

Closing the hepatitis C diagnostic gap: Point-of-care testing, reflex RNA testing and simplified diagnosis

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Abstract

Hepatitis C virus (HCV) infection remains a major public health challenge despite the availability of highly effective direct-acting antiviral therapies. A substantial barrier to elimination is the gap between initial screening, confirmation of active infection and linkage to treatment. Conventional diagnostic pathways commonly require an antibody test followed by nucleic acid testing (NAT) for HCV RNA, creating additional costs, delays and opportunities for loss to follow-up. Emerging approaches, including reflex RNA testing, point-of-care molecular assays, dried blood spot sampling, HCV core antigen detection and self-testing, offer opportunities to simplify diagnosis and extend testing to underserved populations. However, these approaches differ in analytical sensitivity, infrastructure requirements, cost and suitability across clinical settings. This mini-review examines the principal diagnostic barriers and emerging strategies for improving HCV case detection. It argues that diagnostic innovation must be accompanied by decentralised service delivery, quality assurance and effective linkage to care to accelerate progress towards HCV elimination.

Keywords: hepatitis C virus, diagnosis, point-of-care testing, HCV RNA, reflex testing, dried blood spots

1. Introduction

Hepatitis C virus (HCV) infection can cause chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. The introduction of direct-acting antivirals (DAAs), which cure more than 95% of treated patients in appropriate clinical settings, has transformed HCV management [1]. Nevertheless, access to diagnosis and treatment remains uneven, limiting progress towards the World Health Organization's (WHO) goal of eliminating viral hepatitis as a public health threat by 2030 [2].

A central challenge is that HCV diagnosis often requires multiple steps. Initial antibody testing identifies previous exposure but cannot reliably distinguish current infection from spontaneously resolved or successfully treated infection. A reactive antibody result must therefore be followed by a test for active viraemia, usually HCV RNA detection [3]. When testing requires separate appointments, specimen transfers or referrals to central laboratories, patients may be lost before receiving a definitive diagnosis or starting treatment. Simplifying this pathway is consequently an important component of HCV elimination.

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2. Limitations of conventional HCV diagnosis

The conventional diagnostic pathway begins with an HCV antibody test, using a laboratory immunoassay or rapid diagnostic test. Reactive results are followed by nucleic acid testing (NAT) to detect HCV RNA [3]. Detectable RNA indicates current infection, whereas a reactive antibody result with undetectable RNA generally indicates no current infection, although recent exposure and other clinical circumstances may warrant further evaluation [3].

This distinction is fundamental. Antibodies may remain detectable after spontaneous clearance or successful treatment, so seropositivity alone should not be interpreted as evidence of active infection. Conversely, antibody testing may be negative during the early window period following exposure or in some immunocompromised individuals, for whom direct virological testing may be necessary [3–5].

Although the two-step pathway is diagnostically effective, it can impose practical barriers. Patients may need to return for another blood collection, await laboratory results or travel to a specialist facility. These demands disproportionately affect rural communities, people who inject drugs, incarcerated populations and other groups experiencing limited access to healthcare. Diagnostic delays can therefore become failures of linkage to treatment rather than merely technical inconveniences.

3. Emerging approaches to simplified diagnosis

3.1. Reflex HCV RNA testing

Reflex testing addresses an important weakness of conventional diagnosis by automatically performing HCV RNA testing when the initial antibody result is reactive [6]. Laboratory-based reflex testing can use a specimen already collected for antibody testing, reducing the need for an additional visit or blood draw. Clinic-based approaches can similarly arrange immediate collection of a confirmatory specimen following a reactive rapid antibody test [6].

The principal advantage is operational rather than biological: reflex testing does not change what the assays detect, but it reduces the number of actions required from patients and healthcare providers. Its implementation nevertheless depends on laboratory workflows, specimen adequacy, financing and reliable communication of results. Where reflex testing is unavailable, a reactive antibody result may remain unresolved, weakening the effectiveness of screening programmes.

3.2. Point-of-care molecular testing

Point-of-care (POC) molecular assays detect HCV RNA near the site of patient care, potentially allowing diagnosis without referral to a central laboratory [7]. Some platforms use fingerstick capillary blood and provide results within the same clinical encounter [7]. This approach may be especially valuable in community outreach, harm-reduction services, prisons and remote healthcare settings.

A systematic review and meta-analysis by Tang and colleagues found high diagnostic accuracy for POC approach to HCV RNA detection. For the Xpert HCV viral load fingerstick assay, pooled sensitivity and specificity for RNA detection were 98% and 99%, respectively, although implementation requires appropriate training and quality assurance [8]. These findings support the potential of decentralised molecular testing, but performance depends on the assay, specimen type and testing environment.

POC testing also introduces practical considerations, including cartridge costs, equipment maintenance, power supply, consumables and quality-control procedures [9–11]. Its greatest benefit is therefore likely when test placement is matched to local patient needs and when a positive result leads directly to clinical assessment and treatment.

3.3. Dried blood spot sampling

Dried blood spot (DBS) sampling involves collecting a small volume of blood onto specialised filter paper, allowing the specimen to dry before transport and laboratory analysis. It can reduce

dependence on venepuncture, refrigerated transport and conventional blood-processing infrastructure. DBS samples can support antibody testing and, with validated methods, HCV RNA or core antigen testing.

Evidence supports the use of DBS as an alternative specimen pathway, particularly where conventional phlebotomy and laboratory access are difficult [12,13]. However, assay performance varies with the analyte, collection method, storage conditions and laboratory protocol. DBS should therefore be used with appropriately validated assays, and results must be interpreted according to the specific test's performance characteristics.

3.4. HCV core antigen testing

HCV core antigen testing detects a viral protein associated with active infection and offers a potential alternative to RNA-based confirmation [14]. Because it can use immunoassay platforms, core antigen testing may be more accessible in some laboratories than molecular testing. It may also help simplify diagnostic workflows by providing a marker of current infection rather than merely previous exposure [14].

Nevertheless, core antigen assays are generally less analytically sensitive than highly sensitive HCV RNA tests, particularly at low viral loads [15]. A systematic review and meta-analysis of DBS-based HCV core antigen testing reported pooled sensitivity of 85% and specificity of 100%, while noting limitations related to the small number of included studies and heterogeneity [16]. These findings illustrate the trade-off between accessibility and sensitivity. The suitability of core antigen testing depends on the clinical or public health context, and negative results may require further assessment when suspicion of infection remains high.

3.5. HCV self-testing

HCV self-testing allows individuals to collect and interpret their own screening specimens, potentially increasing privacy, convenience and reach among people who do not routinely access healthcare. WHO recommends self-testing as an additional approach to HCV testing services, complementing rather than replacing established diagnostic pathways [17].

Most self-tests detect antibodies, so a reactive result does not establish current infection. Access to confirmatory RNA or another validated test for viraemia remains essential. Self-testing programmes must therefore include clear instructions, accessible confirmatory services, counselling and linkage to treatment. Without these components, increasing screening uptake may not translate into increased treatment coverage.

4. From testing innovation to HCV elimination

No single diagnostic technology is optimal for every population or healthcare system. Centralised laboratory NAT may remain appropriate where infrastructure is reliable, whereas POC testing, reflex testing and DBS sampling may be advantageous where transport, follow-up or venepuncture presents substantial barriers. WHO's consolidated 2026 guidance supports person-centred approaches that integrate testing with decentralised services and treatment pathways [18].

Future progress requires more than improved assay performance. Programmes should monitor the proportion of screened individuals receiving confirmatory testing, time from screening to diagnosis, linkage to treatment, treatment initiation and sustained virological response. Cost-effectiveness, patient acceptability and equity of access should also inform implementation decisions.

Importantly, simplified pathways must preserve diagnostic accuracy. Quality assurance, staff training, validated specimen procedures and appropriate interpretation of discordant results are essential. Research should also evaluate how testing strategies perform across different populations and settings, rather than assuming that findings from well-resourced clinical environments will translate directly to community-based services.

5. Conclusion

The HCV diagnostic gap is increasingly an implementation challenge rather than an absence of effective testing technologies. Reflex RNA testing, point-of-care molecular assays, DBS sampling, core antigen detection and self-testing can reduce barriers at different stages of the diagnostic pathway. Their impact, however, depends on appropriate assay selection, quality assurance and reliable access to confirmatory testing and treatment. Integrating these approaches into decentralised, person-centred services offers a practical route towards earlier diagnosis, reduced loss to follow-up and progress towards HCV elimination.

Data Availability

No new datasets were generated or analyzed for this mini-review. All information discussed in the manuscript are derived from published literature cited in the reference list.

Authors' Contributions

Abubakar Ibrahim: Conceptualization, literature search and synthesis, writing—original draft preparation, supervision, and writing—review and editing. Ola Hamad Salah Rashid: Literature search and synthesis, critical review, and writing—review and editing. Bushra Kanaan Shakir: Literature search and synthesis, critical review, and writing—review and editing. Lukman Yusuf: Conceptualization, supervision, manuscript coordination, and writing—review and editing.

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Competing Interests

The authors declare that they have no competing interests.

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation Process

All content of this manuscript was drafted by the authors, and the grammar was improved using AI. The authors take full responsibility for the accuracy, scientific integrity, and final content of the manuscript.

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