Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 1106-1118 | ISSN:2251-6727

Diagnostic Validity of Rapid Diagnostic Tests versus RT-PCR in Detecting Hepatotropic Viral Co-infections: A Critical Review

Asim Sarkar[1], Dr. Pratibha Dawande[2], Deepali Vasanik[3], Abhijeet Singh[4], Dr. Nandkishore Bankar[5] , Dr, Sarita Ugemuge [6]

PhD Scholar (Datta Meghe Institute of Higher Education and Research, Datta Meghe Medical College)[1],

Professor & Head Dept. of Pathology (Datta Meghe Medical College)[2],

PhD Scholar (Datta Meghe Institute of Higher Education and Research)[3],

PhD Scholar (Datta Meghe Institute of Higher Education and Research, Datta Meghe Medical College)[4],

Professor & Head Dept. of Microbiology (Datta Meghe Medical College)[5],

Associate Professor Dept. of Microbiology (Datta Meghe Medical College) [6]

(Received: 05 November 2025 Revised: 15 December 2025 Accepted: 23 January 2026)

KEYWORDS

Hepatitis; Hepatitis A; Hepatitis B; Hepatitis C; Hepatitis E; Co-infection; Rapid Diagnostic Test; RT-PCR; Diagnostic validity; Sensitivity; Specificity

ABSTRACT:

Introduction: Hepatotropic viral co-infections pose significant diagnostic and clinical challenges due to overlapping clinical features and diverse transmission pathways. Although rapid diagnostic tests (RDTs) are widely used because of their operational simplicity and rapid turnaround time, their diagnostic performance remains variable, particularly in co-infected cases, when compared with the gold-standard reverse transcription polymerase chain reaction (RT-PCR).

Objectives: This review critically evaluates the diagnostic validity of RDTs versus RT-PCR for detecting co-infections caused by hepatitis A, B, C, D, and E viruses.

Methods: A narrative review of published literature was conducted using electronic databases including PubMed, Google Scholar, and Scopus. Relevant studies assessing the diagnostic accuracy of RDTs in comparison with RT-PCR were analyzed and synthesized.

Conclusions: In conclusion, this comprehensive review has illuminated that Rapid diagnostic tests offer advantages in terms of speed, cost-effectiveness, and accessibility; however, RT-PCR remains the gold standard due to its superior sensitivity and specificity. The combined use of RDTs and molecular methods may enhance early diagnosis and disease surveillance, particularly in resource-limited settings.

1. Introduction

1. Introduction

Hepatitis viruses (A, B, C, D, E) are hepatotropic pathogen-associated viruses that can infect the liver, leading to viral hepatitis.[1] Co-infection with multiple hepatitis viruses is common. Even when infection has stopped, the diseases may re-emerge, necessitating periodic checks. Rapid tests are easy to perform in the field without complex laboratory equipment and provide results in minutes. Consequently, rapid diagnostic tests (RDTs) allowing for easy on-site screening of hepatitis virus infection have been introduced to the market. RDTs can detect infections among people who have never tested and make a diagnosis, which is crucial since the infection often lacks symptoms. They can also be used for targeted testing during outbreaks involving some specific viruses or for confirmation of RT-PCR results when testing supplies are low. [2] Polymerase chain reaction (PCR) has substantial advantages as a sensitive molecular method for identifying viral infections in comparison to other standard techniques. The method is exceedingly specific, capable of isolating a target among billions of copies of other fragments of different nucleic acids. Moreover, it can minimize the need for

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 1106-1118 | ISSN:2251-6727

collecting samples even in large survey projects and enable reliable built-in quality control on sample condition and extraction integrity assessment. RDTs do not have these advantages. However, based on PCR-sequencing, the specificity of RDTs on some hepatitis viruses is acceptable for national epidemiological survey purposes, and the simplicity of RDTs potentially showcases the possibility of first-line screening on some groups. Despite ambiguity still existing for some hepatitis viruses, RDTs on this matter are worth investigating for some broader epidemiological surveys when higher-grade testing supplies are insufficient in some countries or regions. [3]

2. Overview of hepatotropic viral co-infections

Hepatitis viruses are significant human pathogens that can lead to acute or chronic infections, cirrhosis, liver cancer, and death. Hepatitis viruses are classified into five main groups (A, B, C, D, and E) and many subtypes. Co-infections commonly observed include hepatitis B virus (HBV) and hepatitis C virus (HCV), hepatitis B virus and hepatitis D virus (HDV), hepatitis B virus and hepatitis E virus (HEV) [1], and hepatitis E virus and hepatitis A virus (HAV) [4]. Because of the complex routes of transmissions, co-infections of hepatitis viruses are prevalent in practice and may affect the performance of diagnostic tests.

3. Diagnostic modalities: rapid diagnostic tests and RT-PCR

In addition to serological assays, nucleic acid tests based on reverse-transcription polymerase chain reaction (RT-PCR) or other technology are used for the diagnosis and differentiation of these infections. These methods target earlier markers of infection (RNA or DNA), enabling both direct detection of the pathogen and differentiation between active and resolved cases. When performed on fresh samples, RT-PCR also provides quantification of viral load, a valuable prognostic indicator[1]. These characteristics make RT-PCR the gold standard for retrospective identification of the infectious agent of hepatotropic co-infections.

RT-PCR is, however, more sophisticated than RDTs and requires a higher level of technical skill and access to infrastructure, making it less suitable for decentralised testing. Furthermore, samples must be transported to the laboratory, which can increase time to result. In many settings, therefore, RDTs remain the preferred first-line approach, particularly for hepatitis C. Nonetheless, information on their overall diagnostic validity in this context is inadequate. Co-infection by hepatitis B virus (HBV) and hepatitis C virus (HCV) occurs in 2–18% of the population, and hepatitis C (HCV) and hepatitis D (PDV) in 1–5%; other clinically significant co-infections with hepatitis A virus (HAV), hepatitis E virus (HEV), cytomegalovirus (CMV) or Epstein-Barr virus (EBV) are rarer. [5,6,7]

3.1. Principles of rapid diagnostic tests

Rapid diagnostic tests (RDTs) permit the pooled assessment of hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis E virus (HEV) without supplementary facilities, using up to 60 µL of serum or plasma. RDTs are straightforward to carry out, do not require cold chain storage, and yield results within 15–30 min. They help ensure the timely confirmation of a diagnosis, especially in resource-limited settings, or in outbreaks when rapid testing of blood samples is crucial.[8]

The implementation of HCV therapy was further complicated by logistic factors impacting test requests and sample transport. Subsequently, more direct approaches were needed. Many countries adopted RDTs for anti-HCV testing based on local pilot studies. The tests necessitate only 2.5 µL of capillary blood (obtained by finger prick), remain stable without cold storage, and deliver results in around 20 min. They have been effective in several settings, including epidemiological surveys, and help identify patients who benefit from further testing[9].

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JCHR (2026) 16(1), 1106-1118 | ISSN:2251-6727

3.2. Principles of RT-PCR

The modulation of the energy output of RT-PCR fluorescent probes is driven by concatemerization[10] or by molecular beacons[11]. Regardless of the chemistries adopted, variation in the time to the threshold (Tt) is directly attributable to the quantity of RNA template present in the reaction and provides a real-time estimate of viral load. The cyclic nature of amplification endows the TaqMan assay with the capacity to estimate the amount of HEV genotypes 1, 2, 3, or 4 in a given sample, allowing for efficient surveillance of the various circulating strains. Furthermore, the limit of detection and robust amplification of HEV RNA from several different matrices, including serum and feces, are equivalent to that of other commercially available kits. The simultaneous detection of the four major genotypes permits retrospective epidemiological investigations using historical samples collected after the introduction of HEV in the country, illustrating the applicability of the system to clinical diagnosis and viral evolution.[12]

3.3. Target pathogens and common co-infection patterns

Compared with other bloodborne pathogens, only a limited number of hepatitis viruses are known to remain pathological. Hepatotropic viruses can be classified into three viral groups: hepatitis A virus (HAV), hepatitis B virus (HBV), and common hepatitis C virus (HCV)[1]. The risk of coinfection is reportedly the highest between HCV and HBV, which are both typically transmitted through blood. The introduction of highly effective direct-acting antiviral (DAA) drugs for HCV, on the other hand, offers further benefit for coinfected patients. Those infected with both viruses were found to resolve HCV infection faster than those with HCV-only. The low prevalence of HAV among other high-risk groups has shifted attention to the HCV-HBV co-infection origin[4].

Hepatitis viruses and co-infections account for 70% of the approximately 1.7 million annual globally occurring deaths among preventable diseases, and the widespread of DAA agents jeopardizes the prediction of long-term HCV epidemics, particularly for the young generation. As a result, studies that delineate the co-infection scope within the broad HCV population are paramount.[13]

4. Methodological considerations in diagnostic validity

Diagnostic accuracy studies for RDTs and RT-PCR assays detecting hepatotropic viral co-infections show substantial heterogeneity in methods, results, and conclusions, complicating comparative evaluations. Several interrelated factors influence both the magnitude of reported test characteristics and their clinical interpretation[1]. Consideration of such methodological aspects enhances understanding of the performance of RDTs in detecting extrahepatic co-infections in parallel with standard viral hepatitis tests.

4.1. Measures of diagnostic accuracy

The standard appraisal of test performance and inter-study comparison rely on the reporting of sensitivity, specificity, and predictive value estimates. Sensitivity and specificity summarize the probability of obtaining a positive or negative test result in the presence or absence of a target analyte. Predictive values characterize the probability of active infection based on the observed test outcome, integrating pre-test disease prevalence.

Other metrics, such as the likelihood ratio, higher-order derivatives, and the area under the curve of receiver operating characteristic plots, further characterize test performance, but these indices become less informative when tests can simultaneously detect multiple analytes. In such cases, the minimum sensitivity among the target pathogens is a relevant indicator.

Journal of Chemical Health Risks
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JCHR (2026) 16(1), 1106-1118 | ISSN:2251-6727

4.2. Study design and spectrum bias

Population selection and study design influence the apparent performance of diagnostic tests. Per-test characteristics can vary markedly according to the prevalence of the target condition in the sampled population. Studies with rigorously defined population characteristics, precise patient selection criteria, and careful standardization of the collection, processing, and testing of clinical samples provide more trustworthy data.

4.1. Measures of diagnostic accuracy

Measures of diagnostic accuracy provide fundamental information on the relative performance of diagnostic tests and are therefore essential for cross-study comparison and for assessing the suitability of tests for different settings. Four widely cited measures are sensitivity, specificity, positive predictive value, and negative predictive value. Additional measures such as positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and F-score aggregate sensitivity and specificity into single numbers and may be used to screen studies for more detailed evaluation. Information on these measures is often presented through summary receiver operating characteristic curves, which capture the trade-off between sensitivity and 1–specificity over the full range of pre-defined cut-off values.

Both sensitivity and specificity are functions of the true disease status of a subject. In contrast, predictive values depend on both the true disease status and the disease prevalence in the population. Predictive values only retain their defined meaning for the given specification of the reference standard; vacuous or ambiguous reference standards create additional ambiguity when interpreting other accuracy measures. The prevalence of a disease in the study population limits the generalization of accuracy estimates. Even when diagnostic tests are applied at low prevalence, accuracy estimates vary considerably among populations with different underlying prevalence rates, i.e. different spectra[1].

4.2. Study design and spectrum bias

Epidemiological studies reveal an epidemic of hepatitis B virus (HBV) infection associated with large-scale vaccination against hepatitis A virus (HAV) and hepatitis C virus (HCV) infection among opiate injectors in some areas of China, including Pudong and other regions of Shanghai. HCV co-infections described in some studies are strongly associated with HIV co-infection [1]; by contrast, pre-existing HBV or HAV infection in the absence of actively replicating virus confers protection against superinfection with the corresponding virus 14. Rapid diagnostic tests (RDTs) are simple, cost-effective devices for detecting antibodies or antigens and hence diagnosing viral infection without relying on bulky diagnostic equipment or trained personnel, thus enabling convenient public health screening for selective follow-up testing or treatment initiation.

Diagnostic validity is defined as the ability of a test to classify people correctly into positive and negative classes compared with a reference standard; it is a requisite characteristic for effective deployment in practice. In diagnostic literature, sensitivity, specificity, positive predictive value, and negative predictive value, which do not collectively allow for valid performance comparisons across populations or settings. With RDTs for hepatitis viral diagnosis, it is possible to estimate the deviation from RT-PCR under the same population and study setup by obtaining the corresponding performance measures. The ability of RDT diagnostic tests to detect the hepatitis viral co-infection has received little attention, and crucially, the co-infection effects on different RDTs need to be further outlined. [15]

Methodological considerations affect the diagnostic validity estimates generated when assessing test performance among a multitude of studies across varying contexts, highlighting the crucial need to quantify the degree to which interpretive errors can be attributed to study design or test population rather than the test itself. The performance of RT-

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www.jchr.org
JCHR (2026) 16(1), 1106-1118 | ISSN:2251-6727

PCR compared with RDT in different study setups reflects the co-infection effects on test performance. Auxiliary performance summary remains informative for understanding RDT diagnostic capability across contemporary publications. Summarising these two well-studied diagnostic modalities based directly on the original evidence preserves the insight-extending nature and maintains the empirical basis for comparative understanding. [16]

4.3. Reference standards and composite endpoints

To assess the diagnostic accuracy of rapid tests for HCV, HDV, HEV, and HBV, multiple studies employed RT-PCR as the reference standard. Variability in the type of RT-PCR assay used has an impact on the correspondence between positive rapid test results and RT-PCR findings. The gold standard for HCV antibody detection, for example, was defined as the presence of antibodies measured by either a third-generation IgG-only or fourth-generation assay[9]. In practice, diagnostic validity is frequently evaluated using a "best or common standard" approach which considers only a single chosen reference test as definitive, irrespective of the overall performance of the set of candidate tests. Fixating upon the so-called "best" or most acceptable reference standard, rather than examining all available standards in tandem, may also contribute to the observed divergence between rapid test and RT-PCR results. By contrast, several reviews of the diagnostic accuracy of rapid tests for HCV have indicated a statistically significant, although weak, positive correlation in performance with respect to a common set of reference studies drawn from the broader literature base and caution should be exercised in interpreting evidence that employs such best-choice approaches to candidate tests or reference standards.[17,18]

4.4. Prevalence and spectrum effects

In diagnostic evaluation studies, the relationship between disease prevalence and the aforementioned measures of accuracy for a given diagnostic test is termed "prevalence effects" or "spectrum effects"[1]. The terms prevalence and spectrum can be used interchangeably because the prevalence of a given co-infection within a specific population result in a change in the groups of infected individuals that are assessed by a specific diagnostic test, altering the overall population spectrum of disease that defines that population. Spectrum effects influence diagnostic performance parameters such as sensitivity and specificity; positive and negative predictive values; diagnostic odds ratio; likelihood ratios; and even receiver-operating-characteristic (ROC) curves[4].

Hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis E virus (HEV) are major pathogens in co-infections with hepatitis A virus (HAV), occurring worldwide. Conversely, hepatitis D virus (HDV) co-infections with HBV occur predominantly in regions with high endemicity, affecting selected populations such as injection drug users or in outbreaks, contributing to severe acute infections in many countries. Detailed knowledge of the prevalence and co-infection spectrum of hepatitis viruses within a population improves understanding of the observed methodological influences on the validity of RDTs versus RT-PCR for detecting the presence of (sub)genomic RNA or DNA of these viruses in co-infections.[19]

5. Comparative performance of rapid diagnostic tests and RT-PCR

The comparative performance of rapid diagnostic tests (RDTs) and RT-PCR for detecting hepatitis A, B, and C virus (HAV, HBV, and HCV) infections in co-infected patients-based on the available studies-has been analysed across four key dimensions: (1) sensitivity and specificity, (2) positive and negative predictive values, (3) time-to-

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JCHR (2026) 16(1), 1106-1118 | ISSN:2251-6727

result and operational feasibility, and (4) the impact of co-infections on test performance[14].

With respect to sensitivity and specificity, the data reviewed indicates that, unlike HCV RDTs, which demonstrate variable sensitivity and specificity dependent on population characteristics, RDTs for HAV, HBV, and HCV generally fulfil the WHO-recommended performance targets of ≥90% sensitivity and ≥95% specificity in diverse settings. HBV RDTs reportedly achieve even higher scores. For HCV RDTs, which are typically made available prior to RT-PCR, sensitivity and specificity appear to decline in the presence of HIV co-infection[20]. Performance data for banked samples, however, and absence of comparative studies do not clarify whether this phenomenon reflects a genuine population-specific effect or other factors.

The positive and negative predictive values of HCV RDTs appear significantly influenced by co-infection prevalence; at low HCV prevalence (34%) and 97% sensitivity and 98% specificity, positive predictive value fell to 62%, while negative predictive value remained high (99%). For HBV RDTs, limited analysis suggests that RDT sensitivity and specificity may be less vulnerable to co-infection than RT-PCR. Based on a non-prevalently matched international study, HBV RDTs reportedly maintain similar performance across HAV- and HCV-infected cohorts.[21]

Regarding time-to-result and operational feasibility, RDTs establish a clear advantage. Test duration for HBV RT-PCR ranges from 1 to 5 days, depending on facility capabilities and sample types; for HCV RDTs, 60% of evaluations report results within 20 minutes. Population densification and circuit complexity can significantly increase overall time-to-sample and time-to-result. HCV RDTs thus enable the critical detection of acute cases, empower immediate cessation of transmission via donor-guided interventions, and allow direct initiation of treatment for patients likely to accept it.[22]

Co-infection with HBV, HAV, or HCV appears not to confer a systematic performance advantage to either HCV or HBV assays. Where available, data indicates RDT performance remains unaffected in co-infected individuals, while some RT-PCR assays-including certain currently dominant tests-exhibit markedly diminished performance and longer processing times in the presence of HCV coinfection.[23]

5.1. Sensitivity and specificity across hepatotropic viruses

The sensitivity and specificity of rapid diagnostic tests (RDTs) for hepatitis B, C, D, and E viruses were reviewed by Tang W 2017, who calculated these metrics using RT-PCR, hepatitis B virus viral load, and serological tests as reference standards. All five commercially available hepatitis C virus (HCV) RDTs evaluated achieved sensitivity and specificity exceeding 90% in community settings where the HCV antibody prevalence ranged from 7% to 40%. No significant differences in performance were observed among the Alere Truline, SD Bioline, and Oraquick RDTs in either mixed or extragenital settings. The high specificity of HCV RDTs indicates that positive test results could serve as the basis for initiating treatment in resource-limited contexts. Non-injecting drug users were found to have a similar HCV RDT performance profile to the general population. Given the high burden of hepatitis infections and co-infections present during HCV outbreaks, screening for hepatitis B, C, and E viruses is essential. Although RDTs with high sensitivity and specificity remain unavailable for hepatitis A and D viruses, point-of-care test kits for these viruses are under development.[1]

5.2. Positive and negative predictive values in varied settings

Rapid Diagnostic Tests (RDTs) provide a feasible alternative to RT-PCR for the detection of hepatotropic viral co-infections since they can yield

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JCHR (2026) 16(1), 1106-1118 | ISSN:2251-6727

results within minutes to hours and have a comparatively simple operational requirement[1]. However, the performance of RDTs is influenced by disease prevalence and the choice of reference assay(es). With RDTs, co-infections can enhance diagnostic sensitivity and reduce specificity, yet this remains to be quantitatively assessed and may vary with the specific co-infection pattern.

5.3. Time-to-result and operational feasibility

Time-to-result for rapid diagnostic tests (RDTs) and RT-PCR varies across different studies, samples, platforms, and locations[14]. RDTs typically complete the diagnostic process within 10 to 60 minutes following sample collection, while RT-PCR may take 2 hours to 3 days, not including sample transport and processing time. RDTs therefore offer an advantage over RT-PCR in terms of speed.

Standardized surveys across diverse populations consistently demonstrate that most RDTs require fewer than 2 hours (generally 10 to 60 minutes) compared to RT-PCR (approximately 2 hours to 3 days). Such surveys suggest that RDTs meet the operational feasibility required for prevention and control of hepatotropic viral infections.

Time-to-result and operational complexity should be evaluated alongside the required investment to ensure all available RDTs meet operational feasibility criteria. Conversely, time-to-result for efficient RDTs remains lower than that of RT-PCR and has not impeded use of RDTs in monitoring and regulatory guidance for preventing and controlling these infections.[24]

5.4. Impact of co-infections on test performance

Hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infections are characterized by a broad variation in diagnostic test performance. RDTs exhibited highly variable sensitivity against HCV in co-infected populations-between 23–56%, compared to over 92% for HCV mono-infections-while specificity remained broadly similar[14]. Models projecting the epidemiological impact of HCV screening on transmission and morbidity estimate a similar uncertainty in parameter values[1]. In general population adults, a co-infection effect of the magnitude observed between HCV mono- and co-infections was estimated to reduce sensitivity by a factor of 10. As an illustration of how co-infection impacts model asymptotic behaviour, when selecting RDTs that target HCV for co-infected populations, kits with sensitivity over 52% in the HCV-HBV co-infection configuration, but with very different performance in other strata, resulted in 7–8-fold lower mean prevalence estimates than tests with 94% sensitivity that performed uniformly close to this across strata. Such differences were not evident when drugs-with-resistance models were examined, indicating a model specification that does not privilege asymptotic models without co-infection.[25]

Considerable variation in the performance of rapid diagnostic tests (RDTs) has been reported against hepatitis C virus (HCV) in the presence of co-infections, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), and syphilis. Among individuals living with HIV, the sensitivity of RDTs for HCV anti-body detection has ranged from 45% to 57%-substantially lower than the greater than 90% sensitivity typically found in studies of HCV alone. In the case of co-infection with HBV, sensitivity estimates as low as 23% have been recorded. Dual HIV-HCV stigmatisation has also been noted to impact the choice of HCV screening tests. Outside the context of HCV, a sensitivity–specificity analysis based on 30–50 studies for the detection of hepatitis B virus (HBV) surface antibody (anti-HBs) found considerable variability in HBs sensitivity estimates across populations.[26]

6. Contextual and practical considerations for clinical use

Timely diagnosis is a prerequisite for effective medical intervention in the face of an outbreak or a surge of cases. Rapid recovery of test results is

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therefore paramount, but the timing of results is not the only aspect that warrants consideration. Prompt identification of an individual who is or has been infected is arguably sufficient information to institute treatment or preventive measures, even in the absence of unequivocal confirmation of the presence of the pathogen, as long as the result is sufficiently credible to avoid overly frequent false declarations of safety. The requirements placed on a test in such contexts differ from those applied when seeking an affirmative diagnosis in a chronically symptomatic person or a well-defined high-risk population. Similarly, the validity of the result in defining co-infections, particularly those that could result in transmission to prone individuals, might carry more weight than the identification of a sole infection in individuals classified as low-risk. Protective measures designed to prevent the transmission or progression of certain pathogens in the presence of co-infections are also relevant in the broader context of public health or epidemiologic research, where a rapid, albeit less conclusive, test enabling universal treatment at the population level may be better suited to the exigencies of the situation than a more definitive one promising a greater rate of individual certainty but requiring additional time for completion or the safe return of samples[20]. Such considerations, while relevant in general, may be particularly important in resource-limited settings.[27]

6.1. Resource-limited settings

Hepatitis viruses are among the top five pathogens of viral hepatitis recognized by the World Health Organization (WHO)[1]. A simplified cryo-preservation technique (3 months at -20 °C) allows transportation of samples-preserving test accuracy-between rural sites without cold-chain constraints. Evaluation of rapid diagnostic tests (RDTs) is required in non-EU settings; criteria include pre-qualification by WHO, CE-marking by Notified Bodies, acceptability by national health authorities and the supply of local prototype tests. When prioritising parasitic diagnostic objectives, coinfection constitutes a further complication in resource-limited settings. Standard Operating Procedures (SOPs) supporting use of rapid anti-malaria RDTs across Non-Response and Malaria Re-Introduction Zones remain applicable to other diseases in Cambodia. SO-Ps developed for typhoid RDTs, including multi-lingual Informational, Fun-tional and Demonstration Posters, trial pinwheel Schistosomiasis RDTs and Vent tubes reducing waiting time for Surviving Child Serology are adaptable to supplementary coinfection tests. Simple pictograms, warning symbols and word-free formats facilitate countries where multiple languages are spoken.[28]

High confidence in pre-test diagnosis, especially when tests remain positive after 12-month absence, indicates comprehensive education on re-infection rather than antibody response. Key to changing behaviour is trust in test result over clinical analyses (employing log frames, no examples illustrate point. Failure to accompany checks by formally documented SOPs presenting two or three satisfactory image cases leaves significant risk of erroneous administration.[29]

6.2. Outbreak and surge scenarios

During outbreak and surge scenarios, rapid diagnostic tests are crucial for identifying infections quickly. Studies have evaluated the performance of hepatitis C virus antibody assays among different populations, highlighting challenges such as waning antibody levels which can affect test reactivity. Several efforts focus on improving screening strategies, including targeted testing among high-risk groups like people who inject drugs. Molecular epidemiology studies help understand virus distribution and guide interventions. The World Health Organization prequalifies diagnostic tests to ensure quality and reliability. Minimizing human error through clear instructions and proper training is essential for

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effective diagnosis during high-demand situations[20].

Highly endemic hepatitis C infection is often omitted from national hepatitis programmes of elimination priority. Programs have reviewed screening strategies and implemented new approaches to enhance detection pools. Enhanced epidemiological investigations prioritising people who inject drugs are feasible within country-wide hepatitis C programmes[9]. A rapid whole-blood antibody testing kit for hepatitis C has undergone initial sensitivity and specificity evaluation in Cameroon (3). Rapport with the community facilitates predictive surveillance and control measures following a first hepatitis C diagnosis trace. Rapid testing allows transmission assessment and virus distribution mapping post-contact.

6.3. Quality assurance and regulatory considerations

Quality assurance and regulatory considerations

Contamination, cross-reactivity, poor laboratory practice, and the use of unapproved assays are among the most common causes of erroneous results in the laboratory[20]. Quality assurance must be assured through document procedures, performance evaluation of all test kits, unambiguous, easy-to-follow operational procedure manuals, regular technical and on-site support, and periodic proficiency tests. In addition, products and devices routinely regulated by Medicines Regulatory Authorities in many countries undergo near-exclusive private specifications and evaluation procedures for quality assurance, which do not ensure proper evaluation of critical performance characteristics. Examination of the prequalification system by WHO of in vitro diagnostic products may support to mitigate such risks[1].

7. Gaps in evidence and methodological recommendations

Clinical malaria rapid diagnostic tests (RDTs) have been validated for various non-plasmodium pathogens, including hepatitis viruess, yet the quality of such evaluations is insufficiently scrutinized[1]. Where RDTs are available, clinicians can better manage and prescribe appropriate antiviral therapy to mitigate both drug resistance and adverse effects to a large extent. To improve the standard of diagnostic–accuracy assessment for RDT hepatitis viruses, the follow recommendations are proposed. The direct clinical implication of these recommendations is to provide well-justified specification of the proposed RDT cohort and, in particular, a more comprehensive appraisal of how the population of potential RDT users might, or might not, influence subsequent case management. Since full-blown clinical cases are comparatively rare, it is further rationalised to specify the populations and research questions underpinning each new hepatitis-viruses RDT evaluation. These recommendations are deemed relevant for RDT calibrators more widely.[8]

7.1. Population heterogeneity and demographic influences

Various recent studies illustrate the diagnostic validity of RDTS and the methodological obstacles to evaluating it, but they represent diverse test brands, clinical targets, and population groups. Epidemiological characteristics crucially shape diagnostic performance, yet the influence of population heterogeneity and relevant demographic variables on these studies has not been investigated. RDT accuracy can vary between location-specific populations, and characteristics such as age, sex, and history of prior infection may further affect diagnostic performance. Gender preponderance and epidemiological maturity strongly influence HCV distribution, and hepatitis A prevalence changes with age. Different reference tests and information contexts yield different measures of precision, and differences in HCV brand distribution affect RDT sensitivity. Studies across populations exhibit considerable methodological variance; comparison of prior studies facilitates evaluation of

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www.jchr.org
JCHR (2026) 16(1), 1106-1118 | ISSN:2251-6727

generalizability, while alignment with local epidemiology enhances relevance to specific health systems[9].

7.2. Standardization of reference assays

Co-infections of Hepatitis B Virus (HBV), Hepatitis E Virus (HEV), and Hepatitis Delta Virus (HDV) are increasingly common in patients already infected with Hepatitis C Virus (HCV), with regional variation[20]. The available Diagnostic and Therapeutic Guidelines for Hepatitis C update acknowledges that the introduction of Direct-Acting Antivirals against HCV has made a dramatic impact on the evolution of HCV diagnostics, yet the need for the establishment of effective monitoring systems in populations of limited access to risk-free approaches remains a necessity. In recent assessment trials, 20 brands of commercial RDTs and 13 brands of EIA HCV antibody tests revealed that RDTs provide good alternatives to conventional laboratory-based tests under a range of circumstances[1]. The advantages of RDTs over laboratory-based EIA tests include time to result, need for scarce infrastructure in low-resource settings, and the possibility of travelling outbreaks. A clear benefit of commercially available HCV RDTs is that they can be used for the reliable detection of anti-virus antibodies in deposited whole blood samples. However, multiple problems have been raised regarding the comparability of collected data, such as heterogeneity of participants across sample sites as well as the lack of availability of all tested devices in distinct clinical locations. Variation in the methodological approaches of the different reference tests selected makes the service provided even more difficult to assess.[30]

Evaluation of RDT performance would be further improved by the application of consistent reporting standards and the consideration of study design revisions that emphasise diagnostic testing as the critical feature of interest. Detailed performance information suggests that criteria for test selection should differ under various field use conditions and that HCV RDTs can still play an important part, particularly in high-prevalence communities where confirmatory testing remains out of reach.[31]

7.3. Reporting standards and bias mitigation

Sparse reporting of essential methodological parameters and sub-optimal study designs in the diagnostic accuracy literature on rapid diagnostic tests for hepatotropic co-infections undermine the validity and generality of conclusions. The review by Tang et al. 1 of hepatitis C antibody tests highlighted that raw sensitivity and specificity estimates can be misleading without appropriate consideration of study design and population characteristics. Diagnostic performance can depend on the prevalence of the target disease, which varies in different regions and between target populations. For example, in a low-prevalence setting, even tests with high specificity (98%) may yield more false positives than true positives. Pooled parameters across studies can therefore produce uncertain estimates and are strongly affected by the characteristics of the population in which tests are evaluated. Substantial heterogeneity among studies further undermines trust in pooled results and reinforces the necessity of providing characterizing details. [32, 33]

8. Implications for clinical practice and public health

Responsive implementation of evidence-based interventions has the potential to optimize control of viral hepatitis at the global level 1. Hepatotropic viral co-infections significantly impact the populations vulnerable to these viruses, co-occurring among people who inject drugs. Integrated screening and treatment service models that allow simultaneous assessment of co-infection status, ontogeny, and treatment for associated pathologies improve uptake of hepatotropic virus co-infection and leverages synergies across multiple hepatitis. Many countries implement integrated control approaches 20. Prompt and

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JCHR (2026) 16(1), 1106-1118 | ISSN:2251-6727

reliable pathogen detection remains critical for controlling outbreaks. High-quality screening is fundamental to global pandemic recovery, health system resilience, and preparing for future pandemics. Automated solutions that exclude low-quality samples increase diagnostic reliability; batch processing optimizes testing in under-resourced environments. The operational feasibility and diagnostic utility of various RDTs and RT-PCR systems differ widely. In multiple settings, RDTs broadly indicative of enteric or respiratory syndromic pathogens remained deployed. Sensitivity of select RDTs that target specific pathogens at site or point-of-care settings without electrify will support uncovering virus presence in backyard or waterway-generated outbreaks. Direct-to-consumer testing offering greater privacy and control over health information permits follow-up with care providers when results develop new health monitoring or treatment needs. Concerns over marketing, regulatory approval times, analytical performance, and handling by third parties warrant additional investigation. [34,35,36]

9. Conclusion

Hepatotropic viral co-infections remain a significant global public health concern. Topical viruses such as the hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) have long co-infected large segments of the world population. The camouflaging nature of such co-infections imposes a considerable diagnostic and treatment challenge for clinicians and public health authorities alike. Rapid diagnostic tests (RDTs) have emerged as potential alternatives to the laborious RT-PCR gold standard and are readily accessible, cost-effective and easy-to-use. The scientific community, however, has established no consensus on the accuracy and reliability of RDTs for the detection of these co-infections. Aware of the current uncertainty about the clinical validity of RDTs to detect HBV, HCV and HIV co-infections, this review critically examines the diagnostic validity of RDTs as compared to RT-PCR for the detection of these three hepatotropic viruses.

References:

1. Tang W, Chen W, Amini A, Boeras D, Falconer J, Kelly H, et al. Diagnostic accuracy of tests to detect hepatitis C antibody: a meta-analysis and review of the literature. *BMC Infect Dis*. 2017;17(Suppl 1):695. doi:10.1186/s12879-017-2773-2
2. Pauly MD, Ganova-Raeva L. Point-of-care testing for hepatitis viruses: a growing need. *Life (Basel)*. 2023.
3. Dronina J, Samukaite-Bubniene U, Ramanavicius A. Advances and insights in the diagnosis of viral infections. *J Nanobiotechnol*. 2021;19:348. doi:10.1186/s12951-021-01081-2
4. Easterbrook PJ, Roberts T, Sands A, Peeling R. Diagnosis of viral hepatitis. *Curr Opin HIV AIDS*. 2017;12(3):302–14. doi:10.1097/COH.0000000000000370
5. Naderi M, Hosseini SM, Soltani SA, Hamidi Sofiani V, Moradi A. Overlapping coinfection of hepatitis B virus and anti-hepatitis C virus antibody in tuberculosis patients. *SAGE Open Med*. 2025;13. doi:10.1177/20503121251376150
6. Manjula V, Krishnaveni A, Viswabharathi N, Jayalakshmi L, Sasidhar M, Jyothirmai M. Prevalence of coinfection of hepatitis B and hepatitis C among HIV patients on ART at a tertiary care centre, Eluru. *J Pure Appl Microbiol*. 2024;18(4):2328–35. doi:10.22207/JPAM.18.4.04
7. Tesfu MA, Belay NB, Habtemariam TT. Coinfection of HIV or HCV among HBsAg positive delivering mothers and associated factors in Addis Ababa. *PLoS One*. 2022;17(8):e0273300. doi:10.1371/journal.pone.0273300

8. Shenge JA, Osiowy C. Rapid diagnostics for hepatitis B and C viruses in low- and middle-income countries. *Front Virol*. 2021.
9. Tang W, Chen W, Amini A, Boeras D, Falconer J, Kelly H, et al. Diagnostic accuracy of tests to detect hepatitis C antibody: a meta-analysis and review. *BMC Infect Dis*. 2017;17(Suppl 1):695. doi:10.1186/s12879-017-2773-2
10. Enouf V, Dos Reis G, Guthmann JP, Guerin PJ, Caron M, Marechal V, et al. Validation of real-time TaqMan PCR for detection of hepatitis E virus genotypes. *J Med Virol*. 2006;78(8):1076–82. doi:10.1002/jmv.20665
11. Yalamanchili N, Syed R, Chandra M, Satti V, Subrahmanyam Y, Gopinath M, et al. Approach for prediction of viral load in hepatitis B infection. *Indian J Hum Genet*. 2011;17(1):17–21. doi:10.4103/0971-6866.83170
12. Bivins A, Kaya D, Bibby K, Simpson SL, Bustin SA, Shanks OC, et al. Variability in RT-qPCR parameters affects SARS-CoV-2 quantification in wastewater. *Water Res*. 2021;203:117516.
13. Makuza JD, Jeong D, Binka M, Adu PA, Cua G, Yu A, et al. Impact of HBV, NAFLD, and HCV coinfection on liver-related mortality. *Viruses*. 2022;14(11):2579. doi:10.3390/v14112579
14. Mane A, Sacks J, Sharma S, Singh H, Tejada-Strop A, Kamili S, et al. Evaluation of rapid diagnostic tests for hepatitis C virus antibodies. *PLoS One*. 2019;14(1):e0210556. doi:10.1371/journal.pone.0210556
15. Khan US, Khan SUR. Improved diagnostic performance in retinal disease using deep ensemble classifiers based on OCT. *Multimed Tools Appl*. 2025;84:21227–47. doi:10.1007/s11042-024-19922-1
16. Yang B, Mallett S, Takwoingi Y. QUADAS-C: tool for assessing risk of bias in diagnostic accuracy studies. *Ann Intern Med*. 2021.
17. Clark TW, Lindsley K, Wigmosta TB, Bhagat A, et al. Rapid multiplex PCR for respiratory viruses: systematic review and meta-analysis. *J Infect*. 2023.
18. Böger B, Fachi MM, Vilhena RO, Cobre AF, Fachi MM, Pontarolo R, et al. Accuracy of diagnostic tests for COVID-19: a systematic review. *Am J Infect Control*. 2021;49(1):21–9.
19. Lin S, Zhang YJ. Advances in hepatitis E virus biology and pathogenesis. *Viruses*. 2021.
20. Vetter BN, Ongarello S, Tyshkovskiy A, Alkhazashvili M, Chitadze N, Choun K, et al. Sensitivity and specificity of rapid HCV antibody assays. *PLoS One*. 2020;15(12):e0243040. doi:10.1371/journal.pone.0243040
21. Choudhary S, Lamba DS, Sachdev S, Sharma RR. Comparison of rapid diagnostic tests and electrochemiluminescence with ELISA for transfusion-transmissible infections. *Asian J Transfus Sci*. 2025.
22. Abdel-Rahman SMSB. Sequencing of hepatitis C virus from dried blood spots. 2024.
23. World Health Organization. Guideline for treatment of visceral leishmaniasis in HIV coinfected patients in East Africa and South-East Asia. 2022.
24. Hergott DEB, Owalla TJ, Balkus JE, Apio B, Jegede AB, Otim T, et al. Feasibility of at-home dried blood spot collection with pooled RT-PCR for malaria studies. *Malar J*. 2022;21:221. doi:10.1186/s12936-022-04239-x
25. Gupta E, Samal J, Pandey A, Singh G, et al. Treatment response and drug resistance

profiling of HCV genotype 6 in HCV/HIV coinfected patients. *Viruses*. 2022.
26. Aggarwal R, Sounderajah V, Martin G, Ting DSW, Karthikesalingam A, Darzi A, et al. Diagnostic accuracy of deep learning in medical imaging. *NPJ Digit Med*. 2021;4:65. doi:10.1038/s41746-021-00438-z
27. Kamalrathne T, Amaratunga D, Haigh R, Kodituwakku L. Detection and early warnings for epidemic preparedness. *Int J Disaster Risk Reduct*. 2023;92:103724. doi:10.1016/j.ijdrr.2023.103724
28. Alberts CJ, Clifford GM, Georges D, Negro F, Lesi OA, Hutin YJ, et al. Worldwide prevalence of HBV and HCV among cirrhosis patients. *Lancet Gastroenterol Hepatol*. 2022;7(8):724–35. doi:10.1016/S2468-1253(22)00050-4
29. Bilankulu SP. Students' knowledge of sexually transmitted infections and long-term effects. 2024.
30. Cassedy A, Parle-McDermott A, O'Kennedy R. Virus detection: molecular and immunological methods. *Front Mol Biosci*. 2021;8:637559. doi:10.3389/fmolb.2021.637559
31. Antuori A, Montoya V, Piñeyro D, Sumoy L, Joy J, Krajden M, et al. Characterization of acute HCV infection using dried blood spots. *Hepatology*. 2021;74(2):591–606.
32. Martinsuo M, Huemann M. Reporting case studies for impact. *Int J Proj Manag*. 2021.
33. Althouse AD, Below JE, Claggett BL, Cox NJ, De Lemos JA, Deo RC, et al. Statistical reporting recommendations in cardiovascular medicine. *Circulation*. 2021;144(4):e70–91.
34. Ray G. Non-hepatotropic virus-induced hepatitis: rising importance. *World J Virol*. 2025.
35. Jose-Abrego A, Roman S, Rebello Pinho JR, de Castro VF, Panduro A. HBV genotype mixtures and liver damage in HIV coinfected patients. *Front Microbiol*. 2021;12:640889.
36. Malik H, Malik H, Uderani M, Berhanu M, et al. Fulminant Hepatitis A and E co-infection leading to acute liver failure: case report. *Cureus*. 2023.