de Espanha e dos Estados Unidos da América.

O diagnóstico reflexo demonstrou aumentar a proporção de indivíduos sujeitos a confirmação de viremia, o custo-eficácia do rastreio, a ligação com os cuidados de saúde e o início de tratamento. O antigénio core demonstrou ser aceitável como alternativa aos métodos moleculares para fins confirmatórios, com custos e tempos de processamento mais baixos (70% e 85% respetivamente). Os testes moleculares *point-of-care* permitem a descentralização do rastreio na comunidade, com uma infraestrutura e conhecimentos técnicos requeridos mínimos. Embora subsistam preocupações quanto ao custo das soluções *point-of-care*, disponibilizá-las permitiria aos serviços que cuidam de populações em risco acrescido realizar testes confirmatórios em tempo útil e aumentar a ligação aos cuidados de saúde.

Há uma forte evidência que apoia o diagnóstico reflexo como padrão para o rastreio em todos os serviços de saúde. O uso de testes confirmatórios de antigénio core ou de testes moleculares *point-of-care* também deve ser considerado em contextos apropriados.

Palavras-chave: Hepatite C/diagnóstico; Hepatite C Crónica/diagnóstico; Hepatite Viral; Sistemas Point-of-Care.

[1]Serviço de Gastrenterologia e Hepatologia, Unidade Local de Saúde de Santa Maria, Faculdade de Medicina, Lisboa, Portugal
[2]Gilead Sciences, Lisboa, Portugal

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Abstract:

Current Portuguese hepatitis C virus (HCV) screening guidance (2017) limits confirmation of antibody-positive test results to hospital specialist visits, using nucleic acid amplification tests (NAAT). No other professionals, healthcare settings, or testing technologies are included, despite innovations toward more patient-centric care.

We performed a focused review of literature published between 2011 and 2021 regarding reflex (in which confirmation of antibody-positive test is done on the initial serum specimen) or single-step testing, HCV core antigen (cAg), and point-of-care (POC) HCV NAAT.

We incorporated a total of 44 relevant papers, mostly from Spain and the United States of America.

Reflex testing workflows have consistently been shown to increase completion of confirmatory testing, screening program cost-effectiveness, patient linkage to care, and treatment initiation. HCV cAg determination has also been established as an acceptable alternative to NAAT for confirmatory testing, with lower cost and lower turnaround time (70% and 85% respectively). Lastly, POC HCV NAAT enables decentralized screening in community settings, with minimal infrastructure and technical expertise required. Although concerns remain regarding the cost of POC NAAT solutions, their availability is crucial in empowering facilities caring for at-risk populations to perform timely confirmatory testing and increase patient linkage to care and treatment.

There is a strong body of evidence to support reflex testing as the standard of care for HCV screening in all healthcare settings. HCV cAg or POC HCV NAAT confirmatory testing should also be supported where appropriate, in alternative to strictly central laboratory-based HCV NAAT.

Keywords: Hepatitis C/diagnosis; Hepatitis C, Chronic/diagnosis; Hepatitis, Viral, Human; Point-of-Care Systems.

Introduction

Achieving the World Health Assembly 'Global Health Sector Strategy on Viral Hepatitis' goal of eliminating viral hepatitis as a public health threat by 2030 will require innovative approaches to screening and linkage to care.[1] According to recent

modulization studies, the COVID-19 pandemic may have further delayed progress toward these important goals.[2] A staple of secondary prevention, screening is an entry point to care and elimination or suppression of disease, improving the health of those tested and preventing further transmission.[1]

An estimated 3.9 million individuals are chronically infected with hepatitis C virus (HCV) in the European Economic Area, with national estimates of HCV antibody (HCV Ab) prevalence in the general population ranging from 0.1%–5.9%.[3] In Portugal, serosurveys have estimated the seroprevalence of HCV in the general population to be 0.5% (IC95%:0.2%-0.9%, 22% viremia) in 2012-2014, and 0.3% (IC95%:0.1%-0.6%) in 2015-2016.[4,5] Recent results from large scale opportunistic screening projects in people aged 18-65 found an HCV seroprevalence of 0.4% (62% viremia) in 2019 among 13,600 emergency department patients in Cascais, and an HCV seroprevalence of 0.7% (32% viremia) in 2020 among 7000 hospital ward patients in Funchal in 2020.[6,7]

Goal setting and knowledge of local epidemiology (data gathering) are key first steps in a public health mission that entails a long cascade of events, from first-line HCV Ab testing to second-line confirmation of active chronic infection (viral replication), patient test result notification, linkage to care, comprehensive health assessment, liver fibrosis staging, treatment initiation, retention in care, confirmation test of cure, and follow-up. It is crucial to know if the patient has cirrhosis, which is an oncogenic situation. Screening programs are more effective if supported by updated national testing strategies.[1] Portuguese HCV screening guidance published in 2017 recommends testing patients with a known history of risk factors or exposure to the virus, as well as 'progressively, extending it according to population disease prevalence.' The guidance accepts using either laboratory-based enzyme-linked immunosorbent assays (ELISA) or point of care (POC) rapid diagnostic tests (RDT) as first-line screening tests. The latter are performed by healthcare professionals or lay workers at the time and place where patient and provider meet, often with blood collected by finger-stick sampling. However, the guidance stipulates that subsequent and mandatory second-line confirmatory testing must be carried out during a specialist visit at a hospital, using nucleic acid amplification tests (NAAT), such as reverse transcription-polymerase chain reaction (RT-PCR).[8] Other professionals, healthcare settings, or testing technologies are not included, despite innovations in HCV screening and diagnosis approaches and technology that would otherwise contribute to more patient-centric care, including reflex or single-step testing, HCV core antigen (HCV cAg) confirmatory testing, and POC HCV NAAT confirmatory testing.

Herein we review innovations in HCV screening and diagnosis that healthcare organizations in Portugal can leverage to provide more efficient and patient-centric approaches to diagnosing the virus.

Methods

We performed a focused electronic search using PubMed, government agencies' websites, and scientific society websites. We retrieved clinical guidelines, literature reviews, original articles, and conference proceedings written in English, Portuguese, Spanish, and French published from 2011 to 2021, covering reflex or single-step HCV testing as a primary intervention, and HCV cAg and POC HCV NAAT confirmatory testing as supporting secondary interventions. We excluded pieces on other HCV diagnostic innovations, such as sampling approaches (e.g., self-testing, oral fluid, and dried blood spot or DBS) and fibrosis staging indices (e.g., aspartate aminotransferase-to-platelet ratio index or APRI, and Fibrosis 4 index or FIB-4). Two authors independently assessed individual evidence pieces for compliance with study objectives, risk of publication bias and inclusion, first by title, then abstract, lastly by full text. Differences were individually addressed.

Results

We incorporated 44 relevant evidence pieces: 10 clinical guidelines, 9 literature reviews, 22 original articles, and 3 conference proceedings. Most literature originates from Western Europe and North America, specifically Spain and the United States of America. A total of 24 evidence pieces addresses the primary intervention of reflex or single-step HCV testing, 12 pieces addresses HCV cAg confirmatory testing, and 16 pieces addresses POC HCV NAAT confirmatory testing. Evidence pieces may address one, a combination of two, or all three of the analyzed interventions. A synthesis of the data is available in Tables 1, and 3.

Discussion

REFLEX OR SINGLE-STEP TESTING

Diagnosis of HCV infection in Portugal is performed in multiple sequential stages, with a blood draw for the first-line detection of HCV Ab, patient interim test result notification, referral to specialist care, a second blood draw for second-line confirmatory HCV NAAT, and patient confirmed test result notification. Because HCV Ab-positive patients may spontaneously resolve the infection or have received previous treatment, this process consumes higher-than-necessary hepatologist specialist care visits.[9]

From the patient perspective, the current process is lengthy, anxiogenic in the interval from interim test results to confirmed test results, and inconvenient as it requires multiple visits to health care services to receive confirmed test results.[10,11] Hurdles in HCV diagnosis lead to poor linkage to care and delayed treatment initiation, particularly among vulnerable populations.[12] A report estimated the delay from HCV Ab-positive test result to HCV NAAT at 3.65 (SD 3.34) weeks, ranging from 1-12 weeks.[13] Studies have also shown how increases in the number of doctor visits before

Table 1: Data synthesis regarding Reflex or single-step HCV testing (primary intervention).

Citation	Type	Key points
NICE 2013[32]	clinical guideline	The same sample used for HCV Ab testing should be reflexed to HCV confirmatory testing without another blood draw or be sent to a laboratory to perform the test.
CDC 2013[33]	clinical guideline	The same sample used for HCV Ab testing should be reflexed to HCV confirmatory testing without another blood draw.
HAS 2014[34]	clinical guideline	The same sample used for HCV Ab testing should be reflexed by a microbiologist to HCV confirmatory testing without another blood draw.
Aparicio 2020[18]	clinical guideline	HCV Ab-positive results must be subject to confirmation in the same sample. Reflex testing improves LTC and decreases time to treatment. Reflex testing increased from 31% in 2017 to 89% in 2019 of surveyed hospitals. Joint consensus recommendations by the country's leading scientific societies contributed to the increase.
Schillie 2020[35]	clinical guideline	CDC encourages reflex testing. HCV testing should be provided on-site when feasible.
ECDC 2018[1]	clinical guideline	Reflex testing should be prioritized where available to increase LTC.
EASL 2020[17]	clinical guideline	Reflex testing increases HCV Ab-positive patients tested for viremia and LTC and should be applied whenever possible to shorten pathways to care.
SEIMC 2016[19]	clinical guideline	Reflex testing reduces LTC time. Although 81% of Spanish hospitals can deploy reflex testing, only 31% do so, which generates 40% losses to follow-up or overloads specialist care with a third of patients who have resolved their infections spontaneously or after previous treatment. Pilot studies in Granada and Santiago concluded that reflex testing increased completion of HCV viral confirmation.
García 2018[20]	clinical guideline	Adoption of reflex testing and alerts for patient referral to specialized care in 2016 increased LTC up from previous 40% estimated losses to follow-up.
Dieterich 2019[29]	clinical guideline	Reflex testing is recommended. Lack of reflex results in second phlebotomy and loss of patients during follow-up.
Kapadia 2018[21]	review	Reflex testing eliminates the need for a second blood draw, simplifies evaluation and improves LTC.
Chevaliez 2019[22]	review	Reflex testing should be considered as a standard of care to eliminate the need for a second blood draw and simplify evaluation and improve LTC.
Patel 2021[49]	review	Reflex POC HCV NAAT streamlines diagnosis in correctional settings.
Chapko 2015[28]	original article	HCV NAAT was performed in 98% of HCV Ab-positive individuals in facilities with reflex testing, but in only 64% in facilities where reflex testing was not performed.
Howes 2016[59]	original article	Reflex testing became standard practice and eliminated the need to arrange repeat blood tests on HCV Ab-positive patients.
Coyle 2016[30]	original article	Reflex testing is feasible in primary care settings and is a success factor in ensuring 88.7% of HCV Ab-positive patients received confirmatory HCV testing.
Moorman 2017[60]	original article	False-positive antibody assays may occur with great frequency, emphasizing the need for "reflex" HCV RNA testing to ascertain current infection status
Crespo 2019[13]	original article	Reflex testing is cost-effective compared to routine clinical practice. Although 81% of hospitals have the means to deploy reflex testing, only 31% do so.
Casas 2019[9]	original article	Reflex testing improves diagnosis by avoiding losses in LTC and avoiding overloading specialist visits with patients who have spontaneously cleared the infection.
Assoumou 2018[23]	original article	Reflex testing should be ensured to reduce loss to follow-up.
Lopez-Martinez 2019[25]	original article	Implementing reflex testing is feasible and significantly increases diagnostic effectiveness of HCV. Reflex testing reduced the traditional six-step to a single-step process, reducing the time to confirmation of HCV infection from weeks or months to 2 days, with demonstrated cost-effectiveness of a universal primary care, general adult population-based screening approach at a low cost toward meeting WHO goals.
Garcia 2019[26]	original article	Reflex testing saves costs, while achieving better health outcomes. A cost-effectiveness analysis in Andalusia, Spain, estimated that with reflex testing, no negative viral load patients would be referred to specialist care versus 540 with standard diagnosis, generating total cost saving of €3,634 per patient.
Casas 2019[24]	original article	Reflex testing improves the shortcomings of a traditional diagnosis workflows by facilitating LTC, treatment and viral elimination. The introduction of reflex testing increased patient LTC from 55% to 83%, reduced mean time from diagnosis to specialist assessment from 70 to 52 days, and obviated unnecessary visits, as patients with resolved infections were not referred to a specialist, with a consequent cost savings.
Crespo 2021[27]	original article	Reflex testing is the most efficient way to screen for HCV. In 2019, 98.4% of 129 surveyed Spanish hospitals believed reflex testing should be the standard of care, and 89% had implemented it, against 31% in 2017. Scientific society recommendations may have contributed to the change and implementation of reflex testing.

HCV = hepatitis C virus, Ab = antibody, LTC = linkage to care, CDC = Centers for Disease Control & Prevention, POC = point of care, NAAT = nucleic acid amplification testing, RNA = ribonucleic acid, WHO = World Health Organization.

Table 2: Data synthesis regarding HCV cAg confirmatory testing (supporting secondary intervention).

Citation	Type	Key points
ECDC 2018[1]	clinical guideline	HCV cAg is an alternative to NAAT for HCV Ab-positive test result confirmation.
EASL 2020[17]	clinical guideline	HCV NAAT or HCV cAg can be used in serum or plasma to diagnose acute infection, chronic infection, or after HCV reinfection. HCV cAg assays are less sensitive than HCV NAAT assays for the diagnosis of viremia (lower limit of detection equivalent to approximately 500 to 3,000 HCV RNA IU/ml). In rare cases of chronic infection, HCV cAg is undetectable in the presence of HCV RNA.
Chevaliez 2019[22]	review	HCV cAg is a surrogate marker of HCV replication when HCV NAAT is not available or not affordable. However, it requires a centralized laboratory.
Peeling 2017[37]	review	HCV cAg was developed as an alternative to HCV NAT, as access to and affordability of confirmatory HCV NAAT assays remains a challenge in resource-limited settings. Advantages of HCV cAg assay in comparison to HCV RNA assays are cost per test, turnaround time and the random access to a platform that does not require specimen batching. However, the most widely used HCV cAg assay (ARCHITECT HCV Ag), requires a floor-standing immunoassay analyzer. HCV cAg can have similar accuracy compared to HCV NAAT for viral load >3000 IU/mL. Data shows high sensitivity and specificity of HCV cAg when compared to HCV RNA for detection of viremia both before treatment and after treatment. The development of a highly sensitive POC platform may prove more cost-effective than NAAT POC tests.
Chevaliez 2018[39]	review	HCV cAg is an alternative marker of viral replication and to assess therapy response (limit of detection equivalent to 500-3,000 HCV RNA IU/mL according to the HCV genotype). Sensitivity for HCV cAg detection from DBS was low in HCV RNA-positive patients (65%), and cannot be used when whole blood is collected on DBS.
Tillmann 2014[42]	review	HCV cAg is a substitute for HCV NAT. As HCV NAAT is still more sensitive, utility of HCV cAg depends on cost saving in a population setting. A big advantage is the ease to allowing reflex testing when the same testing-instrument is used for anti-HCV and HCV cAg.
Freiman 2016[40]	review	HCV cAg is a potential replacement for HCV NAT, particularly if a lower cost per test allows reaching more patients, with similar accuracy to HCV NAAT for identification of active infection when the viral load exceeds 3000 IU/ml. HCV cAg should be explored for POC testing to increase the number of patients diagnosed and streamline the HCV cascade of care. Both the Abbott ARCHITECT HCV Ag test and Ortho ELISA-Ag perform similarly regarding sensitivity—93.4% (90.1, 96.4) vs 93.2% (81.6, 97.7)—and specificity—98.8% (97.4, 99.5) vs 99.2% (87.9, 100). However, the large amount of consistent, homogenous data on the ARCHITECT (33 studies vs six on Ortho) allows for greater precision and more confidence in these estimates.
Easterbrook 2017[61]	review	Following HCV Ab-positive results, quantitative or qualitative RNA NAAT is recommended as the preferred testing strategy to diagnose viremic infection. Detection of HCV cAg has comparable sensitivity and may be considered as an alternative.
Kuo 2012[45]	original article	HCV cAg detection in serum or plasma is useful as an indirect marker of HCV replication due to excellent correlation with HCV RNA concentrations. HCV cAg assays are faster and less expensive to perform than HCV NAT.
Perez-Garcia 2019[41]	original article	Use of HCV cAg as an alternative to HCV NAAT for diagnosis and treatment monitoring is supported by the European Association for the Study of the Liver. The Abbott Diagnostics Architect HCV cAg assay uses the same platform employed in HCV Ab determination, contributing to diagnostic simplification.
Pollock 2020[62]	original article	A cohort study of 744 patients demonstrated HCV cAg sensitivity of 82.1% (95% CI 77.1%-86.2%) and a specificity of 99.8% (95% CI 98.6%-100%) in Scotland from June 2011 to December 2017. Genotype 3 was associated with increased odds of a false-negative result (OR = 3.59, 95% CI: 1.32- 9.71).
Chitadze 2017[63]	conference proceeding	HCV cAg accurately identified >97% of patients with active viremia. HCV cAg can be used as an alternative to HCV NAAT testing.

HCV = hepatitis C virus, cAg = core antigen, NAAT = nucleic acid amplification testing, Ab = antibody, RNA = ribonucleic acid, DBS = dried blood spot, POC = point of care.

treatment initiation directly affect loss for follow-up among vulnerable populations, with a significant proportion of HCV Ab-positive patients failing to receive second-line confirmatory HCV NAAT.[14-16]

Reflex or single-step testing means positive HCV Ab first-line test results automatically trigger confirmatory tests on the same specimen without physician or patient initiation, which obviates the need for patients to return for a second blood draw for follow-up testing.[17] In reflex testing approaches, organizations only communicate positive results to patients after running second-line confirmatory testing. Negative HCV Ab first-line test results do not trigger additional testing. Reflex testing reduces the traditional multi-step to a single-step process, substantially increasing the proportion of HCV Ab-positive patients who are tested for active infection and receive subsequent linkage to care, decreasing time to confirmation of HCV infection from weeks or months to as little as 2 days, with demonstrated cost-effectiveness compared to routine clinical practice, including in the United States of America (USA), the United Kingdom, France, and Spain.[13,17-28] An analysis in Andalusia, Spain, estimated the ability not to refer HCV Ab-positive but HCV RNA-negative patients to specialist care thanks to reflex testing generated 3634 € cost savings per patient.[26]

Concerns regarding the use of reflex testing workflows include the possibility of false-positive diagnoses due to sampling errors or biological contamination, as errors in laboratory systems could cause minimal sample contamination (<15 IU/mL).[25] However, considering most naive patients' viral loads have been previously reported to be >3 logs, this should be considered irrelevant in the context of a new diagnosis.[17,25] The inability to ascertain HCV genotype

Table 3: Data synthesis regarding POC HCV NAAT confirmatory testing (supporting secondary intervention).

Citation	Type	Key points
EASL 2020[17]	clinical guideline	Affordable (<US$10) POC HCV NAAT are needed in low-to middle-income areas and community settings in high-income countries.
Chevaliez 2019[22]	review	Simplification of diagnostic algorithms with the implementation of decentralized testing is needed to enhance HCV screening, LTC and treatment.
Patel 2021[49]	review	There is a drop-off in PWID populations before LTC, because off-site referral to specialty HCV care is not effective in this population. POC HCV NAAT among PWID in Spain showed 98.4% sensitivity compared with gold-standard HCV NAT, with 80% receiving same-day confirmatory results.
Peeling 2017[37]	review	HCV NAAT can be performed on POC devices outside of laboratory settings, allowing for same-day or next-day confirmation of infection and timely LTC. Results take approximately 110 minutes, with some products being capable of running multiple simultaneous parallel tests.
Chevaliez 2018[39]	review	POC HCV NAAT performance of the Xpert® HCV Viral Load assay is comparable to other HCV NAAT assays, with a lower limit of quantification of 100 IU/mL (2 Log10 IU/mL) and an upper limit of linear quantification of 100,000,000 IU/mL (8 Log10 IU/mL).
Applegate 2018[56]	review	POC HCV NAAT detects active infection with high performance, enables "test and treat" approaches and is likely to enhance care in marginalized populations. Further reduction in cost and time are needed.
Grebely 2017[14]	original article	On-site HCV testing improves LTC. The POC HCV NAAT Xpert HCV Viral Load assay showed good sensitivity and specificity in capillary whole blood collected by finger-stick and plasma collected by venipuncture compared with the Abbott RealTime HCV Viral Load assay in drug health and homelessness services.
McHugh 2017[46]	original article	Currently, HCV viral load monitoring must be done in specialist centers, with a turnaround time of up to 1 week due to the need to batch samples. Performance, limited hands-on time (5 min), short run time (105 min), random access testing, and uncomplicated operator input suggest that the Xpert HCV Viral Load assay can have an important role in HCV diagnosis and monitoring. Disadvantages include high cost per test.
Lamoury 2018[64]	original article	In studies from Australia, Canada, and the United States, only 46–73% of HCV Ab-positive individuals received confirmatory HCV NAAT testing. Compared to traditional diagnostic pathways that require multiple visits to a practitioner or off-site phlebotomists, POC HCV NAAT increases testing and LTC, particularly outside the tertiary care setting, such as drug treatment clinics, community health centers, prisons, needle and syringe programs, and supervised consumption rooms. The ability to perform HCV NAAT from finger-stick whole blood provides a major advance in HCV screening. Finger-stick testing is highly acceptable to both patient and provider, avoiding phlebotomy where venous access is difficult. Xpert HCV VL FS sensitivity and specificity in finger-stick whole blood was 100%.
Gupta 2017[50]	original article	The short turnaround time of 105 min (compared to 5h for the current laboratory-based standard), and minimal required infrastructure and technical expertise make POC HCV NAAT ideal for decentralization of NAAT. Cepheid Xpert® HCV Viral Load demonstrated sensitivity of 94.4% and specificity of 100%.
Bajis 2018[48]	original article	An Australian report indicated only 46% of HCV Ab-positive PWID received confirmatory testing. Finger-stick whole-blood POC HCV NAAT was acceptable among PWID. Participants reported a preference for finger-stick over venipuncture, due to venous access difficulties and reduced stress.
Bregenzer 2019[51]	original article	HCV POC NAAT in capillary whole blood is a convenient, rapid, and reliable method to diagnose active HCV infection, monitor treatment response and detect reinfection. For patients with difficult venous access after long-term intravenous drug use, capillary testing removes crucial barriers. Same-day results might improve LTC and allow test-and-treat approaches. The GeneXpert system is easily transportable by car, enabling its use in peripheral drug substitution centers and addiction clinics. Sensitivity and specificity of the Xpert HCV VL test with 100µl capillary whole blood was 97.0% and 94.7%. Sensitivity and specificity of the Xpert HCV VL FS test with 100 µl capillary whole blood was 100% and 88.9%. Discordant results were under treatment when HCV RNA was near the limit of quantification.
Wlassow 2019[47]	original article	Laboratory-based HCV NAAT platforms require batch testing of multiple specimens. POC HCV NAAT Xpert HCV Viral Load assay accurately quantifies HCV RNA in serum and whole blood samples collected on DBS, with performance comparable to that of the real-time PCR platforms used in clinical practice.
Grebely 2020[55]	original article	POC HCV NAAT increases testing uptake and LTC by reducing the time from sample collection to diagnosis in a single visit. Although POC HCV NAAT represents a major advance, 1 hour time to results is still too long in many settings (e.g., drug treatment clinics, community health centers, needle and syringe programs, and pharmacies). Strategies to reduce the time from testing to diagnosis may be an important component of a single visit test and treat model for HCV infection.
Lens 2020[52]	conference proceeding	Availability of POC HCV NAAT technology in outreach services targeting PWID improves linkage to care
Montague 2020[53]	conference proceeding	Integration of POC HCV NAAT technology in outreach services targeting the housing insecure population improves acceptability, if coupled with peer delivered care, or when compared with venipuncture or with the need to attend appointments at the hospital.

HCV = hepatitis C virus, NAAT = nucleic acid amplification testing, LTC = linkage to care, PWID = people who inject drugs, POC = point of care, Ab = antibody, DBS = dried blood spot.

and subtype in reflex workflows is another concern that the advent of effective pan-genotypic treatment regimens has overcome.[29]

Case studies in the USA show that HCV NAAT was performed in 98% of HCV Ab-positive individuals in facilities where reflex testing was performed, in contrast to only 64% in facilities where reflex testing was not available.[28] Reflex testing is also feasible in primary care settings and was credited in a Pennsylvania study as a success factor in ensuring 88.7% confirmatory testing completion among HCV Ab-positive patients.[30]

In Andalusia, Spain, the introduction of reflex testing increased patient linkage to care from 55% to 83%, reduced mean time from diagnosis to specialist assessment from 70 to 52 days, and obviated unnecessary physicians visits with consequent cost savings, as patients with resolved infections were not referred to specialist care.[24] In Catalonia, Spain, reflex testing enabled a cluster of primary care centers and an affiliated hospital to ensure completion of HCV NAAT in 91.3% of HCV Ab-positive patients, providing confirmed diagnoses within a mean of 2 days, down from the weeks and months from before.[25] Completion of HCV NAAT increased from 71.6% to 91.8% in primary care alone and from 18.5% to 94.9% in drug treatment centers.[25] Another study recorded an increase in patient linkage to care from 55% to 83% (p <0.01) with the introduction of reflex testing and referral reminders.24 Implementation of reflex testing in healthcare organizations has increased significantly in Spain, from 31% of 129 surveyed hospitals in 2017 to 89% in 2019, in response to joint consensus recommendations by the country's leading scientific societies.[9,13,18-20,25,27] To the best of our knowledge, only one cluster of hospitals and primary care centers in Madeira, Portugal, has implemented reflex testing, using Abbot Diagnostics Alinity's assays for HCV Ab and Roche Cobas 6800 system for HCV NAAT.[31]

Numerous international public health authorities and scientific societies now recommend that the same sample of venipuncture blood used for first-line HCV Ab testing, if reactive, should be reflexed to second-line HCV confirmatory testing in the same specimen, without another blood draw, or be sent to a laboratory that can perform the test.[1,17,18,29,32-35] Reflex or single-step testing is thus encouraged and deemed the standard of care as it eliminates screening barriers and improves linkage to care.[21-23,36]

HCV cAg CONFIRMATORY TESTING

Access to and affordability of confirmatory HCV NAAT assays remains a challenge in resource-limited settings.[37] HCV cAg was developed as a more affordable alternative to HCV NAAT, an important option for large population screening programs.[38] Alternative tests to HCV NAAT must accurately identify active infection and assess patient therapeutic response.[38] HCV cAg has similar diagnostic accuracy to HCV NAAT for identifying active infection for viral load >3000 IU/mL.[37,39,40] For viral load cut-off points between 3000-10 000 IU/mL, HCV cAg determines 97.2% of active infections and 100% of therapeutic failures, and for cut-off points above 10 000 IU/mL, approximately 90% of active infections.[41,42] In cases of chronic infection with very low viral loads <3000 IU/mL (estimated at 5.3% of treatment-naïve persons), HCV cAg is undetectable in the presence of HCV RNA.[17,43] Although HCV cAg has a lower sensitivity than first-line antibody tests and a lower detection limit than NAAT (i.e., it requires higher quantities of viral nucleic acid to yield positive results), it maintains the very high specificity desired of second-line tests to confirm HCV active infection, as high as 99.98% in some estimates.[17,42] HCV cAg testing is also useful in predicting sustained viral response 12 weeks off therapy, showing similar kinetics when compared to HCV RNA (p <0.001) during treatment and after follow-up.[44]

Different tests have been introduced in the market to determine HCV cAg, based on chemiluminescence (Abbott ARCHITECT HCV cAg, EIKEN Lumispot HCV cAg, Fujirebio Lumipulse® Ortho HCV cAg) or on enzyme-linked immunosorbent assays (ORTHO® ELISA HCV cAg, Bio-RAD Monolisa HCV Ag-Ab ULTRA, Hunan Jynda HCV cAg ELISA, DiaSorin Murex Ag/Ab EIA).[38] The Abbott ARCHITECT HCV cAg assay can be processed in the same floor-standing analyzer platform used for HCV Ab testing, contributing to diagnostic simplification and enabling reflex testing workflows following HCV Ab-positive tests.[37,41,42]

Advantages of HCV cAg assays in comparison to HCV NAAT are: 70% lower cost per test (8-15 *vs* 30-50 €/unit), stability at room temperature (allowing unrefrigerated transportation), 85% lower turnaround time (45 minutes *vs* 5 hours), and random access to a platform that does not require specimen batching.[37,45] Disadvantages are: the inability to process samples collected in DBS, unavailability in POC form, and requirement of a central laboratory.[22,39]

The overall favorable profile of HCV cAg has prompted scientific societies to recommend that HCV Ab-positive patients be subject to either HCV NAAT or HCV cAg confirmatory testing in serum or plasma.[1,17]

POC HCV NAAT CONFIRMATORY TESTING

In Portugal, HCV Ab-positive test results are usually subject to confirmatory HCV NAAT in hospital settings, upon specialist physician initiative (even if they originated in POC RDT from community-based settings), with a turnaround time of up to one week, due to laboratory-based platforms' need to batch multiple samples.[46,47] Innovative POC testing strategies are needed to address the frequent drop-off following first-line HCV Ab-positive test result notification, enhancing second-line HCV confirmatory testing completion.[48,49] In reports from Australia, Canada, Spain, and USA, only 46%-73% of HCV Ab-positive individuals received

subsequent HCV confirmatory testing, or as low as 18.5% for persons who inject drugs (PWID) in Spain.[25,48,50]

HCV NAAT can now be performed outside of central laboratories, at the point of care, reducing the time from sample collection to diagnosis in a single visit, enabling same-day or next-day confirmation of infection and timely initiation of treatment in test-and-treat approaches.[51] POC HCV NAAT technology enables decentralized screening in community settings, namely primary healthcare centers and facilities caring for high-prevalence populations (e.g., community-based organizations, prisons, addiction treatment centers, needle and syringe programs, mobile outreach services). The ability to perform on-site second-line confirmatory testing empowers formal and informal healthcare organizations to implement reflex testing workflows, regardless of the availability of a central laboratory, accelerating decision-making processes and patient linkage to care. Indeed, POC HCV NAAT has been shown to improve linkage to care, particularly outside the tertiary care setting, for PWID, for the housing insecure population, and for people in prisons — simplifying screening workflows in correctional facilities is particularly important due to high turnover.[14,49,50,52,53] Use of POC HCV NAAT in Spain increased HCV confirmatory testing completion among PWID to 80% of patients receiving same-day confirmatory results.[49]

Different POC NAAT tests have been introduced in the market to determine HCV RNA: Epistem Genedrive®, Molbio® TruenatTM, Cepheid® GeneXpert® HCV Viral Load, and Cepheid® GeneXpert® HCV VL Finger Stick.[37,54] HCV POC NAAT in capillary whole blood by finger-stick sampling is a convenient confirmatory testing method that is highly acceptable to both patients and providers, reducing stress by avoiding venipuncture where venous access is difficult, such as in long-term PWID.[48,50,51] Performance of the Cepheid® GeneXpert® HCV Viral Load and Cepheid® GeneXpert® HCV VL Finger Stick assays in serum, in peripheral venipuncture whole blood, and in capillary whole blood is comparable to that of laboratory-based real-time PCR platforms used in clinical practice (e.g., Abbott RealTime HCV Viral Load, Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV Test 2.0, Siemens VERSANT® HCV Genotype 2.0), with 100% specificity, a lower quantification limit of 100 IU/mL (2 Log10 IU/mL) and an upper limit of linear quantification of 100 000 000 IU/mL (8 Log10 IU/mL).[14,39,46,47,50] Discordant results (i.e., false negatives) have only been recorded in patients under treatment when HCV RNA is near the limit of quantification.[51]

Advantages of POC HCV NAAT assays in comparison to laboratory-based HCV NAAT are: minimal required infrastructure and technical expertise, portability in mobile outreach services, the possibility of finger-stick sampling, limited hands-on time (5 minutes), the short run time (60-105 minutes *vs* 5 hours), multiple simultaneous parallel processing with random access testing, and increased patient linkage to care.[37,46,50,51] Further reduction in time and cost are needed: time to result is still challenging for some settings, and test cartridge cost (30-80 €/unit) and platform cost (9420-71 850 US$ analyzer plus 1896-7800 US$/year warranty, contingent on test cartridge quantity discount contracts) are not affordable for most organizations.[17,46,55-58]

UNEXAMINED DIAGNOSTIC APPROACHES

Our review did not investigate other HCV diagnostic innovations, such as sampling approaches (i.e., self-testing, oral fluid, and DBS) and APRI or FIB-4 fibrosis staging indices. While HIV self-testing is effective for first-line testing, the experience with HCV self-testing is still very limited, and the two realities are not equivalent. On the other hand, self-testing sample volumes are insufficient for second-line HCV testing. Oral fluid is also effective for first-line testing but inadequate for second-line HCV testing in the same sample. DBS testing is effective but arguably more labor-intensive. It requires central laboratories to process samples and patients to return for a second visit to receive their results, often creating delays in result reporting. In summary, despite the merits of these interventions, they are logistically challenging to integrate into reflex testing protocols, which led to the decision to exclude them from the present study. APRI and FIB-4 were excluded because they are not HCV screening tools per se but rather liver fibrosis staging indices, which fall outside the scope of this work.

POLICY AND PRACTICE CHANGE RECOMMENDATIONS FOR HCV DIAGNOSIS IN PORTUGAL

Among the three HCV diagnostic innovations analyzed—reflex or single-step testing, HCV cAg, and POC HCV NAAT—reflex testing emerges as the most immediately viable option for Portugal. Its implementation requires minimal workflow adjustments and technological changes at the ground level. This is particularly noteworthy given its proven effectiveness in the Autonomous Region of Madeira and in neighboring Spain. Reflex testing not only expedites diagnosis but also enhances the linkage to care, effectively addressing delays in treatment initiation. While HCV cAg offers cost benefits, it is constrained by compatibility with a limited number of manufacturers' platforms. POC HCV NAAT delivers quick results but comes with a higher implementation cost. Given these factors, the nationwide adoption of reflex testing could substantially improve HCV diagnosis and care in Portugal, making it a top priority for policy change.

Conclusion

Our review builds on a growing body of evidence pointing to the need to update, implement and monitor HCV screening guidance in Portugal and elsewhere to establish single-step or reflex testing as the standard of care for HCV screening in all healthcare settings. Moreover, updates should also include provisions to allow for the use of HCV cAg or POC HCV NAAT confirmatory testing in alternative to HCV NAAT where appropriate, as a substitute to strictly laboratory-based HCV NAAT. ■

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ICR, IB, SB - Conceção e desenho do estudo, revisão da literatura, análise e interpretação dos dados, redação do manuscrito, revisão crítica do artigo e aprovação da versão final

DM, RAF- Conceção e desenho do estudo, revisão crítica do artigo e aprovação da versão final.

FS - Revisão crítica do artigo e aprovação da versão final

Todos os autores aprovaram a versão final a ser submetida.

Contributorship Statement

ICR, IB, SB - Study conception and design. Literature review. Data analysis and interpretation. Draft of the manuscript. Critical review of the paper. Approval of the final version

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Todos os dados gerados ou analisados durante este estudo estão incluídos neste artigo e nos seus ficheiros de material suplementar. Outras questões podem ser dirigidas ao autor correspondente.

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Correspondence / Correspondência:

Inês Botto ines.botto@gmail.com

Serviço de Gastrenterologia e Hepatologia, Unidade Local de Saúde de Santa Maria, Faculdade de Medicina, Lisboa, Portugal

Av. Prof. Egas Moniz MB, 1649-028 Lisboa

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