Journal of Clinical Virology 185 (2026) 105953

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

A lateral flow immunoassay combining HCV antigen and antibody detection for improved early diagnosis

Lay Sin Teoh [a], Kaosalya Murali [b], Muhammad Firdaus Bin Md Ibrahim [b], Nurul Huda Binti Mohamed Rashidi [b], Haris Ong [a], Matthew Mak [a], Yang Du [a], Narcisse Mary A/P Sither Joseph Vesudian [c], Syafinaz Amin-Nordin [b,c], Niazlin Mohd Taib [b,c,*], Zhihai Zhao [a,**], Na Xu [a,**]

[a] Department of Research and Development, MP Biomedicals Asia Pacific Pte Ltd, Singapore, Singapore
[b] Department of Medical Microbiology, Hospital Sultan Abdul Aziz Shah (HSAAS), Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia
[c] Department of Medical Microbiology, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia

ARTICLE INFO

Keywords:
Hepatitis C virus (HCV)
HCV antibodies
HCV core antigen
Rapid test
Lateral flow immunoassay
Diagnostic performance

ABSTRACT

Background: Rapid and accurate detection of hepatitis C virus (HCV) infection is essential for early diagnosis, timely intervention, and cost-effective disease management. Conventional antibody-based assays may fail to detect infection during the acute phase, highlighting the need for diagnostic tools capable of identifying both HCV antibodies and antigens.

Methods: A total of 2501 serum and plasma specimens (778 HCV-positive, 1723 HCV-negative) were evaluated using the ASSURE HCV Ab/Ag Rapid Test. Diagnostic performance was assessed against reference assays, including an independent clinical evaluation in Malaysia. Analytical sensitivity, cross-reactivity, interference, seroconversion performance and specimen equivalency were also examined.

Results: The assay demonstrated 100% sensitivity (778/778), 99.83% specificity (1720/1723), and near-perfect agreement with reference assays (Kappa = 0.998). Analytical evaluation confirmed robust antibody and antigen detection across serial dilutions. No cross-reactivity was observed across 30 antibody panels, and no interference was detected from 19 endogenous or exogenous substances. Seroconversion analysis identified infection ~17 days earlier than antibody-only comparators. Performance was fully equivalent across serum, plasma, venous whole blood, and finger-prick whole blood.

Conclusions: The ASSURE HCV Ab/Ag Rapid Test demonstrated high sensitivity and specificity for HCV screening, combining antibody and antigen detection to support earlier diagnosis including acute-phase infections, though prospective real-world validation is warranted before population-level deployment.

1. Introduction

Hepatitis C virus (HCV) infection remains a leading cause of severe liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1]. Despite its substantial global public health burden, no preventive vaccine is currently available [2]. Moreover, a large proportion of infected individuals remain undiagnosed due to the frequently asymptomatic nature of the infection, particularly during the early stages [3]. According to the World Health Organization (WHO), an estimated 50 million people worldwide are living with chronic HCV infection, and at least 240,000–250,000 deaths occur annually caused by HCV-related liver complications [3,4].

Acute HCV infection is estimated to account for approximately 15–20% of diagnosed cases, reflecting the proportion of infections identified during the early phase prior to full seroconversion, while 50–80% of individuals infected during the post-acute phase progress to chronic infection [5,6]. Among chronically infected patients, approximately 20% develop cirrhosis if left untreated, and a subset of these individuals may subsequently develop hepatocellular carcinoma [4]. Early diagnosis of HCV infection is therefore critical for identifying both

* Correspondence to: Serdang, Selangor 43400, Malaysia.
** Correspondence to: 2 Pioneer Place, 627885, Singapore.
E-mail addresses: niazlin@upm.edu.my (N. Mohd Taib), zhihai.zhao@mpbio.com (Z. Zhao), delynn.xu@mpbio.com (N. Xu).

https://doi.org/10.1016/j.jcv.2026.105953
Received 26 March 2026; Received in revised form 11 May 2026; Accepted 19 May 2026
Available online 25 May 2026

acute and chronic cases, enabling timely initiation of antiviral therapy and improving clinical outcomes [3,7,8]. In particular, detection of infection during the acute phase is essential for achieving favorable prognoses and preventing long-term complications.

HCV antigen becomes detectable approximately 2–3 weeks after viral exposure, appearing nearly simultaneously with HCV RNA [9,10]. HCV antigen detection assays have been applied in various clinical settings, especially among high-risk populations, immunocompromised individuals, and patients requiring early diagnosis [9,11]. Detection of HCV core antigen, a highly conserved protein across all major HCV genotypes, which serves as a surrogate marker for HCV RNA, has proven effective in shortening the diagnostic window period and confirming active infection [12,13].

HCV was initially identified as the primary causative agent of parenterally transmitted non-A, non-B (NANB) hepatitis [14]. Currently, HCV antigen detection relies largely on enzyme-linked immunosorbent assays (ELISA). Although effective, these assays are costly, time-consuming, and require specialized laboratory equipment and trained personnel, making them unsuitable for point-of-care testing (POCT). Consequently, their use is limited in resource-constrained regions or areas without access to centralized laboratories. There is a clear need for a rapid, affordable HCV antigen-based POCT that enables timely diagnosis and expands testing accessibility in decentralized healthcare settings.

To address this diagnostic gap, the ASSURE HCV Ab/Ag Rapid Test was developed to provide a simple, user-friendly solution for immediate HCV screening, particularly in emergency and decentralized healthcare settings. This lateral flow immunoassay simultaneously detects both HCV core antigen and anti-HCV antibodies, offering a more comprehensive and efficient screening approach. The present study evaluates the diagnostic performance of the ASSURE HCV Ab/Ag Rapid Test, including its sensitivity, specificity, and applicability across various sample types, and highlights its potential as a reliable point-of-care diagnostic tool in settings without access to centralized laboratory facilities.

2. Materials and methods

2.1. Clinical specimens

2.1.1. Specimen collection and characterization

A total of 778 HCV-positive serum and plasma specimens were sourced from commercial suppliers and collaborating institutions. These comprised 489 samples confirmed positive by the Abbott PRISM HCV Assay, 12 samples characterised using the Ortho HCV 3.0 ELISA Test System, and 277 samples in which HCV antigen was determined using the Abbott ARCHITECT HCV Antigen Assay, with corresponding HCV antibody status verified by the Abbott ARCHITECT Anti-HCV assay, or Abbott PRISM HCV Assay, or Ortho HCV 3.0 ELISA Test System or SD Bioline HCV Rapid Test.

Among these, 531 samples were supplied as anti-HCV antibody-positive specimens with accompanying genotype information provided in the certificates of analysis (CoA) by the suppliers. These genotype assignments were based on prior clinical, or laboratory records associated with the donors and were not independently confirmed by HCV RNA testing on the specific aliquots used in this study. The remaining 247 HCV-positive samples were determined by serological reference assays but did not have genotype information available from the suppliers.

The HCV-negative panel consisted of 1723 serum and plasma specimens, including 1025 samples from healthy donors, 153 potentially cross-reactive specimens, 135 specimens containing interfering substances, 210 samples from pregnant women, and 200 samples from hospitalised patients. The number of specimens (153 for cross-reactivity and 135 for interference testing) follows standard regulatory guidance for in vitro diagnostic device validation (e.g., CLSI EP07 and IVDR common technical specifications).

Two commercial performance panels, the AccuSet™ Worldwide HCV Performance Panel and the AccuSet™ Low Titer HCV Performance Panel, were obtained from SeraCare Life Sciences (Milford, MA, USA). For seroconversion analysis, 31 commercially sourced HCV seroconversion panels were evaluated, comprising 19 panels from SeraCare Life Sciences and 12 panels from ZeptoMetrix (Buffalo, NY, USA).

2.1.2. Cross-reactivity evaluation

Potentially cross-reactive specimens were obtained from individuals with known seropositivity or clinical conditions that could interfere with HCV antibody detection. The analytical specificity panel comprised 153 specimens, including: anti-*Candida albicans* IgG (n = 5), anti-*Chlamydia trachomatis* IgG/IgM (n = 6), anti-Dengue IgG/IgM (n = 6), anti-Hepatitis A virus (HAV) IgG/IgM (n = 7), anti-Hepatitis B virus (HBV) IgG/IgM (n = 8), anti-Hepatitis E virus (HEV) IgM (n = 5), anti-HIV I/II IgG/IgM (n = 10), anti-*Helicobacter pylori* IgG (n = 6), anti-Herpes Simplex Virus (HSV) IgG (n = 5), alcoholic hepatitis-associated IgG/IgM (n = 4), influenza vaccine recipients (n = 5), anti-influenza A/B IgG/IgM (n = 7), anti-*Toxoplasma* IgG/IgM (n = 10), anti-*Chlamydia pneumoniae* IgG/IgM (n = 5), anti-B19 parvovirus IgG/IgM (n = 6), anti-Rubella IgG (n = 5), anti-Syphilis IgG/IgM (n = 5), anti-Tuberculosis (TB) IgG (n = 8), anti-HTLV I/II IgG/IgM (n = 7), yellow fever virus post-immunization IgG (n = 4), anti-Malaria IgG/IgM (n = 5), anti-Cytomegalovirus (CMV) IgG/IgM (n = 5), anti-Epstein–Barr virus (EBV) IgM (n = 5), anti-Epstein–Barr virus (EBV) IgG (n = 2), anti-Varicella-Zoster Virus (VZV) IgG (n = 7), and anti-Zika virus IgG/IgM (n = 5).

2.1.3. Interference testing

Specimens for interference testing were collected from individuals with conditions or substances known to potentially affect assay performance. These included diabetes, elevated hemoglobin A1c, high bloodstream E. coli, elevated ethanol levels, hemolysis, icteric, hyperlipidemia, elevated bilirubin, increased total protein, high triglycerides, presence of rheumatoid factor (RF), multiple transfusions, antinuclear antibodies (ANA), high alpha-fetoprotein (AFP), homozygous sickle-cell anemia, Human Anti-Mouse Antibodies (HAMA), biotin interference, elevated Streptolysin O (ASO), and autoimmune hepatitis. A 7-way anticoagulant-matched set was also evaluated, consisting of one serum sample and six corresponding plasma samples collected in Acid Citrate Dextrose (ACD), K-Oxalate, Citrate Phosphate Dextrose (CPD), Lithium Heparin (Li Hep), Sodium Citrate (Na Citrate), and Ethylenediaminetetraacetic acid (EDTA) tubes.

2.1.4. Specimen sources

The cross-reactive and interference specimens were sourced commercially from Access Biologicals (Vista, CA, USA), Precisions (Houston, TX, USA), AbBaltis (Dartford, UK), SeraCare Life Sciences (Milford, MA, USA), Aalto Bio Reagents (Dublin, Ireland), SLR Research Corporation (Carlsbad, CA, USA), ABRI (Rockville, MD, USA), Boca Biolistics (Pompano Beach, FL, USA), Advanced BioResources (Rockville, MD, USA), SSI Diagnostica (Hillerød, Denmark), Boston Biomedica (West Bridgewater, MA, USA), Bio Plasma Services (Everett, WA, USA), Discovery Life Sciences (Huntsville, AL, USA), and Trina Bioreactives (Nänikon, Switzerland).

2.1.5. Clinical evaluation

An independent clinical evaluation of the antibody detection component of the ASSURE HCV Ab/Ag Rapid Test was performed at Hospital Sultan Abdul Aziz Shah (HSAAS), University Putra Malaysia (UPM), Serdang, Malaysia. A total of 79 clinical specimens were included, comprising 24 HCV-antibody-positive and 55 HCV-antibody-negative samples, as determined by the ARCHITECT Anti-HCV chemiluminescent immunoassay (CLIA). All specimens were obtained from patients receiving care at HSAAS. As HCV antigen or RNA testing is not

routinely performed at this site, no HCV antigen-positive specimens were available for inclusion in the study.

2.2. ASSURE HCV Ab/Ag rapid test

The MP Diagnostics ASSURE HCV Ab/Ag Rapid Test is a lateral flow immunochromatographic assay for the simultaneous qualitative detection of anti-HCV antibodies and HCV core antigen in human serum, plasma, and whole blood. The test device yields two independent results: (1) the antibody component detects anti-HCV immunoglobulins (IgG and IgM) directed against HCV Core antigen; and (2) the antigen component detects circulating HCV core antigen (HCVcAg) using anti-HCV core monoclonal antibodies conjugated to gold nanoparticles (Fig. 1).

For the ASSURE HCV Ab test, 10 μL of specimen was dispensed into the sample well, whereas 30 μL of specimen was added for the ASSURE HCV Ag test. This was followed by the addition of 3 drops of chase buffer to the sample well. Upon initiation of the assay, the colored conjugate migrated across the result window of the test device. Test results were interpreted between 15 and 20 min after sample application, and results read beyond 20 min were considered invalid.

2.3. Diagnostic performance analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the ASSURE HCV Ab/Ag Rapid Test were calculated using MedCalc statistical software (MedCalc Software Ltd., Ostend, Belgium).

2.4. Analytical sensitivity study/ limit of detection (LoD)

Analytical sensitivity was evaluated using two anti-HCV antibody–positive specimens obtained from commercial suppliers, and the WHO 1st International Standard for HCV Core Antigen (Paul-Ehrlich-Institut, Germany; PEI code 129096/12). The limit of detection (LoD) of the ASSURE HCV Ab/Ag Rapid Test was determined for each material by testing two-fold serial dilutions prepared in HCV-negative plasma as appropriate. Dilution series were assessed to establish the lowest concentration at which HCV antibodies or HCV core antigen could be consistently detected.

2.5. Specimen equivalency

Specimen equivalency was evaluated using matched sets of serum, EDTA plasma, venous whole blood, and finger-prick whole blood collected from the same healthy donors and tested in parallel. For the antibody detection component, samples were spiked with anti-HCV antibodies to generate 50 positive and 50 negative specimens per matrix. For the antigen detection component, samples were spiked with the WHO 1st International Standard for HCV Core Antigen to generate 50 positive and 50 negative specimens per matrix. All specimens within each matched set were tested using the ASSURE HCV Ab/Ag Rapid Test according to the standard procedure, and qualitative results were compared across sample types to assess inter-matrix concordance.

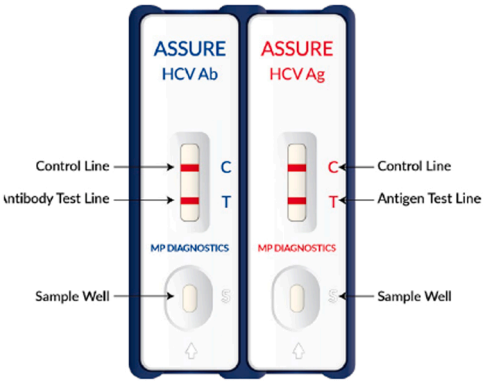

Fig. 1. Representative results of the ASSURE HCV Ab/Ag Rapid Test demonstrate positive detection of HCV antibodies and HCV antigen, indicated by the presence of test lines (T) on the ASSURE HCV Ab and ASSURE HCV Ag devices, respectively. The appearance of control lines (C) on both devices confirms the validity of the test results.

3. Results

As shown in Table 1, the overall diagnostic performance of the ASSURE HCV Ab/Ag Rapid Test was

100% sensitivity (95% Confidence Interval, 99.53–100.00%) and 99.83% specificity (95% Confidence Interval, 99.49–99.96%). The Kappa value of 0.998 indicated a nearly perfect agreement between the ASSURE HCV Ab/Ag Rapid Test and the reference HCV assays. The calculated positive likelihood ratio (LR+) of 588.24 and negative likelihood ratio (LR−) of 0.00 (conservative estimate 0.0047 based on the lower bound of the 95% CI for sensitivity) further confirm the strong discriminatory performance of the assay (Table 1).

As shown in Table 2, the antibody component was evaluated on all 766 specimens that were confirmed positive for anti HCV antibodies by reference assays (Abbott PRISM HCV, Ortho HCV 3.0 ELISA, or Abbott ARCHITECT Anti HCV). The ASSURE antibody component detected all 766, giving 100% sensitivity (766/766; 95% CI: 99.52–100.00%). The 12 specimens that were negative by reference antibody assays represent the acute pre seroconversion window period; these were correctly identified as negative by the antibody component. Regarding the 277 specimens that were confirmed positive for HCV core antigen by the Abbott ARCHITECT HCV Ag assay, the ASSURE antigen component detected 107 of these 277 specimens, giving 38.63% sensitivity (107/277; 95% CI: 32.86–44.64%).

Of the 778 specimens evaluated, 607 (78.02%) were positive by the antibody component alone (antigen-negative), consistent with chronically infected individuals who had undergone seroconversion but had antigen levels below the assay's detection threshold. A further 159 (20.44%) were positive for both antibody and antigen, indicating active infection with high-level viraemia in the context of either ongoing seroconversion or established chronic infection. Notably, 12 (1.54%) were positive by the antigen component alone (antibody-negative),

Table 1
Evaluation of In-house Overall Diagnostic Performance of ASSURE HCV Ab/Ag Rapid Test.

Diagnostic Performance	Performance	95% Confidence Interval
Sensitivity[a]	100% (778/778)	99.53–100.00%
Specificity	99.83% (1720/1723)	99.49–99.96%
Positive Predictive Value	99.62%	98.82–99.88%
Negative Predictive Value	100%	99.79–100.00%
Kappa Value	0.998	0.996–1.000
Positive Likelihood Ratio (LR+)	588.24	N/A[b]
Negative Likelihood Ratio (LR−)	0.00[c]	N/A[b]

[a] Sensitivity is calculated based on the combined outcome, whereby a specimen is considered positive if the antibody (Ab) and/or antigen (Ag) component yields a reactive result.

[b] 95% confidence interval not applicable for likelihood ratios calculated from a qualitative binary assay with a single fixed operating point.

[c] LR− = 0.00 is a mathematical consequence of 100% sensitivity (zero false negatives observed). Using the lower bound of the 95% CI for sensitivity (99.53%), the conservative estimate of LR− = 0.0047. LR+ : Positive Likelihood Ratio = Sensitivity / (1 − Specificity) = 1.0000 / 0.0017 = 588.24. LR−: Negative Likelihood Ratio = (1 − Sensitivity) / Specificity.

Table 2
Sensitivity of the ASSURE HCV Ab/Ag Rapid Test by Detection Component.

Detection Component	Reference Positive Specimens (n)	Sensitivity	95% Confidence Interval
Antibody Test	766	100% (766/766)	99.52–100.00%
Antigen Test	277	38.63% (107/277)	32.86–44.64%

representing specimens collected during the pre-seroconversion window period of acute infection, when viral antigen is detectable but antibodies have not yet developed.

To further characterize the functional threshold of the ASSURE Ag component, we analysed ARCHITECT HCV Ag levels (fmol/L) across the 277 HCV Ag positive specimens (Table 3). Detection rates increased progressively with antigen concentration, from 13% at < 700 fmol/L to 38% at 700–2000 fmol/L and 52% at 2000–5000 fmol/L, rising to 76% at 5000–10,000 fmol/L and plateauing at 75% at > 10,000 fmol/L. The effective detection threshold of the ASSURE Ag component is therefore estimated at approximately 5000–10,000 fmol/L.

The ASSURE HCV Ab/Ag Rapid Test demonstrated 100% sensitivity across 531 samples representing HCV genotypes 1–6, correctly detecting all cases. In comparison, the SD Bioline HCV Rapid Test showed slightly lower overall sensitivity (97.18%, 516/531), with reduced detection observed in several genotypes. Detailed genotype-specific results are provided in Supplementary Table S1.

Analytical sensitivity assessment showed that the antibody component detected anti-HCV antibodies at dilutions up to 1:6400 in one sample and 1:1600 in another, while the reference assay detected these samples at 1:6400 and 1:3200, respectively. For antigen detection, the ASSURE HCV Ab/Ag Rapid Test detected WHO standard HCV core antigen down to 3.125 IU/mL, demonstrating sensitivity comparable to the reference immunoassay. Full dilution series and signal intensity data are presented in Supplementary Table S2.

The cross-reactivity of the ASSURE HCV Ab/Ag Rapid Test was evaluated using an extensive panel of serum and plasma specimens reactive with a wide range of antibodies as stated in Materials and Methods Section. Despite this broad panel of potentially cross-reactive antibodies, the ASSURE HCV Ab/Ag Rapid Test yielded no false-positive reactions across 153 potentially cross-reactive specimens (Supplementary Table S3). Furthermore, evaluation of 19 categories of potentially interfering substances demonstrated that none produced false-positive or false-negative results with the ASSURE HCV Ab/Ag Rapid Test (Supplementary Table S4). These results confirm that the assay maintains high specificity and robustness even in the presence of diverse antibodies or in specimens with conditions.

Seroconversion panels comprise sequential specimens collected from individuals during the early phase of HCV infection, ranging from the pre-seroconversion stage, in which antibodies are not yet detectable, to the post-seroconversion stage, characterized by the presence of anti-HCV antibodies. Evaluation using these panels enables assessment of assay performance throughout the full course of early infection. Due to the incorporation of HCV antigen detection, the ASSURE HCV Ab/Ag Rapid Test was able to identify HCV infection ~17 days earlier than antibody-only assays, including SD Bioline HCV Test and HCV Blot 3.0 (Table 4).

As shown in Table 5, the independent clinical evaluation of the ASSURE HCV Ab/Ag Rapid Test (antibody component) at HSAAS demonstrated excellent diagnostic performance, achieving 100% sensitivity (24/24) and 100% specificity (55/55). The results indicate complete concordance with the reference ARCHITECT Anti-HCV CLIA assay, confirming the reliability of the antibody detection component of the ASSURE HCV Ab/Ag Rapid Test for accurate HCV antibody detection in clinical specimens.

Since no HCV antigen-positive clinical specimens were available at HSAAS, additional evaluation using well-characterized HCV performance panels was conducted to assess the antigen detection capability of the ASSURE HCV Ab/Ag Rapid Test. The assay was tested using the SeraCare AccuSet™ worldwide and low-titer HCV performance panels (Table 6). In the worldwide panel, the test demonstrated 100% sensitivity (7/7) for antibody detection and 90% sensitivity (18/20) for antigen detection. In the low-titer panel, the ASSURE HCV Ab/Ag Rapid Test showed superior sensitivity compared with the SD BioLine HCV Rapid Test. Specifically, the antibody component achieved 94.74% sensitivity (18/19) versus 84.21% (16/19) for the comparator, while the antigen component demonstrated 75% sensitivity (3/4), highlighting its capability to detect low-level antigenemia in challenging samples. All negative samples were correctly identified as negative. The RNA-stratified results from the worldwide panel were shown in Supplementary Table S5.

To further assess performance across different specimen types, the ASSURE HCV Ab/Ag Rapid Test demonstrated 100% agreement (100/100) across serum, plasma, venous whole blood, and finger-prick whole blood, supporting the suitability of the assay for point-of-care testing across diverse clinical settings (Supplementary Table S6).

Table 3
Detection rate of ASSURE HCV Ag component by ARCHITECT HCV Ag concentration (n = 277).

ARCHITECT HCV Ag (fmol/L)	No. of Samples (n)	Ag Positive (n)	Detection Rate	95% CI
< 700	98	13	13%	7.56–20.84%
700–2000	72	27	38%	27.43–49.70%
2000–5000	58	30	52%	39.47–64.20%
5000–10,000	33	25	76%	58.79–88.20%
> 10,000	16	12	75%	50.55–91.34%
Total	**277**	**107**	**38.63%**	**32.86–44.64%**

4. Discussion

The inclusion of the HCV antigen detection component provides a significant advantage over conventional rapid tests that detect only anti-HCV antibodies. By detecting HCV core antigen, the ASSURE HCV Ab/Ag Rapid Test enables the identification of acute or early-phase HCV infections, which may not yet elicit detectable antibody responses. This dual detection strategy advances the diagnostic window, allows for earlier intervention, and improves the overall sensitivity of HCV screening, particularly in individuals recently infected with HCV or immunocompromised patients. These results indicate that the ASSURE HCV Ab/Ag Rapid Test combines the rapidity and convenience of lateral flow immunoassays with the clinical benefit of detecting both HCV antibodies and antigen, making it a valuable tool for early and accurate HCV diagnosis. The added clinical benefit of antigen detection was further highlighted through seroconversion panel analysis. The ASSURE HCV Ab/Ag Rapid Test detected 73.5% of panel members and enabled diagnosis approximately ~17 days earlier than antibody-only rapid test

Table 4
Comparative Early Detection Performance of ASSURE HCV Ab/Ag Rapid Test and Comparator Assays Using HCV Seroconversion Panels.

	ASSURE HCV Ab/Ag Rapid Test	SD Bioline HCV	HCV Blot 3.0
No. of panels tested	31 HCV Seroconversion Panels		
Detected panel members / total panel members	222/302	152/302	131/302
Detection rate	73.50%	50.30%	43.40%
1st day of Detection of Infection	Reference	+ 16.9 days	+ 17.2 days

Table 5
Independent Clinical Evaluation of the Antibody Detection Component of the ASSURE HCV Ab/Ag Rapid Test at HSAAS, Malaysia.

Diagnostic Performance	Performance	95% Confidence Interval
Sensitivity	100% (24/24)	85.75–100.00%
Specificity	100% (55/55)	93.51–100.00%
Positive Predictive Value	100%	85.75–100.00%
Negative Predictive Value	100%	93.51–100.00%
Kappa Value	1.000	0.946–1.000

Note: As no HCV antigen–positive specimens were available at this clinical site, only the antibody detection performance was evaluated.

Table 6
Comparative performance of ASSURE HCV Ab/Ag Rapid Test against SD BioLine HCV Rapid Test using one (1) anti-HCV low titer and one (1) worldwide panels.

Source of Panels	Panel No.	Number of samples	ASSURE HCV Ab/Ag Rapid Test	SD BioLine HCV Rapid Test
SeraCare AccuSet™ Worldwide HCV Performance Panel	0810–0230	20 Positive + 2 Negative	Antibody: 7/7 (100%); Antigen: 18/20 (90%)*	Antibody: 7/7 (100%)
SeraCare AccuSet™ Low Titer HCV Performance Panel	0810–0256	19 Positive + 1 Negative	Antibody: 18/19 (94.74%) Antigen: 3/4 (75%)*	Antibody: 16/19 (84.21%)

* The presence of antigen was characterized by Abbott ARCHITECT HCV Ag Test.

and Western blot assay. Such earlier detection facilitates timely clinical intervention, rapid linkage to care, and the implementation of public health measures to reduce onward transmission.

The exceptionally high sensitivity (100%) and specificity (99.83%) observed in this study can be attributed to several key design features of the ASSURE HCV Ab/Ag Rapid Test. For antibody detection, the assay employs recombinant HCV Core antigen covering the regions known to elicit strong and early immune responses across all major genotypes. This broad antigenic coverage minimises the risk of false negatives due to genotype-specific epitope variation. For antigen detection, highly specific monoclonal antibodies targeting the conserved HCV core protein ensure minimal non-specific binding. Furthermore, the dual-channel architecture (independent Ab and Ag test lines on a single device) with separate conjugate-buffer systems reduces the risk of cross-reaction between the two detection systems. Together, these design elements account for the robust analytical and clinical performance observed across a diverse specimen panel encompassing six genotypes and multiple specimen matrices.

Consistent with World Health Organization (WHO) recommendations, effective HCV screening assays must demonstrate high sensitivity to minimize missed infections and high specificity to reduce unnecessary follow-up testing and patient anxiety. The diagnostic performance observed in this study meets and exceeds these criteria, including in decentralized and resource-limited settings where access to molecular diagnostics may be constrained. In addition, the assay demonstrated excellent analytical specificity, with no false-positive results observed in extensive cross-reactivity studies involving a wide spectrum of viral, bacterial, and parasitic antibodies, post-vaccination samples, or specimens containing common endogenous and exogenous interfering substances. These findings underscore the robustness and reliability of the assay in real-world clinical settings, where co-infections and underlying medical conditions are frequently encountered.

The absence of HCV antigen–positive specimens in the independent clinical evaluation represents a limitation in assessing the antigen detection performance of the ASSURE HCV Ab/Ag Rapid Test under real-world clinical conditions. However, data from characterized antigen panels provide insight into this aspect. Three antigen-positive samples were not detected by the antigen component of the ASSURE HCV Ab/Ag Rapid Test across the evaluated panels. These missed samples were associated with low antigen concentrations, as characterized by the Abbott ARCHITECT HCV Ag (CMIA) assay, with levels below 700 fmol/L, a concentration range that challenges even laboratory-based immunoassays. Several factors may contribute to the reduced sensitivity in this range: (i) at very low antigen concentrations, the probability of antigen-antibody-conjugate complex formation on the nitrocellulose membrane decreases non-linearly; (ii) the qualitative nature of the lateral flow readout means that faint test lines may be subjectively interpreted as negative; and (iii) competitive binding between free circulating HCV core antigen and naturally occurring anti-core antibodies in chronically infected specimens may reduce effective free antigen concentration available for detection. These findings indicate that while the assay demonstrates strong overall performance, its sensitivity for HCV antigen detection may be affected in samples with low viral antigen levels, a factor that should be considered when interpreting results, particularly in early or low-viremia infections.

This study has several limitations that should be considered when interpreting the findings. First, the independent clinical evaluation was conducted using a relatively small sample size from a single clinical site, which may limit the generalizability of the results to broader and more diverse populations. Second, due to the unavailability of HCV antigen–positive specimens at the clinical site, the antigen detection performance of the ASSURE HCV Ab/Ag Rapid Test could not be assessed in real-world clinical samples, and conclusions regarding this component rely on characterized panels rather than routine clinical specimens. Ideally, validation of the antigen detection component requires testing of specimens from patients with confirmed active HCV viraemia (RNA-positive, antigen-positive). The reliance on characterised commercial panels for antigen performance data, while providing useful analytical information, does not fully substitute for real-world clinical validation. Lastly, the study did not include detailed stratification by disease stage, viral load, or co-infection status, which may influence test performance, particularly in early or low-viremia infections. Further multi-centre studies with larger sample sizes, inclusion of HCV antigen-positive clinical specimens from patients with acute or untreated chronic infection, and broader patient representation are warranted to fully validate the performance and utility of the assay in diverse clinical settings.

5. Conclusions

The ASSURE HCV Ab/Ag Rapid Test demonstrated high sensitivity and specificity for HCV detection across a large and diverse specimen panel, with equivalent performance across serum, plasma, venous whole blood, and finger-prick whole blood. The inclusion of HCV core antigen detection enables earlier identification of infection compared to antibody-only assays. The assay shows considerable promise as a point-of-care tool in decentralized and resource-limited environments, and its clinical value warrants further investigation in prospective multi-centre trials.

CRediT authorship contribution statement

Muhammad Firdaus Bin Md Ibrahim: Validation, Investigation, Data curation. **Nurul Huda Binti Mohamed Rashidi:** Validation, Investigation, Data curation. **Lay Sin Teoh:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Conceptualization. **Kaosalya Murali:** Validation, Investigation, Data curation. **Niazlin Mohd Taib:** Writing – review & editing, Formal analysis, Data curation. **Na Xu:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **ZHAO Zhihai:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Narcisse Mary A/P Sither Joseph Vesudian:**

Validation, Investigation, Data curation. **Syafinaz Amin-Nordin:** Writing – review & editing, Validation, Formal analysis, Data curation. **Yang Du:** Writing – review & editing. **Haris Ong:** Writing – review & editing. **Matthew Mak:** Writing – review & editing.

Ethical statement

The study was conducted in accordance with the requirements of local ethics, biosafety, and investigational committees. Human serum and plasma specimens were obtained and managed under the regulations of the Singapore Biosecurity and Biological Agents Act (BATA). The acquisition, handling, and storage of human blood samples were approved by the Ministry of Health, Singapore. All experimental protocols received approval from MP Biomedicals Asia Pacific Pte Ltd. Informed consent was obtained from all participants and/or their legal guardians, where applicable.

The study conducted in Malaysia was approved by the relevant institutional review board and carried out in full compliance with all applicable local regulatory and ethical requirements.

Declaration of Generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used CHATGPT and CLAUDE AI in order to improve readability and language clarity. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Funding

MP Biomedicals Asia Pacific Pte Ltd covered all the expenses generated during the study.

Declaration of Competing Interest

LST, ZZ, HO, MM, YD, and NX are employees of MP Biomedicals Asia Pacific Pte Ltd, the manufacturer of the ASSURE HCV Ab/Ag Rapid Test evaluated in this study. The remaining authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank all project team members for their support throughout the product development process. The authors are grateful to Muhammad Firdaus Bin Md Ibrahim, Kaosalya Murali, and Nurul Huda Binti Mohamed Rashidi for their contributions to the processing of HCV-antibody-positive and HCV-antibody-negative samples at Hospital Sultan Abdul Aziz Shah.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jcv.2026.105953.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

[1] S. L.A, F. G.D, L. Eric, M. Claudia, T. E.G, G. Reem, R. Robert, R. Vinod, M. Fiona, W.-R. Megan, P. Anna, Z. Kurt, I. D.D, E. Timothy, G. Tong, M. G.D, P. Claudio, Preliminary study of two antiviral agents for hepatitis C genotype 1, N. Engl. J. Med. 366 (2012) 216–224, https://doi.org/10.1056/NEJMoa1104430.

[2] K.S. Abdelwahab, Z.N.A. Said, Status of hepatitis C virus vaccination: recent update, World J. Gastroenterol. 22 (2016) 862–873, https://doi.org/10.3748/wjg.v22.i2.862.

[3] Global Hepatitis Report, Action for Access in Low- and Middle-Income Countries, 2024, World Health Organization, 2024. Available at: ⟨https://www.who.int/publications/i/item/9789240091672⟩.

[4] World Health Organization, Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2024, WHO, Geneva, 2024. Available at: ⟨https://www.who.int/publications/i/item/9789240027077⟩.

[5] D.L. Thomas, Hepatitis C epidemiology, Curr. Top. Microbiol Immunol. 242 (2000) 25–41.

[6] V. Narayanamurthy, Z.E. Jeroish, K.S. Bhuvaneshwari, F. Samsuri, Hepatitis C virus (HCV) diagnosis: Via microfluidics, Anal. Methods 13 (2021) 740–763, https://doi.org/10.1039/d0ay02045a.

[7] J.M. Pawlotsky, F. Negro, A. Aghemo, M. Berenguer, O. Dalgard, G. Dusheiko, F. Marra, M. Puoti, H. Wedemeyer, EASL recommendations on treatment of hepatitis C: Final update of the series, J. Hepatol. 73 (2020) 1170–1218, https://doi.org/10.1016/j.jhep.2020.08.018.

[8] W. Tang, W. Chen, A. Amini, D. Boeras, J. Falconer, H. Kelly, R. Peeling, O. Varsaneux, J.D. Tucker, P. Easterbrook, Diagnostic accuracy of tests to detect Hepatitis C antibody: a meta-analysis and review of the literature, BMC Infect. Dis. 17 (2017), https://doi.org/10.1186/s12879-017-2773-2.

[9] G.L. Flores, J.C. Mota, L.T. Da Silva Andrade, R.S. Lopes, F.I. Bastos, L.M. Villar, Performance of HCV antigen testing for the diagnosis and monitoring of antiviral treatment: a systematic review and meta-analysis, Biomed. Res. Int. 2022 (2022), https://doi.org/10.1155/2022/7348755.

[10] M.P. Manns, M. Buti, E. Gane, J.M. Pawlotsky, H. Razavi, N. Terrault, Z. Younossi, Hepatitis C virus infection, Nat. Rev. Dis. Prim. 3 (2017), https://doi.org/10.1038/nrdp.2017.6.

[11] A.S. Baber, B. Suganthan, R.P. Ramasamy, Current advances in Hepatitis C diagnostics, J. Biol. Eng. 18 (2024), https://doi.org/10.1186/s13036-024-00443-2.

[12] J.K. Rockstroh, J.J. Feld, S. Chevaliez, K. Cheng, H. Wedemeyer, C. Sarrazin, B. Maasoumy, C. Herman, J. Hackett, D.E. Cohen, G.J. Dawson, G. Cloherty, J. M. Pawlotsky, HCV core antigen as an alternate test to HCV RNA for assessment of virologic responses to all-oral, interferon-free treatment in HCV genotype 1 infected patients, J. Virol. Methods 245 (2017) 14–18, https://doi.org/10.1016/j.jviromet.2017.03.002.

[13] Y. Wang, W. Jie, J. Ling, H. Yuanshuai, HCV core antigen plays an important role in the fight against HCV as an alternative to HCV-RNA detection, J. Clin. Lab. Anal. 35 (2021), https://doi.org/10.1002/jcla.23755.

[14] G. Kuo, Q.-L. Choo, H.J. Alter, G.L. Gitnick, A.G. Redeker, R.H. Purcell, T. Miyamura, J.L. Dienstag, M.J. Alter, C.E. Stevens, G.E. Tegtmeier, F. Bonino, M. Colombo, W.-S. Lee, C. Kuo, K. Berger, J.R. Shuster, L.R. Overby, D.W. Bradley, M. Houghton, An Assay for Circulating Antibodies to a Major Etiologic Virus of Human Non-A, Non-B Hepatitis, n.d. ⟨www.sciencemag.org⟩.