DIAGNOSTIC PERFORMANCE OF DRIED BLOOD SPOT HEPATITIS C VIRUS CORE ANTIGEN TESTING FOR HEPATITIS C SCREENING: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Title page

Title: Diagnostic performance of dried blood spot HCVcAg testing for hepatitis C screening: a systematic review and meta-analysis

Running head: HCVcAg screening in DBS samples

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Abstract

Objectives: Dried blood spot (DBS) sampling is increasingly used for hepatitis C virus (HCV) screening. HCVcAg testing offers a faster and more streamlined approach to diagnosing HCV infection. We conducted a systematic review and meta-analysis to assess the diagnostic performance of the Abbott ARCHITECT HCV Ag assay for screening active HCV infection using DBS samples.

Methods: Eight studies (n=1,229) were selected among all published studies available up to Oct 4, 2024, in different databases with a search strategy registered (PROSPERO: CRD42022363975). The gold standard method was the HCV PCR test. Data were analyzed using the MIDAS module in STATA with a random effects model.

Results: Combined diagnostic accuracy measures were as follows: sensitivity 85%, specificity 100%, positive likelihood ratio (PLR) 233.1, negative likelihood ratio (NLR) 0.15, and summary receiver operating characteristic (SROC) 0.99. Likelihood ratios and Fagan's nomogram suggested that the HCVcAg assay with DBS samples can confirm or rule out active HCV infection with over 92% accuracy in high-prevalence settings (≥5%). However, in low-prevalence settings (≤1%), a confirmatory test must be required for positive results. The ability of the test to identify people without HCV infection was high regardless of HCV prevalence, with an error rate of less than 3%. This meta-analysis is subject to limitations, particularly due to the number of included studies and significant heterogeneity among them.

Conclusions: HCV screening using the Abbott ARCHITECT HCV Ag assay with DBS samples showed excellent diagnostic performance, but its external validity may be limited when HCV prevalence is low (≤1%).

Keywords

Hepatitis C; Screening; Diagnostic performance; Dried blood spot; HCV core antigen

Introduction

Hepatitis C virus (HCV) infection has a worldwide distribution but is uneven across regions. The World Health Organization (WHO) estimated that globally, 58 million people were diagnosed with chronic hepatitis C infection in 2019, representing 0.75% of the population. During the same year, there were 1.5 million new infections and 290,000 deaths due to hepatitis C [1]. Therefore, HCV infection represents a significant global health concern due to its associated morbidity and mortality [2,3]. To address this issue, the WHO approved a Global Strategy to eliminate hepatitis C by 2030, whose primary objectives are to reduce the incidence of new hepatitis C cases by 90%, decrease hepatitis C-related deaths by 65%, and treat 80% of eligible individuals with chronic HCV infections [4]. However, one of the main barriers to achieving these goals is the high percentage of undiagnosed infections, leading to many cases remaining undiagnosed for decades until liver damage occurs [5].

The current HCV diagnostic process involves two steps [6]. Firstly, a serological test detects anti-HCV antibodies (anti-HCV) using immunoassays. Secondly, patients with reactive antibodies undergo a nucleic acid amplification test (NAAT) to confirm the presence of HCV RNA. While reflex testing for HCV RNA can be implemented to expedite confirmation of HCV infection in anti-HCV positive cases, this process can still be time-consuming in some settings due to factors such as laboratory capacity and resource limitations [7]. To overcome current limitations and support the WHO's 2030 targets of simplifying the diagnostic approach, the HCV core antigen (HCVcAg) test offers a single-step alternative for detecting active HCV infection, particularly in low- and middle-income countries (LMICs) and among high-risk populations within high-income countries with limited healthcare access [8,9]. Moreover, the HCVcAg test provides results in a single step and may be quicker than some NAATs due to its simpler technology and fewer processing steps. However, it is not as widely available as HCV RNA testing and may not be suitable for cases with very low or undetectable HCVcAg levels. The HCVcAg test has demonstrated 100% specificity and reliable sensitivity in cases of low viremia (HCV RNA >500-3,000 IU/ml) and is emerging as a potential alternative, especially since most cases exceed the cutoff (~50,000 IU/ml) [10]. The HCVcAg test is generally more affordable than the RNA test, especially in LMICs, although costs may vary based on location and laboratory setup [11-13]. Additionally, HCVcAg is more stable than HCV RNA at room temperature [14]. HCVcAg, a well-preserved protein across all HCV genotypes, is detectable in peripheral blood within 12 to 15 days of HCV infection [9]. Its presence indicates active hepatitis C and is often detected 4 to 6 weeks before the development of anti-HCV. Early detection of HCVcAg could lead to a significant benefit by allowing for prompt initiation of antiviral treatment, which can prevent the spread of the virus to others and reduce the risk of long-term liver damage. Notably, there is a direct correlation between HCVcAg levels and HCV-RNA load: HCV-RNA can be detected in serum/plasma 1–2 weeks after HCV exposure, while HCVcAg appears 1–2 days after HCV-RNA detection [9].

The HCVcAg test performs well on both plasma and serum samples [15]. This makes the Abbott ARCHITECT HCV Ag test a highly reliable method for identifying active HCV infection in people with chronic hepatitis C and treatment monitoring and assessing cure (sustained virological response, SVR) [15,16]. However, using plasma/serum samples requires qualified personnel, specific facilities, and centralized systems [9,17,18]. Dried blood sampling (DBS) using finger-stick collection is a promising alternative for diagnosing active HCV infection in difficult-to-access settings. DBS simplifies sample collection, does not require specialized personnel, allows reflex testing for multiple samples, and is stable at room temperature, facilitating easy mailing and decentralized diagnostics for hepatitis C and other infectious diseases [19,20]. Thus, the ARCHITECT HCV Ag assay with DBS could be a promising initial screening tool for active HCV infection, particularly in LMICs and marginalized populations with high prevalence, streamlining the entire patient care process and reducing losses during follow-up.

Objective

We aimed to perform a systematic review and meta-analysis to assess the diagnostic performance of the Abbott ARCHITECT HCV Ag assay for screening active HCV infection using DBS samples.

Material and Methods

The systematic review was carried out according to the guidelines described in the *Cochrane Handbook for Diagnostic Test Accuracy Reviews* [21] and the Preferred Reporting Items for Systematic Reviews and Meta-analysis of Diagnostic Test Accuracy (PRISMA-DTA) statement [22]. The PRISMA-DTA checklist can be found in **Supplementary File 1**.

Search strategy

We conducted a systematic search across multiple databases, including Medline/PubMed, EMBASE, SCOPUS, Web of Science (WoS), and Cochrane Library, up until Oct 4, 2024. No restrictions were imposed on language, publication year, study design, or geographic location. Moreover, we meticulously examined the references of eligible studies to identify any potentially relevant studies that may have been overlooked during the initial database search. A detailed description of the search criteria and the literature retrieved from each database can be found in **Supplementary File 2**. To ensure a comprehensive and efficient literature search, we employed EndNote X9 (Clarivate, Philadelphia, PA, USA). This software allowed us to systematically organize references, track search terms, and extract relevant information. Moreover, all search results and cited references were exported to Microsoft Excel 2016 spreadsheets (Microsoft Corporation, Redmond, WA, USA) for further processing. This protocol was established a priori and registered on the International Prospective Register of Systematic Reviews [23] (PROSPERO ID: CRD42022363975).

Study selection

To be considered for inclusion, studies had to meet the following criteria: i) Assessment of diagnostic accuracy of the Abbott ARCHITECT HCV Ag assay using DBS samples; ii) comparison between the Abbott ARCHITECT HCV Ag assay and a reference method (HCV-RNA) using serum, plasma, or whole-blood samples; and iii) availability of sufficient data to construct a 2x2 contingency table, enabling the estimation of sensitivity, specificity, and other relevant statistical parameters. The exclusion criteria were as follows: i) publications without data, including reviews, meta-analyses, studies with unavailable full-text, or data published in chapter books, conference proceedings, editorial comments, and case reports; ii) studies with data, but we could not extract the data necessary for the meta-analysis and which were not provided by the authors after reasonable request; iii) studies with small sample sizes ($n \leq 10$) to minimize bias in the random-effects model; and iv) studies involving commercial samples, non-human subjects, or tests not available on the market.

Data extraction

All studies underwent screening and selection based on their title and abstract by two investigators (AT-N and DS-C), with independent verification by third-party reviewers (JMB and SR). In cases where the data within the studies were deemed insufficient, one investigator (DS-C) contacted the authors via email for clarification. Studies were subsequently excluded if authors could not be contacted after three attempts.

Quality assessment

Each eligible study underwent evaluation for potential risk of bias and applicability, guided by the four key domains outlined in the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) framework [24]: patient selection, index test, reference standard, and flow of participants through the study, including the timing of tests. Patient selection assesses aspects such as inclusion and exclusion criteria and whether there was selection bias or if the sample was not representative of the target population. The index test evaluates whether the investigators were blinded to the reference test results to avoid verification bias. The reference test examines the validity and reliability of the reference test (gold standard) used to confirm or rule out the clinical condition of interest. The flow and timing evaluate whether all patients enrolled in the study received both the index and reference tests, and if there was an appropriate time interval between the two tests, as well as ensures that there were no loss to follow-up or inappropriate exclusions (for more detail, see **Supplementary File 3**).

The methodological quality assessment was conducted by two reviewers (AT-N and DS-C), with any discrepancies resolved by a third reviewer (SR). Risk of bias was assessed across all domains, while applicability concerns were focused on the first three domains. Ratings for risk of bias and applicability were categorized as 'low', 'high', or 'unclear', with the 'unclear' category applied only when insufficient data were available to make a definitive judgment.

Statistical analysis

All statistical analyses were conducted using STATA 15.0 (STATA Corp., College Station, TX, USA) with the MIDAS module and random-effects models [21,25]. Sensitivity and specificity, along with their corresponding 95% confidence intervals (95%CI), were calculated using the true positive (TP), false positive (FP), false negative (FN), and true negative (TN) rates derived from the 2x2 tables of each eligible study. A bivariate random-effects model was employed to determine the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and the area under the summary receiver operating characteristic (AUC-SROC) curve, given TP+FN>0 and FP+TN>0. Moreover, a univariate analysis was performed, including studies with TP+FN=0 or FP+TN=0.

The accuracy of the diagnostic test was assessed by estimating the AUC-SROC, categorized as follows: 0.50-0.60 (failure); 0.60-0.70 (poor); 0.70-0.80 (fair); 0.80-0.90 (good); and 0.90-1 (excellent) [26]. The likelihood ratio scatter matrix provides estimates of clinical validity for the HCVcAg test based on the placement of PLR and NLR values within four quadrants [24]: i) left upper quadrant (LUQ; PLR >10, NLR <0.1): favorable for both confirming and excluding; ii) right upper quadrant (RUQ; PLR >10, NLR >0.1): primarily useful for confirmation; iii) left lower quadrant (LLQ; PLR <10, NLR <0.1): primarily useful for exclusion; and iv) right lower quadrant (RLQ; PLR <10, NLR >0.1): not useful for confirmation or exclusion. Fagan's nomogram was employed to assess the clinical utility of the HCVcAg assay for diagnosis, considering the estimated prevalence or pre-test probability (0.1-15%) and the PLR and NLR values [27].

Heterogeneity among the studies was assessed using the chi-squared test and Higgins' inconsistency index (I^2) [28,29]. Levels of heterogeneity were categorized as follows: ≤30% (might not be important), 30–60% (moderate), 60–75% (substantial), and ≥75% (considerable). The chi-squared test statistic was denoted as Q (Cochran-Q method), and a corresponding p-value was calculated to qualitatively evaluate heterogeneity, with a p≤0.10 considered statistically significant [21]. Galbraith's test [30], bivariate boxplots (bagplot) [31], and meta-regression analysis [32] were additionally performed to explore the impact of studies or variables on heterogeneity.

The meta-regression analysis examined the influence of the following factors on measures of diagnostic accuracy (p≤0.10) [21]: year of publication (Yes: >2017; No: ≤2017), LMICs (Yes/No), collection of venous (Yes/No) or capillary (Yes/No) blood specimens, sample size (Yes: <120; No: ≥120), HCV prevalence (Yes: >75%; No: ≤75%), COBAS Ampliprep/COBAS TaqMan HCV Real-time polymerase chain reaction (PCR) (Yes/No), overall QUADAS-2 risk (Yes: low/unclear; No: high), risk of bias in QUADAS-2 (Yes: low/unclear; No: high), and applicability concerns in QUADAS-2 (Yes: low/unclear; No: high) were considered.

Publication bias was evaluated using Deeks' funnel plot asymmetry [33], with a p≤0.10 indicating significant publication bias [27].

Results

Search results

A summary of the study selection strategy in this meta-analysis is presented in **Figure 1A**. Initially, 116 relevant studies were identified through the primary search of databases. Following a meticulous review of titles and abstracts, 91 duplicate or irrelevant records, as well as studies unrelated to HCV or review articles focusing on HCV diagnostic tests, were excluded. Moreover, 17 papers were eliminated from the 25 potentially relevant studies due to the absence of evaluation of the chosen diagnostic assay performance. Consequently, eight articles reporting data on HCVcAg detection with DBS samples were selected for further analysis [34-41].

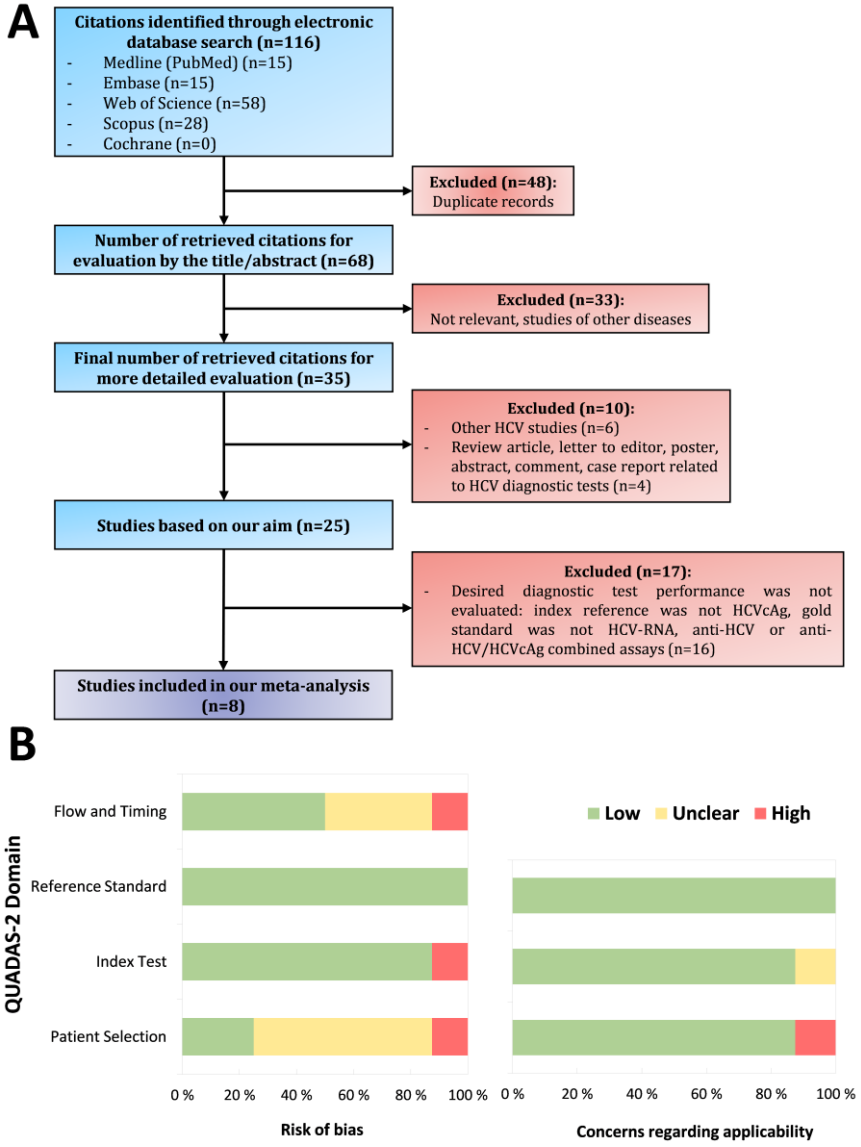

Figure 1. Flow diagram illustrating the search strategy **(A)** and quality assessment of included studies based on QUADAS-2 guidelines **(B)**. In Figure B, green means low risk, orange shows unclear risk, and red indicates high risk. **Abbreviations**: cAg = core antigen; HCV = hepatitis C virus; RNA, ribonucleic acid; QUADAS = quality assessment of diagnostic accuracy study.

Article characteristics

Table 1 summarizes the main characteristics of the eight articles included in the meta-analysis [34-41]. These articles were published between 2016 and 2023, and only three studies were conducted in LMICs [37-39]. Overall, the combined sample size for HCV screening encompassed 1,229 individuals, yielding a pooled prevalence of active HCV infection at 73.1%. In this regard, it should be noted

that seven studies had a cross-sectional design, and one was identified as a case-control study [41], studies with populations selected to evaluate the HCVcAg test, not to study prevalence. Moreover, many of the individuals included were from high-risk populations, such as people who inject drugs (PWID) [35,36,41], people with HIV and/or HBV coinfection [34,37,38], and individuals living in areas with high rates of HCV transmission [37-39]. The average age of participants across the studies was 48.4 years, with men constituting 74.1% of the sample. One study did not provide information on HCV genotypes among infected individuals [35]. Regarding sample collection methods, the majority (n=6; 75%) used DBS samples obtained via venous blood on filter paper [36-41], with one study employing capillary blood [35] and another using both venous and capillary blood [34].
All articles employed the ARCHITECT HCVcAg assay (Abbott Diagnostics) to assess HCVcAg levels via chemiluminescence immunoassay. For the diagnosis of HCV viremic patients, the gold standard method was predominantly the COBAS Ampliprep/COBAS TaqMan HCV (Roche Diagnostics), with the exception of two studies using the Abbott RealTime HCV Assay (Abbott Diagnostics) [35,39], and another using the POC Xpert HCV Viral Load Assay (Cepheid) [41].

Table 1. Summary of studies included in meta-analysis for detection of HCV core antigen in DBS samples

Author (year)	Country	Study design	Participants (No.)	Age (years)	Males (%)	HCV prevalence (%)	HCV genotype	HIV (%)	HBV (%)	DBS samples
Soulier et al. (2016)	France	Cross-sectional	511	53.3	55.2	61.6	1, 2, 3, 4, 5, and 6	N/A	N/A	Venous blood
Mohamed et al. (2017)	Tanzania	Cross-sectional	153	38	92.2	75.8	1 and 4	42.5	9.8	Venous blood
Lamoury et al. (2018)	Australia	Cross-sectional	120	N/A	N/A	78.3	1, 2, 3 and 6	N/A	N/A	Venous blood
Nguyen et al. (2018)	Vietnam	Cross-sectional	101	34.3	96	85.1	1, 3 and 6	100	N/A	Venous blood
Biondi et al. (2019)	Canada	Cross-sectional	59	55.8	59	83.1	1, 2, 3, 4, 5, and 6	N/A	1.7	Venous and capillary blood
Catlett et al. (2019)	Australia	Cross-sectional	186	46	84	29.0	N/A	N/A	N/A	Capillary blood
Ranjan et al. (2019)	India	Cross-sectional	29	51	N/A	100.0	1, 3 and 4	N/A	N/A	Venous blood
Troyano-Hernáez et al. (2023)	Spain	Case-control	70	60.5	58	71.4	1, 2, 3 and 4	N/A	N/A	Venous blood

Abbreviations: DBS = dried blood spot; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; N/A = not available

Assessment of risk of bias

A visual representation of the QUADAS-2 risk assessment is provided in **Figure 1B** (for further details, see **Supplementary File 3**). Only one study (12.5%) [35] received a low risk of bias rating and demonstrated low concerns regarding the applicability across all review questions. In the domain of patient selection, one study (12.5%) [40] was deemed to have a high risk of bias and raised high concerns regarding applicability. Five studies (62.5%) [34,36,38,39,41] were categorized as having an unclear risk of bias primarily due to insufficient information about study design, patient selection criteria, and exclusions. Concerning the index test domain, one study (12.5%) [39] was rated as high risk due to the interpretation of the index test with knowledge of the reference standard results.

Regarding applicability, one study (12.5%) [34] raised unclear concerns regarding the application and understanding of the HCVcAg test. In the flow and timing domain, three studies (37.5%) [34,37,40] were deemed to have an unclear risk of bias, while one study (12.5%) [39] was rated as having high risk due to inadequately described intervals between the index and reference tests. All articles (100%) were assessed as having a low risk of bias and minimal concerns regarding the applicability of the reference standard employed. This judgment was based on the high sensitivity and minimal variability of HCV-RNA tests, which made it unlikely for bias to be introduced even if the reference standard resulted in knowledge of the index test result.

Diagnostic performance

A pooled bivariate analysis conducted on seven studies with 1,200 samples estimated an overall sensitivity of 0.85 (95%CI = 0.76-0.92) (**Figure 2A**) and a specificity of 1.00 (95%CI = 0.96-1.00) (**Figure 2B**), respectively. From the pooled sensitivity and specificity, the PLR was 233.1 (95%CI: 19.9-2718.2) (**Figure 2C**), and the NLR was 0.15 (95%CI: 0.09-0.25) (**Figure 2D**). A pooled univariate analysis (n=8 studies) yielded a similar result for sensitivity (0.87, 95%CI: 0.78-0.93), slightly higher than that of the bivariate analysis (**Supplementary Figure 1**). The AUC-SROC was 0.99 (95%CI = 0.98-1.00), indicating excellent diagnostic accuracy (**Supplementary Figure 2**). The Ranjan et al. study [39] only included individuals identified as "HCV positive" using the gold standard. Therefore, it was not possible to calculate the specificity of the HCVcAg assay in this specific study.

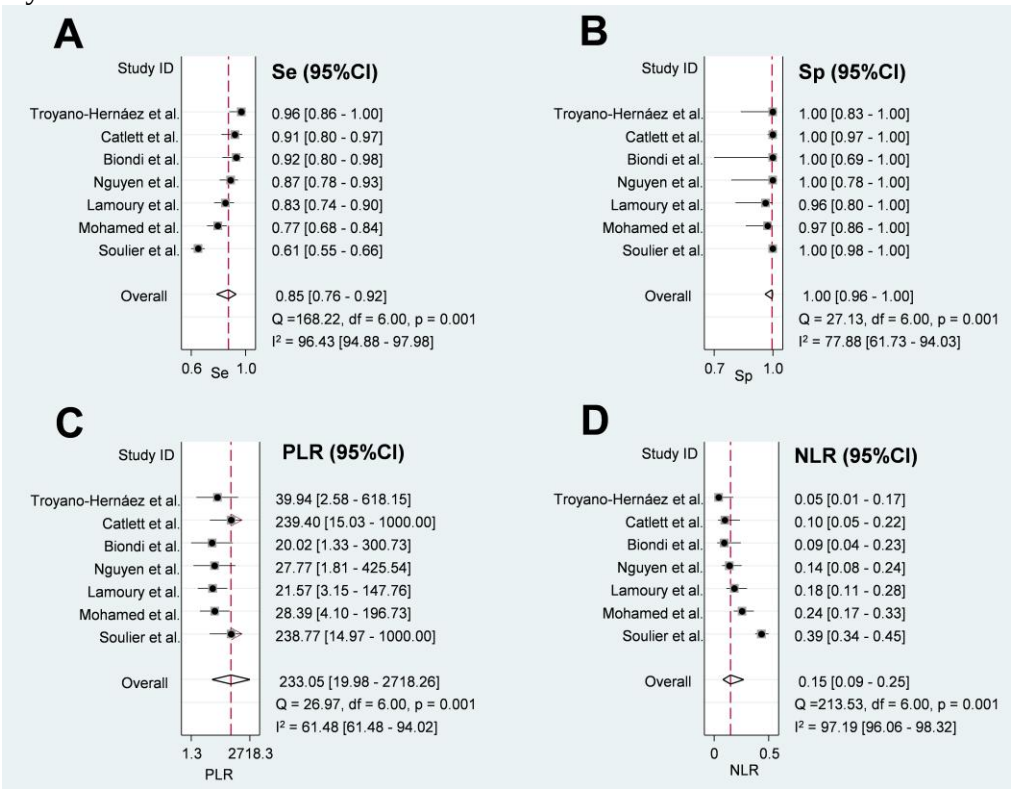

Figure 2. Forest plot showing sensitivity (A), specificity (B), PLR (C), and NLR (D) for detecting active HCV infection with DBS using the Abbott ARCHITECT HCV Ag assay compared to a confirmatory nucleic acid test using bivariate analysis. Abbreviations: 95%CI = 95% confidence interval; DBS = dried blood spot; df = degrees of freedom; I2 = inconsistency index; NLR = negative likelihood ratio; PLR = positive likelihood ratio; Q = Cochran's Q test; Se = sensitivity; Sp = specificity

Clinical application

The four-quadrant likelihood ratio scatters matrix shows that both PLR and NLR were in the RUQ, indicating that the HVCcAg test is useful only to confirm active HCV infection (**Supplementary Figure 3**). A simulation for several prevalence values of active hepatitis C (0.1%, 0.5%, 1%, 5%,

10%, and 15%) using Fagan's plots (**Figure 3**) was performed, showing that for low prevalence (≤1%), the post-test probability that an individual with a positive test result was a TP ranged from 19% to 70%, indicating that a confirmatory test must be necessary. However, the simulation for high prevalence (5-15%) showed a post-test probability between 92% and 98%, indicating that a confirmatory test should not be necessary (**Figure 3**). The probability that a person with a negative test result was an FN was close to zero in all cases (**Figure 3**), indicating that active HCV infection should be completely ruled out, regardless of HCV prevalence.

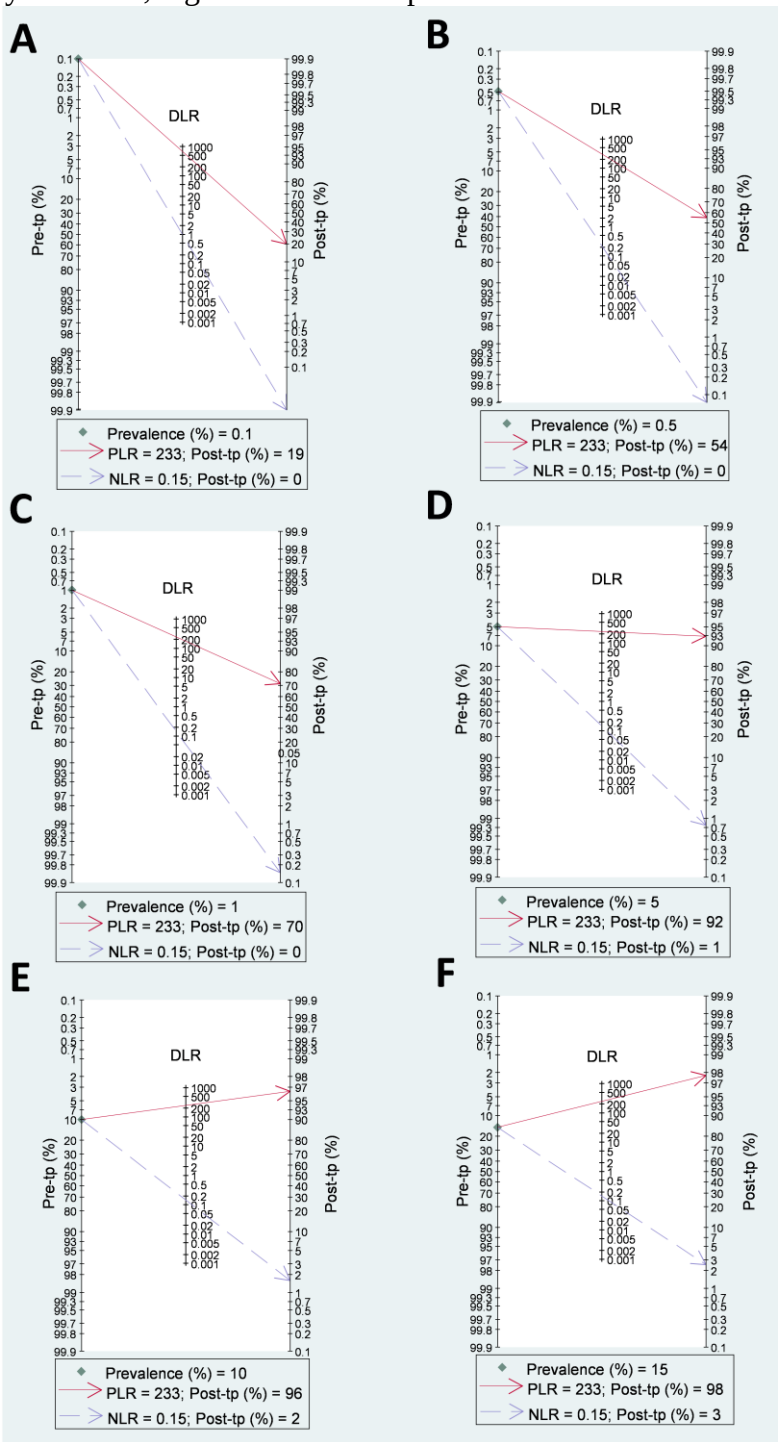

Figure 3. Fagan's plot illustrating the detection of active HCV infection with DBS using the Abbott ARCHITECT HCV Ag assay compared to a confirmatory nucleic acid test across various HCV prevalence percentages: 0.1% **(A)**, 0.5% **(B)**, 1% **(C)**, 5% **(D)**, 10% **(E)**, and 15% **(F)**. The graph features three scales: The left scale represents the pre-tp or prevalence; the central scale represents the DLR; and the right scale represents the post-tp, which is the outcome of the graph and indicates the probability of detecting or not detecting the HCV infection after performing the test. Knowing

the prevalence and the DLR values, a straight line is drawn (red for the PLR and blue for the NLR) from the pre-tp to the DLR. The point where the line crosses the right-hand scale will give us the post-tp. **Abbreviations**: DLR = diagnostic likelihood ratio; HCV = hepatitis C virus; NLR = negative likelihood ratio; PLR = positive likelihood ratio; Post-tp = post-test probability; Pre-tp = pre-test probability.

Exploration of heterogeneity

The heterogeneity tests showed values of I^2 = 96.4% (p<0.001) for sensitivity (substantial heterogeneity), I^2 = 77.9% (p<0.001) for specificity (considerable heterogeneity), I^2 = 61.5% (p<0.001) for PLR (moderate heterogeneity), and I^2 = 97.2% (p<0.001) for NLR (substantial heterogeneity) (**Figure 2**). One study [35] in the Galbraith plot (**Figure 4A**) and another one [34] in the bivariate box plot fell outside the 95%CI (**Figure 4B**), suggesting potential sources of heterogeneity for these studies. A sensitivity analysis was conducted through the sequential exclusion of each study to assess their influence on heterogeneity and diagnostic performance. While one article [41] had a greater impact on performance and diagnostic precision results, none of them showed a significant impact on heterogeneity (**Supplementary Table 1**).

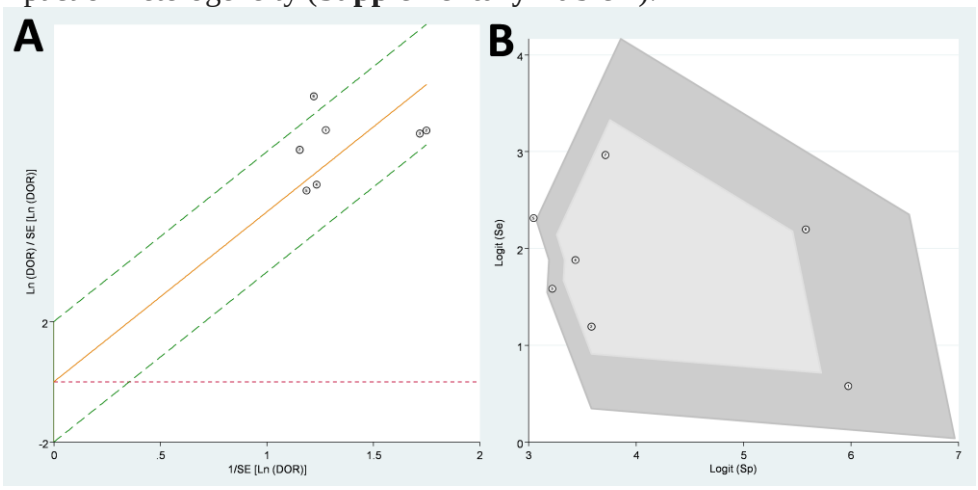

Figure 4. Galbraith plot (radial) **(A)** and bagplot **(B)** were used to evaluate the heterogeneity of the bivariate meta-analysis. The Galbraith analysis (**A**) examines the relationship between effect size (x-axis) and precision (y-axis) of studies. The orange line depicts the overall trend between these two variables, with dashed green lines representing their 95% confidence intervals. The bagplot (**B**) visualizes the joint distribution of sensitivity (y-axis) and specificity (x-axis), with shading indicating performance levels. Each study is plotted as a point. Outliers in either plot highlight variability between studies. **Abbreviations**: DOR = diagnostic odds ratio; Ln = natural logarithm; Se = sensitivity; SE = standard error; Sp = specificity

The meta-regression analysis showed that seven factors had a significant impact on heterogeneity (p≤0.10): year of publication, sample size, HCV prevalence, use of COBAS Ampliprep/COBAS TaqMan HCV Real-time PCR, QUADAS-2 overall risk, risk of bias and applicability concerns in QUADAS-2 (**Supplementary Table 2**). Both sample size and the use of COBAS Ampliprep/COBAS TaqMan HCV Real-time PCR significantly affected sensitivity and specificity (p≤0.10) (**Supplementary Table 3**). Meanwhile, year of publication, the method of sample collection (venous or capillary), HCV prevalence, QUADAS-2 overall risk, and the risk of bias and applicability concerns in QUADAS-2 had a significant effect only on specificity (p≤0.10) (**Supplementary Table 3**). However, these significant differences in sensitivity and specificity are not clinically relevant, mainly due to very close values between the groups and the small number of articles included in the study.

Based on Deeks' funnel plot, publication bias was not observed (p=0.38), indicating that there was no potential impact on heterogeneity (**Supplementary Figure 4**).

Discussion

This study evaluated the diagnostic accuracy of the HCVcAg detection test with DBS samples using the Abbott ARCHITECT HCV Ag assay. Our findings indicate that the ARCHITECT HCV Ag assay with DBS offers excellent diagnostic accuracy for screening active HCV infection. The assay demonstrated a combined sensitivity of 85%, specificity of 100%, and an AUC-SROC of 99%. Remarkably, the diagnostic utility of the HCVcAg assay with DBS samples was good for high-prevalence settings (≥5%) but limited in low-prevalence settings (≤1%).

Three recent meta-analyses have demonstrated excellent diagnostic performance for detecting active HCV infection with DBS samples [42-44]. Among them, only Carty et al. [42] evaluated the diagnostic accuracy of the HCVcAg test with DBS samples. Our meta-analysis has distinctive features compared to Carty's work [42], providing several value-adding assets. First, we included data from eight articles, three more than those initially included by Carty et al. [42], nearly doubling the sample size (1,229 *vs.* 643). Second, our meta-analysis and that of Carty et al.'s [42] achieved very similar results in terms of sensitivity (85% *vs.* 86%), specificity (100% *vs.* 98%), and NLR (both 0.15). However, the combined PLR value in our meta-analysis was 61 times higher than that of Carty et al. (233.1 *vs.* 5.5), and the AUC-SROC was slightly higher (99% *vs.* <90%), probably due to the inclusion of three additional articles. This improvement elevated the diagnostic performance of the HCVcAg assay with DBS samples from good to excellent and demonstrates that the test is 61 times more likely to yield a positive result in HCV-infected individuals compared to the results reported by Carty et al. [42]. Finally, while Carty et al. [42] did not provide comprehensive data on diagnostic utility or clinical applicability, merely indicating the limited utility of the HCVcAg test with DBS specimens in low-prevalence settings, our study evaluated its diagnostic utility across various HCV prevalence scenarios and its potential integration into existing testing programs. Our findings could be extrapolated to real-life scenarios in different at-risk populations and endemic areas.

Our meta-analysis found solid evidence supporting the use of the Fagan nomogram to confirm or rule out active HCV infection. The WHO prioritizes simpler and more affordable HCV diagnostics through single-step tests. However, our data suggests limitations for the ARCHITECT HCV Ag assay with DBS as a standalone tool in low-prevalence settings (≤1%). In such scenarios, it might be most effective to use a first-line screening test, followed by a confirmatory NAAT to confirm positive results obtained with the HCVcAg test. In contrast, the assay could be a highly interesting alternative to the current HCV double-step algorithm in high-prevalence settings (≥5%). Moreover, we observed an insignificant impact of HCV prevalence on negative HCVcAg results. A negative test carries a very low probability (<3%) of active HCV infection, effectively ruling it out with high confidence.

Therefore, the Abbott ARCHITECT HCV Ag assay with DBS can be used in marginalized populations and regions with high HCV prevalence. High HCV prevalence is often found in high-risk adult populations, such as PWID, people experiencing homelessness, men who have sex with men (MSM), sex workers, and individuals in prison, among others [2]. Additionally, these vulnerable populations are at a higher risk of HCV reinfection following successful direct-acting antiviral (DAA) treatment due to high-risk behaviors that can lead to potential virus transmission. Moreover, hepatitis C has a worldwide distribution, but its prevalence varies significantly across regions. As of 2024, some countries exhibit high prevalence rates (>2%), especially in Eastern Europe (Ukraine, Russia, Moldova, Bulgaria, and Latvia), Africa (Gabon, Burundi, Sudan, Libya, Ghana, and Burkina Faso), the Middle East (Syria and Qatar), the South Caucasus (Georgia, Armenia, and Azerbaijan), and Central Asia (Pakistan, Uzbekistan, Mongolia, Tajikistan, Kyrgyzstan, and Turkmenistan) [45]. Many of these countries are LMICs that often lack adequate infection control and clinical hygiene procedures. Additionally, these regions often have populations vulnerable due to poverty, limited healthcare personnel, facilities, or restricted access to rapid HCV screening tests [3,46]. Early detection and management of HCV infection are essential in these settings to minimize virus transmission.

This study evaluated the diagnostic accuracy of the Abbott ARCHITECT HCV Ag assay with DBS for screening for active HCV infection. While the established assay in serum/plasma samples offers higher sensitivity compared to DBS (96% *vs.* 85%) [15], it requires trained phlebotomists for venipuncture, creating logistical hurdles in sample collection, storage, and transport. In contrast, the HCVcAg assay with DBS is a valuable tool for early detection and reducing loss-to-follow-up, particularly in LMICs and hard-to-reach populations. DBS collection is easier, requiring minimal training, and allows for simpler storage and transportation at ambient temperature. Moreover, the less invasive finger-stick collection likely improves patient acceptance, offering a potential compromise between accessibility and ensuring accurate diagnosis. On the other hand, we did not evaluate the assay's potential application for treatment monitoring and SVR assessment due to limited data and research. The current reliance on HCV RNA detection by NAATs for treatment response and SVR assessment, particularly in remote areas, can hinder the establishment of hepatitis C treatment and testing services [47]. A recent study demonstrated the effectiveness of the Abbott ARCHITECT HCV Ag test for treatment monitoring and SVR assessment after DAA completion using plasma/serum samples [16]. However, data on HCVcAg with DBS for treatment monitoring and SVR assessment remains scarce, so its use as a substitute for NAATs cannot be currently recommended.

Despite the existence of a safe and highly effective oral cure for hepatitis C, many people remain undiagnosed. The current HCV testing algorithm, coupled with limited healthcare access, leads to many individuals lost to follow-up after initial. Thus, they never receive a definitive diagnosis and treatment. In this context, point-of-care (PoC) testing offers a more efficient solution for diagnosing HCV infection and rapidly linking patients to treatment and follow-up care [48]. Recently, the US Food and Drug Administration authorized the marketing of the first PoC test for HCV RNA detection, the Cepheid Xpert HCV test with the GeneXpert Xpress System [49]. This test expands access to diagnosis for at-risk individuals in settings with a certificate of waiver under the clinical laboratory improvement amendments. The Cepheid Xpert HCV test, using a finger-stick blood sample, eliminates the need for two-step laboratory testing (immunoassay plus PCR) by detecting HCV RNA and providing results within an hour, enabling same-day testing, diagnosis, and treatment initiation. However, the Cepheid Xpert HCV test faces limitations in resource-limited settings. The GeneXpert machine and its cartridges can be expensive, posing a significant barrier. Besides, operating, maintaining, and calibrating the machine requires trained personnel, which may be scarce in LMICs with limited healthcare budgets. Additionally, extreme environmental conditions, like high humidity or temperature, can affect the Xpert HCV test's performance by compromising the stability and function of reagents. In contrast, the HCVcAg assay using DBS samples offers several advantages for HCV screening in LMICs and hard-to-reach areas. It improves accessibility by reducing costs and simplifying logistics, making it a valuable tool in the fight against HCV.

The accuracy of a systematic review is closely linked to the quality of the included studies. Although the overall quality of the studies in this meta-analysis was moderately high, only one article had low risk across all categories. The remaining articles had some elements that were unclear or missing information related to the risk of bias. The main drawbacks included unclear descriptions of whether the studies used consecutive or random samples, patient flow, and study duration. The patient selection process in these studies often lacks clarity regarding sampling methods, case-control design, and inclusion/exclusion criteria, which can introduce confounding biases and limit the generalizability of the findings. Moreover, a significant percentage of DBS samples were not collected at the point of care for the patients, raising concerns about their real-world applicability. Regarding the applicability of our meta-analysis, the quality was considered high as six studies had a low risk across all three categories. Finally, it is important to note that all studies had a low risk of bias and applicability concerns in the reference standard domain, which is crucial as it is unlikely that biases would be introduced even if HCVcAg test results were known beforehand. Furthermore, while the accuracy of NAAT as a reference standard is not absolute, these tests are highly sensitive and show minimal variability. In summary, five articles were classified as

high quality (62.5%, with 6 or more categories having low risk out of 7). Consequently, an analysis based solely on high-quality studies could not be conducted.

Heterogeneity is another crucial factor to consider when assessing a meta-analysis, typically evaluated using Cochran's Q test and the I^2 statistic [29]. In this case, the I^2 statistic is more appropriate for assessing heterogeneity because it is independent of the number of included studies and easier to interpret [29]. Our meta-analysis showed moderate to substantial heterogeneity, which is common in this type of diagnostic test-based meta-analysis due to the challenges in controlling all potential confounders. Consequently, a random-effects analysis was conducted, assuming heterogeneity among studies [50]. Moreover, a meta-regression was performed to identify possible confounders that significantly impacted heterogeneity, such as year of publication, sample size, HCV prevalence, HCV-RNA assay manufacturer, and quality of studies (QUADAS-2). In addition to these factors, we must not rule out the impact of other variables not evaluated in this study, such as HCV viral load, HCV subtype, DBS elution method, co-infection with HBV and/or HIV, RNA extraction methods, or regions of the HCV genome for amplification. It was also observed that out of the 10 analyzed confounders, nine (except LMICs) had an impact on sensitivity and/or specificity, although this impact was clinically irrelevant.

A strength of our work is that it was conducted in a standardized manner according to a predefined protocol. The systematic review was carried out following an a priori protocol across several international biomedical databases, including PubMed, SCOPUS, EMBASE, WoS, and the Cochrane Library, without language restrictions. Moreover, article selection, data extraction, and quality assessment were performed by two independent researchers. In cases of disagreement or uncertainty, a consensus was reached with the involvement of a third researcher.

Limitations

This meta-analysis has several limitations that need to be considered when interpreting the results. Firstly, the number of included articles was small, highlighting the need for more published studies evaluating the diagnostic performance of HCVcAg with DBS samples. Secondly, the majority of the included studies were conducted in Western countries, so the results may not be directly applicable to LMICs. Thirdly, not all studies used the same gold standard and cutoff point, which could introduce biases. Fourthly, the lack of clarity in patient selection criteria across multiple studies could also introduce a risk of selection bias, limiting the generalizability of the results. Fifthly, only two articles evaluated the diagnostic performance in capillary samples obtained through a finger-stick. Finally, we did not conduct a cost-effectiveness study, which is an important consideration for most public health interventions, especially in LMICs.

Conclusions

In conclusion, HCV screening using the Abbott ARCHITECT HCV Ag assay with DBS samples showed excellent diagnostic performance, but its external validity may be limited when HCV prevalence is low (≤1%). This approach could be valuable for implementing national programs for HCV infection control in LMICs and hard-to-reach populations. Nevertheless, further studies are needed to determine the diagnostic accuracy of this assay in real-world settings.

Declarations

Ethics approval and consent to participate

This study was approved by the "Instituto de Salud Carlos III" Ethics Committee (Ref.: CEI PI 13_2021). This study involves clinical-epidemiological data of the patients from the published articles, so the informed consent signed by the patients was unnecessary.

Consent for publication

Not applicable.

Data Availability statement

The data that supports the findings of this study are available in the paper and supplementary material of this article

Conflict of interest statement

No conflict of interest declared

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Author contribution statement:

DSC: funding acquisition, investigation, resources, writing – original draft
AT: investigation, resources, writing – original draft, and editing
JMB: investigation, methodology, formal analysis
HC: investigation, writing – review, and editing
RAS: investigation, writing – review, and editing
MQD: investigation, writing – review, and editing
PR: writing – review, and editing
IM: funding acquisition, writing – original draft
SR: funding acquisition, conceptualization, formal analysis, writing – original draft, supervision.

Authors' information (optional)

Not applicable.

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Supplementary Materials

Title: Diagnostic performance of dried blood spot HCVcAg testing for hepatitis C screening: a systematic review and meta-analysis

Short title: HCVcAg screening in DBS samples

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Supplementary File 1: PRISMA-DTA Checklist and Abstracts Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	3-4
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	4
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	5
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Suppl. File 2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	5-6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	5-6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	5-6
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	5-6
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	6
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	6-7
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	6-7
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	6-7
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	6-7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	6-7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	7

Section and Topic	Item #	Checklist item	Location where item is reported
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	6
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	6
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	6-7
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	8
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	8
Study characteristics	17	Cite each included study and present its characteristics.	Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	8-9
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Fig. 2 and Suppl. Fig. 1
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	8-9
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	10
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	10
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	10
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	10
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	10
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	11
	23b	Discuss any limitations of the evidence included in the review.	12-14
	23c	Discuss any limitations of the review processes used.	14-15
	23d	Discuss implications of the results for practice, policy, and future research.	11-15
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	5
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	5
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	5
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	16
Competing	26	Declare any competing interests of review authors.	16

Section and Topic	Item #	Checklist item	Location where item is reported
interests			
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	16

Topic	No.	Item for Abstracts	Reported?
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesize results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	No
Registration	12	Provide the register name and registration number.	Yes

Supplementary File 2: Search strategy

Search strategy PubMed

("hepatitis c"[MeSH Terms] OR "hepacivirus"[MeSH Terms] OR ("hepatitis c"[Title/Abstract] OR "hepatitis c virus"[Title/Abstract] OR "hepatitis c viruses"[Title/Abstract] OR "hepatitis c like virus"[Title/Abstract] OR "hepatitis c like viruses"[Title/Abstract] OR "hepatitis virus type c"[Title/Abstract] OR "hcv"[Title/Abstract] OR "h c v"[Title/Abstract] OR "vhc"[Title/Abstract] OR "v h c"[Title/Abstract] OR "hepacivirus"[Title/Abstract] OR "hepaciviruses"[Title/Abstract] OR "hcv viral"[Title/Abstract] OR "hcv infected"[Title/Abstract] OR "hcv infection"[Title/Abstract] OR "hcv rna"[Title/Abstract] OR "hepatitis c virus rna"[Title/Abstract] OR "parenterally transmitted non a non"[Title/Abstract] OR "pt nanbh"[Title/Abstract])) AND ("diagnosis"[MeSH Terms] OR "diagnostic techniques and procedures"[MeSH Terms] OR "clinical laboratory techniques"[MeSH Terms] OR "mass screening"[MeSH Terms] OR "nucleic acid amplification techniques"[MeSH Terms] OR "rna"[MeSH Terms] OR "rna, viral/blood"[MeSH Terms] OR ("clinical laboratory diagnoses"[Title/Abstract] OR "clinical laboratory diagnostic"[Title/Abstract] OR "clinical laboratory techniques"[Title/Abstract] OR "clinical laboratory testing"[Title/Abstract] OR "diagnose"[Title/Abstract] OR "diagnoses"[Title/Abstract] OR "diagnosis of hcv"[Title/Abstract] OR "diagnosis"[Title/Abstract] OR "diagnostic techniques and procedures"[Title/Abstract]OR "diagnostic"[Title/Abstract] OR "hcv infection diagnosis"[Title/Abstract] OR "hcv testing"[Title/Abstract] OR "mass screening"[Title/Abstract] OR "mass screenings"[Title/Abstract] OR "molecular diagnostic techniques"[Title/Abstract] OR "screening approach"[Title/Abstract] OR "screening"[Title/Abstract] OR "testing diagnostic"[Title/Abstract] OR "plasma levels"[Title/Abstract] OR "sera"[Title/Abstract] OR "serum levels"[Title/Abstract] OR "dried blood filter" [Title/Abstract] OR "dried blood spot"[Title/Abstract] OR "dried blood" [Title/Abstract] OR "dried sample" [Title/Abstract] OR "filter paper" [Title/Abstract] OR "Whatman" [Title/Abstract] OR "DBS" [Title/Abstract] OR "assay kits"[Title/Abstract] OR "hcv assays"[Title/Abstract] OR "hcv pcr assay"[Title/Abstract] OR "hcv pcr method"[Title/Abstract] OR "hcv pcr"[Title/Abstract] OR "hcv rna levels"[Title/Abstract] OR "hcv rna"[Title/Abstract] OR "hcv rna quantification"[Title/Abstract] OR "hepatitis c markers"[Title/Abstract] OR "hepatitis markers"[Title/Abstract] OR "immunoassay"[Title/Abstract] OR "quantitative assays"[Title/Abstract] OR "quantitative reverse transcription pcr"[Title/Abstract] OR "real time pcr"[Title/Abstract] OR "rna levels"[Title/Abstract] OR "roche cobas taqman assays"[Title/Abstract] OR "roche cobas taqman hcv"[Title/Abstract])) AND ("hepatitis c antigens"[MeSH Terms] OR ("antigens"[Title/Abstract] OR "core antigen assay"[Title/Abstract] OR "core antigen assays"[Title/Abstract] OR "core antigen test"[Title/Abstract] OR "core antigen"[Title/Abstract] OR "hcv ag assay"[Title/Abstract] OR "hcv ag detection"[Title/Abstract]OR "hcv ag"[Title/Abstract] OR "hcv antigen testing"[Title/Abstract] OR "hcv antigen"[Title/Abstract] OR "hcv core antigen assay"[Title/Abstract] OR "hcv core antigen assays"[Title/Abstract] OR "hcv core antigen detection"[Title/Abstract] OR "hcv core antigen determination"[Title/Abstract] OR "hcv core antigen testing"[Title/Abstract] OR "hcv core antigen"[Title/Abstract] OR "hcv core protein"[Title/Abstract] OR "hcv core region"[Title/Abstract] OR "hcv cp"[Title/Abstract] OR "hcvcoreag"[Title/Abstract] OR "hepatitis c antigens"[Title/Abstract] OR "hepatitis c virus core antigen"[Title/Abstract] OR "hepatitis c virus core"[Title/Abstract] OR "hepatitis non a non b antigen"[Title/Abstract] OR "viral core proteins"[Title/Abstract])) AND ("accuracy"[Title/Abstract] OR "correlation"[Title/Abstract] OR "correlations"[Title/Abstract] OR "negative predictive power"[Title/Abstract] OR "negative predictive value"[Title/Abstract] OR "negative predictive values"[Title/Abstract] OR "NPV"[Title/Abstract] OR "positive predictive power"[Title/Abstract] OR "positive predictive value"[Title/Abstract] OR "positive predictive values"[Title/Abstract] OR "PPV"[Title/Abstract] OR "receiver operating characteristics"[Title/Abstract] OR "regression analysis"[Title/Abstract] OR "ROC"[Title/Abstract] OR "sensitive"[Title/Abstract] OR "sensitivities"[Title/Abstract] OR "sensitivity"[Title/Abstract] OR "specific"[Title/Abstract] OR "specificity"[Title/Abstract] OR "Abbott ARCHITECT HCV Ag assay" OR "Abbott ARCHITECT HCV Ag test" OR "Abbott ARCHITECT HCV Antigen assay" OR "Abbott ARCHITECT i2000SR" OR "Abbott ARCHITECT test" OR "Abbott Diagnostics" OR "Abbott HCV Ag" OR "Abbott HCV core antigen" OR "Abbott Laboratories" OR "ARCHITECT" OR "ARCHITECT ci8200" OR

“Architect core antigen” OR “Architect HCV Ag” OR “ARCHITECT HCV Core antigen” OR “ARCHITECT HCVAg” OR “ARCHITECT i2000SR” OR “ARCHITECT system” OR “ARCHITECTHCVAg” OR “ARCHITECT-i2000R” OR "cleia method" OR “chemiluminescence immunoassay”) AND (“DBS” [Title/Abstract] OR "Whatman” [Title/Abstract] OR "filter paper” [Title/Abstract] OR "Dried blood filter” [Title/Abstract] OR "Dried blood” [Title/Abstract] OR "Dried sample” [Title/Abstract] OR "dried blood spot"[Title/Abstract]) NOT ("review"[Publication Type]) NOT ("meta-analysis"[Publication Type]) NOT ("systematic review"[Publication Type])

Search strategy Embase

#1 'hepatitis c'/exp OR 'hepacivirus'/exp OR 'hepatitis c virus':ti,ab,kw OR 'hepatitis c viruses':ti,ab,kw OR 'hepatitis c like viruses':ti,ab,kw OR 'hepatitis virus type c':ti,ab,kw OR hcv:ti,ab,kw OR 'h c v':ti,ab,kw OR vhc:ti,ab,kw OR 'v h c':ti,ab,kw OR hepacivirus:ti,ab,kw OR hepaciviruses:ti,ab,kw OR 'parenterally transmitted non a non':ti,ab,kw

#2 'diagnosis'/exp OR ('diagnostic techniques'/exp AND 'procedures'/exp) OR 'clinical laboratory techniques'/exp OR 'nucleic acid amplification techniques'/exp OR 'rna'/exp OR 'clinical laboratory diagnostic':ti,ab,kw OR 'clinical laboratory techniques':ti,ab,kw OR 'clinical laboratory testing':ti,ab,kw OR diagnose:ti,ab,kw OR diagnoses:ti,ab,kw OR 'diagnosis of hcv':ti,ab,kw OR diagnosis:ti,ab,kw OR ('diagnostic techniques':ti,ab,kw AND procedures:ti,ab,kw) OR diagnostic:ti,ab,kw OR 'hcv testing':ti,ab,kw OR 'mass screening':ti,ab,kw OR 'mass screenings':ti,ab,kw OR 'screening approach':ti,ab,kw OR screening:ti,ab,kw OR 'plasma levels':ti,ab,kw OR sera:ti,ab,kw OR 'serum levels':ti,ab,kw OR 'dried blood filter':ti,ab,kw OR 'dried blood spot':ti,ab,kw OR 'dried blood':ti,ab,kw OR 'dried sample':ti,ab,kw OR 'filter paper':ti,ab,kw OR whatman:ti,ab,kw OR dbs:ti,ab,kw OR 'assay kits':ti,ab,kw OR 'hcv assays':ti,ab,kw OR 'hcv pcr assay':ti,ab,kw OR 'hcv pcr':ti,ab,kw OR 'hcv rna levels':ti,ab,kw OR 'hcv rna quantification':ti,ab,kw OR 'hepatitis c markers':ti,ab,kw OR 'hepatitis markers':ti,ab,kw OR immunoassay:ti,ab,kw OR 'quantitative assays':ti,ab,kw OR 'quantitative reverse transcription pcr':ti,ab,kw OR 'real time pcr':ti,ab,kw OR 'rna levels':ti,ab,kw OR 'roche cobas taqman':ti,ab,kw

#3 'hepatitis c antigens'/exp OR antigens:ti,ab,kw OR 'cleia method':ti,ab,kw OR 'core antigen assay':ti,ab,kw OR 'core antigen assays':ti,ab,kw OR 'core antigen test':ti,ab,kw OR 'core antigen':ti,ab,kw OR 'hcv ag assay':ti,ab,kw OR 'hcv ag detection':ti,ab,kw OR 'hcv ag':ti,ab,kw OR 'hcv antigen testing':ti,ab,kw OR 'hcv antigen':ti,ab,kw OR hcvcoreag:ti,ab,kw OR 'hepatitis non a non b antigen':ti,ab,kw OR 'viral core proteins':ti,ab,kw

#4 'accuracy':ti,ab,kw OR 'correlation':ti,ab,kw OR 'correlations':ti,ab,kw OR 'negative predictive power':ti,ab,kw OR 'negative predictive value':ti,ab,kw OR 'negative predictive values':ti,ab,kw OR 'NPV':ti,ab,kw OR 'positive predictive power':ti,ab,kw OR 'positive predictive value':ti,ab,kw OR 'positive predictive values':ti,ab,kw OR 'PPV':ti,ab,kw OR 'receiver operating characteristics':ti,ab,kw OR 'regression analysis':ti,ab,kw OR 'ROC':ti,ab,kw OR 'sensitive':ti,ab,kw OR 'sensitivities':ti,ab,kw OR 'sensitivity':ti,ab,kw OR 'specific':ti,ab,kw OR 'specificity':ti,ab,kw OR 'Abbott ARCHITECT HCV Ag assay' OR ' Abbott ARCHITECT HCV Ag test' OR 'Abbott ARCHITECT i2000SR' OR 'Abbott ARCHITECT test' OR 'Abbott Diagnostics' OR 'Abbott HCV Ag' OR 'Abbott HCV core antigen' OR 'Abbott Laboratories' OR 'ARCHITECT' OR 'Architect core antigen' OR 'ARCHITECT i2000SR'

#5 'DBS':ti,ab,kw OR 'Whatman':ti,ab,kw OR 'filter paper':ti,ab,kw OR 'Dried blood filter ':ti,ab,kw OR 'Dried blood':ti,ab,kw OR 'Dried sample':ti,ab,kw OR 'Dried blood spot':ti,ab,kw

#6 #1 AND #2 AND #3 AND #4 AND #5

#7 #6 AND 'Article'/it

Search strategy Web of Knowledge (WoS)

#1 ((((((((((((((((TS=(hepatitis c)) OR TS=(hepatitis c virus)) OR TS=(hepatitis c viruses)) OR TS=(hepatitis c like virus)) OR TS=(hepatitis c like viruses)) OR TS=(hepatitis virus type c)) OR TS=(hcv)) OR TS=(h c v)) OR TS=(vhc)) OR TS=(v h c)) OR TS=(hepacivirus)) OR TS=(hepaciviruses)) OR TS=(hcv viral)) OR TS=(hcv infected)) OR TS=(hcv infection)) OR TS=(hcv rna)) OR TS=(hepatitis c virus rna)) OR TS=(parenterally transmitted non a non)) OR TS=(pt-nanbh)

#2 ((TS=(clinical laboratory diagnoses)) OR TS=(clinical laboratory diagnostic)) OR TS=(clinical laboratory techniques)) OR TS=(clinical laboratory testing)) OR TS=(diagnose)) OR TS=(diagnoses)) OR TS=(diagnosis of hcv)) OR TS=(diagnosis)) OR TS=(diagnostic techniques and procedures)) OR TS=(diagnostic)) OR TS=(hcv infection diagnosis)) OR TS=(hcv testing)) OR TS=(mass screening)) OR TS=(mass screenings)) OR TS=(molecular diagnostic techniques)) OR TS=(screening approach)) OR TS=(screening)) OR TS=(testing diagnostic)) OR TS=(plasma levels)) OR TS=(sera)) OR TS=(serum levels)) OR TS=(dried blood filter)) OR TS=(dried blood spot)) OR TS=(dried blood)) OR TS=(dried sample)) OR TS=(filter paper)) OR TS=(Whatman)) OR TS=(DBS)) OR TS=(assay kits)) OR TS=(hcv assays)) OR TS=(hcv pcr assay)) OR TS=(hcv pcr method)) OR TS=(hcv pcr)) OR TS=(hcv rna levels)) OR TS=(hcv rna quantification assays)) OR TS=(hcv rna quantification)) OR TS=(hepatitis c markers)) OR TS=(hepatitis markers)) OR TS=(immunoassay)) OR TS=(quantitative assays)) OR TS=(quantitative reverse transcription pcr)) OR TS=(real time pcr)) OR TS=(rna levels)) OR TS=(roche cobas taqman assays)) OR TS=(roche cobas taqman hcv)

#3 ((((((((((((((((((((((((TS=(antigens)) OR TS=(cleia method)) OR TS=(core antigen assay)) OR TS=(core antigen assays)) OR TS=(core antigen test)) OR TS=(core antigen)) OR TS=(hcv ag assay)) OR TS=(hcv ag detection)) OR TS=(hcv ag)) OR TS=(hcv antigen testing)) OR TS=(hcv antigen)) OR TS=(hcv core antigen assay)) OR TS=(hcv core antigen assays)) OR TS=(hcv core antigen detection)) OR TS=(hcv core antigen determination)) OR TS=(hcv core antigen testing)) OR TS=(hcv core antigen)) OR TS=(hcv core protein)) OR TS=(hcv core region)) OR TS=(hcv cp)) OR TS=(hcvcoreag)) OR TS=(hepatitis c antigens)) OR TS=(hepatitis c virus core antigen)) OR TS=(hepatitis c virus core)) OR TS=(hepatitis non a non b antigen)) OR TS=(viral core proteins)

#4 ((TS=(accuracy)) OR TS=(correlation)) OR TS=(correlations)) OR TS=(negative predictive power)) OR TS=(negative predictive value)) OR TS=(negative predictive values)) OR TS=(NPV)) OR TS=(positive predictive power)) OR TS=(positive predictive value)) OR TS=(positive predictive values)) OR TS=(PPV)) OR TS=(receiver operating characteristics)) OR TS=(regression analysis)) OR TS=(ROC)) OR TS=(sensitive)) OR TS=(sensitivities)) OR TS=(sensitivity)) OR TS=(specific)) OR TS=(specificity)) OR TS=(Abbott ARCHITECT HCV Ag assay)) OR TS=(Abbott ARCHITECT HCV Ag test)) OR TS=(Abbott ARCHITECT HCV Antigen assay)) OR TS=(Abbott ARCHITECT i2000SR)) OR TS=(Abbott ARCHITECT test)) OR TS=(Abbott Diagnostics)) OR TS=(Abbott HCV Ag)) OR TS=(Abbott HCV core antigen)) OR TS=(Abbott Laboratories)) OR TS=(ARCHITECT)) OR TS=(ARCHITECT ci8200)) OR TS=(Architect core antigen)) OR TS=(Architect HCV Ag)) OR TS=(ARCHITECT HCV Core antigen)) OR TS=(ARCHITECT HCVAg)) OR TS=(ARCHITECT i2000SR)) OR TS=(ARCHITECT system)) OR TS=(ARCHITECTHCVAg)) OR TS=(ARCHITECT-i2000R)) OR TS=(cleia method)) OR TS=(chemiluminescence immunoassay)

#5 (((((((TS=(dried blood filter)) OR TS=(dried blood spot)) OR TS=(dried blood)) OR TS=(dried sample)) OR TS=(filter paper)) OR TS=(Whatman)) OR TS=(DBS))

#6 #1 AND #2 AND #3 AND #4 AND #5

#7 #6 NOT (DT==("REVIEW" OR "EDITORIAL MATERIAL"))

Search strategy SCOPUS

(TITLE-ABS-KEY ("hepatitis c virus") OR TITLE-ABS-KEY ("hepatitis c like virus") OR TITLE-ABS-KEY ({hepatitis virus type c}) OR TITLE-ABS-KEY ({hcv}) OR TITLE-ABS-KEY ({h c v}) OR TITLE-ABS-KEY ({vhc}) OR TITLE-ABS-KEY ({v h c}) OR TITLE-ABS-KEY ("hepacivirus") OR TITLE-ABS-KEY ({hcv viral}) OR TITLE-ABS-KEY ({hcv infected}) OR TITLE-ABS-KEY ({hcv infection}) OR TITLE-ABS-KEY ("hcv rna") OR TITLE-ABS-KEY ({pt nanbh}) OR TITLE-ABS-KEY ({parenterally transmitted non a non})) AND (TITLE-ABS-KEY ({clinical laboratory diagnoses}) OR TITLE-ABS-KEY ({clinical laboratory techniques}) OR TITLE-ABS-KEY ({clinical laboratory testing}) OR TITLE-ABS-KEY ("diagnose") OR TITLE-ABS-KEY ({diagnostic techniques and procedures}) OR TITLE-ABS-KEY ({hcv infection diagnosis}) OR TITLE-ABS-KEY ({hcv testing}) OR TITLE-ABS-KEY ("mass screening") OR TITLE-ABS-KEY ({molecular diagnostic techniques}) OR TITLE-ABS-KEY ("screening*") OR TITLE-ABS-KEY ({testing diagnostic}) OR TITLE-ABS-KEY ({plasma levels}) OR TITLE-ABS-KEY ({sera}) OR TITLE-ABS-KEY ({serum levels}) OR TITLE-ABS-KEY ("dried blood*") OR TITLE-ABS-KEY ("dried sample*") OR TITLE-ABS-KEY ({DBS}) OR TITLE-ABS-KEY ({filter paper}) OR TITLE-ABS-KEY ({Whatman}) OR TITLE-ABS-KEY ({assay kits}) OR TITLE-ABS-KEY ("hcv assay") OR TITLE-ABS-KEY (hcv pcr*) OR TITLE-ABS-KEY ({hcv rna levels}) OR TITLE-ABS-KEY ("hcv rna quantification*") OR TITLE-ABS-KEY ("hepatitis C markers") OR TITLE-ABS-KEY ({immunoassay}) OR TITLE-ABS-KEY ("quantitative assay") OR TITLE-ABS-KEY ({quantitative reverse transcription pcr}) OR TITLE-ABS-KEY ({real time pcr}) OR TITLE-ABS-KEY ({rna levels}) OR TITLE-ABS-KEY ({roche cobas taqman})) AND (TITLE-ABS-KEY ({antigens}) OR TITLE-ABS-KEY ({cleia method}) OR TITLE-ABS-KEY ("core antigen*") OR TITLE-ABS-KEY ("hcv ag*") OR TITLE-ABS-KEY ("hcv antigen*") OR TITLE-ABS-KEY ("hcv core antigen*") OR TITLE-ABS-KEY ("hcv core*") OR TITLE-ABS-KEY ("hcv cp") OR TITLE-ABS-KEY ("hcvcoreag") OR TITLE-ABS-KEY ("hepatitis c antigen") OR TITLE-ABS-KEY ({viral core proteins})) AND (TITLE-ABS-KEY ({ accuracy }) OR TITLE-ABS-KEY ({ correlation }) OR TITLE-ABS-KEY ({ correlations }) OR TITLE-ABS-KEY ({ negative predictive power }) OR TITLE-ABS-KEY ({ negative predictive value }) OR TITLE-ABS-KEY ({ negative predictive values }) OR TITLE-ABS-KEY ({ NPV}) OR TITLE-ABS-KEY ({ positive predictive power }) OR TITLE-ABS-KEY ({ positive predictive value }) OR TITLE-ABS-KEY ({ positive predictive values }) OR TITLE-ABS-KEY ({ PPV}) OR TITLE-ABS-KEY ({ receiver operating characteristics }) OR TITLE-ABS-KEY ({ regression analysis }) OR TITLE-ABS-KEY ({ ROC }) OR TITLE-ABS-KEY ({ sensitive }) OR TITLE-ABS-KEY ({ sensitivities }) OR TITLE-ABS-KEY ({ sensitivity }) OR TITLE-ABS-KEY ({ specific }) OR TITLE-ABS-KEY ({ specificity }) OR ALL ({ Abbott ARCHITECT HCV Ag assay }) OR ALL ({ Abbott ARCHITECT HCV Ag test }) OR ALL ({ Abbott ARCHITECT HCV Antigen assay }) OR ALL ({ Abbott ARCHITECT i2000SR }) OR ALL ({ Abbott ARCHITECT test }) OR ALL ({ Abbott Diagnostics }) OR ALL ({ Abbott HCV Ag }) OR ALL ({ Abbott HCV core antigen }) OR ALL ({ Abbott Laboratories }) OR ALL ({ ARCHITECT }) OR ALL ({ ARCHITECT ci8200}) OR ALL ({ Architect core antigen }) OR ALL ({ Architect HCV Ag }) OR ALL ({ ARCHITECT HCV Core antigen }) OR ALL ({ ARCHITECT HCVAg }) OR ALL ({ ARCHITECT i2000SR }) OR ALL ({ ARCHITECT system }) OR ALL ({ ARCHITECTHCVAg }) OR ALL ({ ARCHITECT-i2000R })) AND (TITLE-ABS-KEY ({ DBS }) OR TITLE-ABS-KEY ({ Whatman }) OR TITLE-ABS-KEY ({ filter paper }) OR TITLE-ABS-KEY ({ Dried blood filter }) OR TITLE-ABS-KEY ({ Dried blood }) OR TITLE-ABS-KEY ({ Dried sample }) OR TITLE-ABS-KEY ({ Dried blood spot })) AND (EXCLUDE (DOCTYPE , "re") OR EXCLUDE (DOCTYPE , "le") OR EXCLUDE (DOCTYPE , "no"))

Search strategy Cochrane

#1 MeSH descriptor: [Hepatitis C] explode all trees
#2 MeSH descriptor: [Hepacivirus] explode all trees
#3 ("hepatitis c"):ti,ab,kw
#4 ("hepatitis c virus"):ti,ab,kw
#5 ("hepatitis c viruses"):ti,ab,kw
#6 ("hcv"):ti,ab,kw
#7 ("h c v"):ti,ab,kw
#8 ("vhc"):ti,ab,kw
#9 ("hepacivirus"):ti,ab,kw
#10 ("hcv viral"):ti,ab,kw
#11 ("hcv infected"):ti,ab,kw
#12 ("hcv infection"):ti,ab,kw
#13 ("hcv rna"):ti,ab,kw
#14 ("hepatitis c virus rna"):ti,ab,kw
#15 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14
#16 MeSH descriptor: [Diagnosis] explode all trees
#17 MeSH descriptor: [Diagnostic Techniques and Procedures] explode all trees
#18 MeSH descriptor: [Clinical Laboratory Techniques] explode all trees
#19 MeSH descriptor: [Mass Screening] explode all trees
#20 MeSH descriptor: [Nucleic Acid Amplification Techniques] explode all trees
#21 MeSH descriptor: [RNA] explode all trees
#22 ("clinical laboratory diagnoses"):ti,ab,kw
#23 ("clinical laboratory diagnostic"):ti,ab,kw
#24 ("clinical laboratory techniques"):ti,ab,kw
#25 ("clinical laboratory testing"):ti,ab,kw
#26 ("diagnose"):ti,ab,kw
#27 ("diagnoses"):ti,ab,kw
#28 ("diagnosis of hcv"):ti,ab,kw
#29 ("diagnosis"):ti,ab,kw
#30 ("diagnostic techniques and procedures"):ti,ab,kw
#31 ("diagnostic"):ti,ab,kw
#32 ("hcv infection diagnosis"):ti,ab,kw
#33 ("hcv testing"):ti,ab,kw
#34 ("mass screening"):ti,ab,kw
#35 ("mass screenings"):ti,ab,kw
#36 ("molecular diagnostic techniques"):ti,ab,kw
#37 ("screening approach"):ti,ab,kw
#38 ("screening"):ti,ab,kw
#39 ("testing diagnostic"):ti,ab,kw
#40 ("plasma levels"):ti,ab,kw
#41 ("sera"):ti,ab,kw
#42 ("serum levels"):ti,ab,kw
#43 ("dried blood"):ti,ab,kw
#44 ("dried sample"):ti,ab,kw
#45 ("filter paper"):ti,ab,kw
#46 ("Whatman"):ti,ab,kw
#47 ("DBS"):ti,ab,kw
#48 ("assay kits"):ti,ab,kw
#49 ("hcv assays"):ti,ab,kw
#50 ("hcv pcr"):ti,ab,kw

#51 ("hcv rna levels"):ti,ab,kw
#52 ("hcv rna quantification"):ti,ab,kw
#53 ("hepatitis markers"):ti,ab,kw
#54 ("immunoassay"):ti,ab,kw
#55 ("quantitative assays"):ti,ab,kw
#56 ("quantitative reverse transcription pcr"):ti,ab,kw
#57 ("real time pcr"):ti,ab,kw
#58 ("rna levels"):ti,ab,kw
#59 ("roche cobas taqman"):ti,ab,kw
#60 #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51 or #52 or #53 or #54 or #55 or #56 or #57 or #58 or #59
#61 MeSH descriptor: [Hepatitis C Antigens] explode all trees
#62 ("antigens"):ti,ab,kw
#63 ("cleia method"):ti,ab,kw
#64 ("core antigen assays"):ti,ab,kw
#65 ("core antigen"):ti,ab,kw
#66 ("hcv ag"):ti,ab,kw
#67 ("hcvcAg"):ti,ab,kw
#68 ("hcv antigen"):ti,ab,kw
#69 ("hcv core antigen"):ti,ab,kw
#70 ("hcv core protein"):ti,ab,kw
#71 ("hcv core region"):ti,ab,kw
#72 ("hepatitis c antigens"):ti,ab,kw
#73 ("hepatitis c virus core antigen"):ti,ab,kw
#74 ("hepatitis c virus core"):ti,ab,kw
#75 ("viral core proteins"):ti,ab,kw
#76 #61 or #62 or #63 or #64 or #65 or #66 or #67 or #68 or #69 or #70 or #71 or #72 or #73 or #74 or #75 8960
#77 ("accuracy"):ti,ab,kw
#78 ("correlation"):ti,ab,kw
#79 ("correlations"):ti,ab,kw
#80 ("negative predictive power"):ti,ab,kw
#81 ("negative predictive value"):ti,ab,kw
#82 ("negative predictive values"):ti,ab,kw
#83 ("NPV"):ti,ab,kw
#84 ("positive predictive power"):ti,ab,kw
#85 ("positive predictive value"):ti,ab,kw
#86 ("positive predictive values"):ti,ab,kw
#87 ("PPV"):ti,ab,kw
#88 ("receiver operating characteristics"):ti,ab,kw
#89 ("regression analysis"):ti,ab,kw
#90 ("ROC"):ti,ab,kw
#91 ("sensitive"):ti,ab,kw
#92 ("sensitivities"):ti,ab,kw
#93 ("sensitivity"):ti,ab,kw
#94 ("specific"):ti,ab,kw
#95 ("specificity"):ti,ab,kw
#96 ("Abbott ARCHITECT i2000SR"):ti,ab,kw
#97 ("Abbott Diagnostics"):ti,ab,kw

#98 ("Abbott Laboratories"):ti,ab,kw
#99 ("ARCHITECT"):ti,ab,kw
#100 ("ARCHITECT ci8200"):ti,ab,kw
#101 ("ARCHITECT i2000SR"):ti,ab,kw
#102 ("ARCHITECT system"):ti,ab,kw
#103 ("cleia method"):ti,ab,kw
#104 ("chemiluminescence immunoassay"):ti,ab,kw
#105 #77 or #78 or #79 or #80 or #81 or #82 or #83 or #84 or #85 or #86 or #87 or #88 or #89 or #90 or #91 or #92 or #93 or #94 or #95 or #96 or #97 or #98 or #99 or #100 or #101 or #102 or #103 or #104
#106 ("dried blood"):ti,ab,kw
#107 ("dried sample"):ti,ab,kw
#108 ("filter paper"):ti,ab,kw
#109 ("Whatman"):ti,ab,kw
#110 ("DBS"):ti,ab,kw
#111 #106 or #107 or #108 or #109 or #110
#112 #15 and #60 and #76 and #105 and #111

Supplementary File 3: Risk of bias assessment adapted from QUADAS-2

Domain 1: Patient Selection

1.1 Risk of Bias: Could the selection of patients have introduced bias?

Signaling questions and answer guidelines

Signaling question 1: Was a consecutive or random sample of patients or specimens enrolled?

- Yes: the study enrolled a consecutive or random DBS sample of eligible patients
- No: the study selected patients by selection or convenience
- Unclear: the study did not report how the patient selection was

Signaling question 2: Was a case-control design avoided?

- Yes: the study is not a case-control design
- No: the study is a case-control design
- Unclear: the study design was not reported, or we were unable to identify from the text

Signaling question 3: Did the study avoid inappropriate exclusions?

- Yes: the study enrolled consecutive or random DBS samples of eligible patients
- No: the study excluded samples based on their prior testing, as these exclusions significantly reduce the generalizability of a study's findings
- Unclear: the study did not report exclusion criteria, or we were unable to identify from the text

Risk of Bias was evaluated as 'low risk' if studies scored 'yes' on all the questions or two questions were answered with 'yes' and one with 'unclear'; 'high risk' if two or more questions were answered with 'no' or one question was answered with 'no' and two with 'unclear'; and 'unclear risk' if studies scored 'unclear' on all the questions, two questions are answered with 'unclear' and one with 'yes', two questions were answered with 'yes' and one with 'no', or each question was answered with 'yes', 'no' and 'unclear'

1.2 Applicability: Are there concerns that the included patients and setting do not match the review question?

- Low concern: the study enrolled a broad study population in any setting
- High concern: the study inappropriately included healthy or blood donors only
- Unclear concern: the population was not well characterized, or we could not identify if a study's patients did not match our review question.

Domain 2: Index Test

2.1 Risk of Bias: Could the conduct or interpretation of the index test have introduced bias?

Signaling question 1: Were the index test results interpreted without knowing the reference standard results?

- Yes: results of the reference standard (HCV-RNA) test were blinded. Studies where the HCVcAg test was reported blinded to the HCV-RNA test or if it was clear that the HCVcAg test was reported before the results of the HCV-RNA test were available
- No: results of reference standard were unblended. The results of the HCVcAg test were reported on previous knowledge of the HCV-RNA test
- Unclear: we were unable to identify whether DBS samples were tested or the HCVcAg test results were interpreted without knowledge of the HCV-RNA test results

Signaling question 2: If a threshold was used, was it pre-specified?

- Yes: the limit of detection for commercially available HCVcAg tests was pre-specified by the manufacturer
- No: the threshold of the HCVcAg test was personally selected to optimize sensitivity and specificity, leading to over-optimistic estimates of test performance

- Unclear: we could not determine whether the threshold of the HCVcAg test was pre-specified or not

Risk of Bias was evaluated as 'low risk' if studies scored 'yes' on all the questions, or one question was answered with 'yes' and the other one with 'unclear'; 'high risk' if studies scored 'no' on all the questions, or one question was answered with 'no' and another one with 'unclear'; and 'unclear risk' if studies scored 'unclear' on all the questions; or questions were answered with 'yes' and 'no'

2.2 Applicability: Are there concerns that the index test, its conduct, or interpretation differ from the review question?

- Low concern: the HCVcAg test was performed according to the manufacturer's recommendations
- High concern: the HCVcAg test procedure was inconsistent with the manufacturer recommendations (i.e., additional processing steps were added), or there was a delayed assessment of DBS samples to perform the HCVcAg test
- Unclear concern: the HCVcAg test was not discussed in the study, or we were unable to determine how the HCVcAg test was conducted or interpreted

Domain 3: Reference standard

3.1 Risk of Bias: Could the reference standard, its conduct, or its interpretation have introduced bias?

Signaling question 1: Is the reference standard likely to classify the target condition correctly?

- Yes: the reference standard for HCV-RNA testing was a nucleic acid amplification test
- No: the reference standard for HCV-RNA testing was not a nucleic acid amplification test, or a combination of different nucleic acid amplification tests was used
- Unclear: there is insufficient information about which was reference standard for HCV-RNA testing used, or we were unable to identify from the text

Signaling question 2: Were the reference standard results interpreted without knowing the index test results?

- Yes: studies where the HCV-RNA test was interpreted blindly to the results of the HCVcAg test
- No: studies where the HCV-RNA test was not interpreted blindly to the results of the HCVcAg test
- Unclear: we were unable to identify whether DBS samples were tested or if the HCV-RNA test results were interpreted without knowledge of the HCVcAg test results

3.2 Applicability: Are there concerns that the target condition as defined by the reference standard does not match the question?

- Low concern: the HCV-RNA test was performed according to the manufacturer's recommendations
- High concern: the HCV-RNA test procedure was inconsistent with the manufacturer recommendations, or there was a delayed assessment of DBS samples to perform the HCV-RNA test
- Unclear concern: the HCV-RNA test was not discussed in the study, or we were unable to determine how the HCV-RNA test was conducted or interpreted

Domain 4: Flow and timing

4.1 Risk of Bias: Could the patient flow have introduced bias?

Signaling question 1: Was there an appropriate interval between the index test and reference standard?

- Yes: DBS samples for HCVcAg and reference standards tests did obtain at the same time
- No: DBS samples for HCVcAg and reference standards tests did not obtain at the same time

- Unclear: it was not discussed in the study, or we were unable to determine when HCVcAg and reference standards tests were conducted or interpreted

Signaling question 2: Did all patients in the study receive the same reference standard?

- Yes: the study used the same rt-PCR for all samples
- No: the study used different types of rt-PCR to analyze all samples
- Unclear: it was not defined in the study, or we were unable to interpret the used rt-PCR

Signaling question 3: Were all patients included in the analysis?

- Yes: the whole population recruited into the study was included in the analysis, or any exclusion was adequately described
- No: participants were missing, or the study excluded samples without a given reason
- Unclear: not enough information was given to assess why participants were excluded from the analysis, or we were unable to find an explanation for the exclusion of samples

Risk of Bias was evaluated as 'low risk' if studies scored 'yes' on all the questions or two questions were answered with 'yes' and one with 'unclear'; 'high risk' if two or more questions were answered with 'no' or one question was answered with 'no' and two with 'unclear'; and 'unclear risk' if studies scored 'unclear' on all the questions, two questions are answered with 'unclear' and one with 'yes', two questions were answered with 'yes' and one with 'no', or each question was answered with 'yes', 'no' and 'unclear'

Summary of the quality assessment by using QUADAS-2

	Risk of bias				Concerns regarding applicability		
Author (year)	**Patient selection**	**Index test**	**Ref. standard**	**Flow and timing**	**Patient selection**	**Index test**	**Ref. standard**
Soulier et al. (2016)	H	L	L	UC	H	L	L
Mohamed et al. (2017)	L	L	L	UC	L	L	L
Lamoury et al. (2018)	UC	L	L	L	L	L	L
Nguyen et al. (2018)	UC	L	L	L	L	L	L
Biondi et al. (2019)	UC	L	L	UC	L	UC	L
Catlett et al. (2019)	L	L	L	L	L	L	L
Ranjan et al. (2019)	UC	H	L	H	L	L	L
Troyano-Hernáez et al. (2023)	UC	L	L	L	L	L	L

H= high; L= low; Ref = reference; UC = unclear

Supplementary Tables

Supplementary Table 1. Sensitivity analysis for diagnostic performance measures

Deleted study	Se [95%CI]	Sp [95%CI]	PLR [95%CI]	NLR [95%CI]
Soulier et al. (2016)	0.88 [0.81-0.92]	1.00 [0.93-1.00]	223.1 [11.7-4244.6]	0.12 [0.08-0.19]
Mohamed et al. (2017)	0.87 [0.77-0.93]	1.00 [0.87-1.00]	640.3 [5.7-71490.0]	0.13 [0.07-0.24]
Lamoury et al. (2018)	0.86 [0.75-0.93]	1.00 [0.85-1.00]	852.9 [437-153480.6]	0.14 [0.07-0.25]
Nguyen et al. (2018)	0.85 [0.74-0.92]	1.00 [0.96-1.00]	231.0 [18.8-2840.4]	0.15 [0.08-0.27]
Biondi et al. (2019)	0.84 [0.74-0.91]	1.00 [0.96-1.00]	224.7 [20.3-2485.7]	0.16 [0.09-0.27]
Catlett et al. (2019)	0.86 [0.76-0.92]	1.00 [0.99-1.00]	246.2 [19.4-3131.1]	0.14 [0.09-0.24]
Troyano-Hernáez et al. (2023)	0.83 [0.74-0.89]	1.00 [0.96-1.00]	195.1 [20.8-1835.0]	0.17 [0.11-0.27]

Abbreviations: 95%CI = 95% confidence interval; NLR = negative likelihood ratio; PLR = positive likelihood ratio; Se = sensitivity; Sp = specificity.

Supplementary Table 2. Results of bivariate meta-regression (inconsistency index) in subgroup analysis for detecting active HCV infection in DBS using Abbott ARCHITECT HCVcAg assay compared to a confirmatory nucleic acid test.

Parameter	**Category**	**I^2 [95%CI]**	**X^2**	***p*-value**
Year of publication	Yes: >2017 No: ≤2017	76 [47-100]	8.22	**0.02**
LMIC	Yes No	0 [0-100]	1.36	0.51
Biological sample type: Venous	Yes No	12 [0-100]	2.26	0.32
Biological sample type: Capillary	Yes No	48 [0-100]	3.85	0.15
Sample size	Yes: <120 No: ≥120	60 [10-100]	4.99	**0.08**
HCV prevalence	Yes: >75% No: ≤75%	67 [27-100]	6.13	**0.05**
COBAS Ampliprep/COBAS TaqMan HCV Real-time PCR	Yes No	71 [36-100]	6.90	**0.03**
QUADAS-2 low/unclear overall risk	Yes: Low/unclear No: high	66 [25-100]	5.95	**0.05**
Low/unclear risk of bias QUADAS-2	Yes: Low/unclear No: high	81 [59-100]	10.54	**0.01**
Low/unclear applicability concerns QUADAS-2	Yes: Low/unclear No: high	81 [59-100]	10.54	**0.01**

Abbreviations: 95%CI = 95% confidence interval; cAg = core antigen; HCV = hepatitis C virus; IU = international units; I^2 = inconsistency index; LMIC = low- or middle-income country; QUADAS-2 = Quality Assessment of Diagnostic Accuracy Studies-2; X^2 = Pearson's chi-squared test.

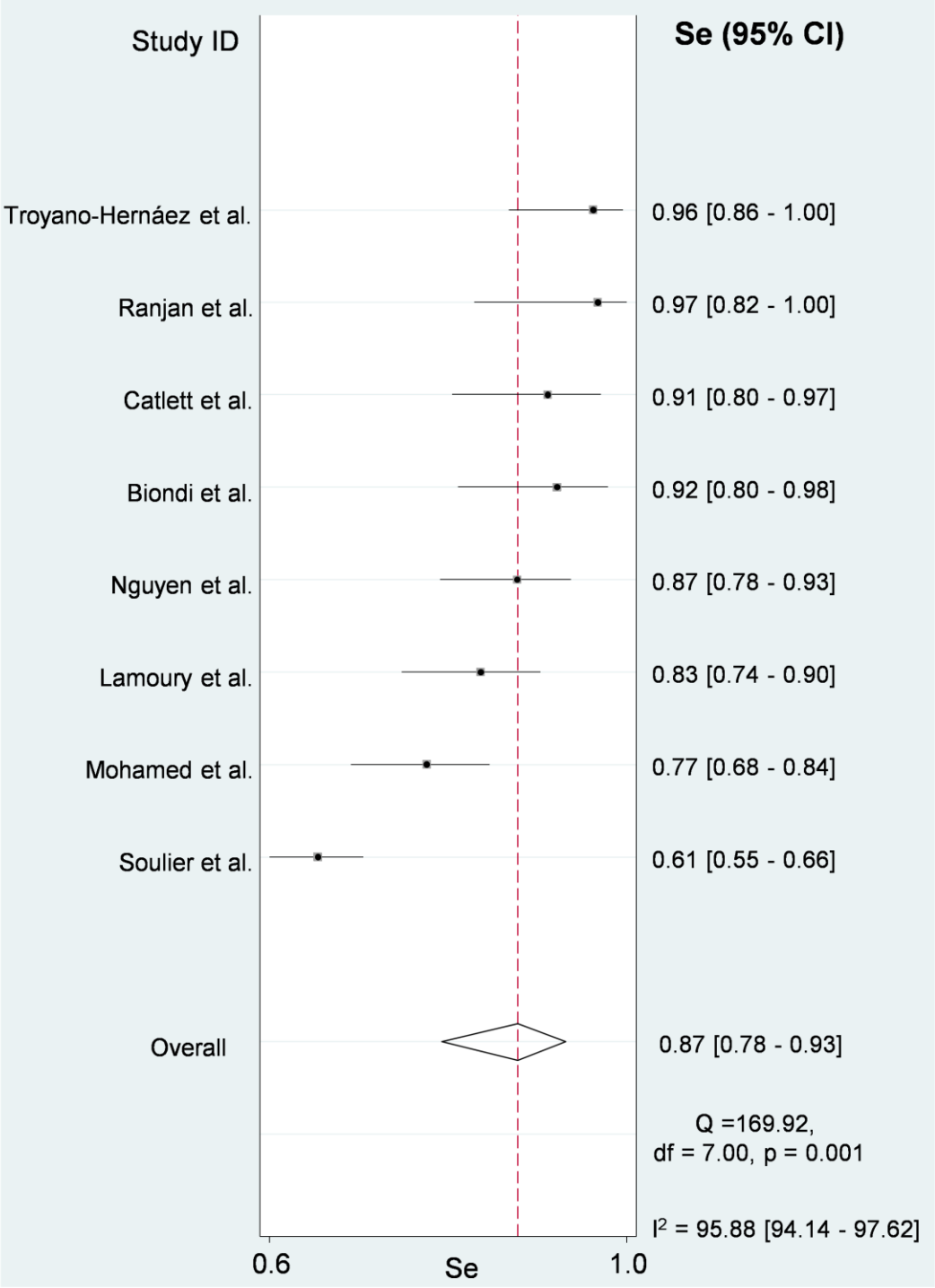
Supplementary Table 3. Results of bivariate meta-regression (sensitivity and specificity) and subgroup analysis in detecting active HCV infection in DBS using Abbott ARCHITECT HCVcAg assay compared to a confirmatory nucleic acid test.

Parameter	Category	No.	Se [95%CI]	*p*-value	Sp [95%CI]	*p*-value
Year of publication	Yes: >2017	5	0.89 [0.85-0.94]	0.84	1.00 [0.99-1.00]	<**0.001**
	No: ≤2017	2	0.68 [0.58-0.78]		1.00 [0.98-1.00]	
LMIC	Yes	2	0.83 [0.67-0.98]	0.25	0.98 [0.94-1.00]	0.99
	No	5	0.86 [0.78-0.95]		1.00 [0.99-1.00]	
Biological sample type: Venous	Yes	6	0.84 [0.76-0.92]	0.33	0.99 [0.98-1.00]	<**0.001**
	No	1	0.91 [0.78-1.00]		1.00 [1.00-1.00]	
Biological sample type: Capillary	Yes	2	0.92 [0.84-1.00]	0.97	1.00 [1.00-1.00]	<**0.001**
	No	5	0.82 [0.73-0.91]		0.99 [0.97-1.00]	
Sample size	Yes: <120	4	0.78 [0.68-0.88]	<**0.001**	1.00 [0.99-1.00]	<**0.001**
	No: ≥120	3	0.92 [0.86-0.98]		1.00 [1.00-1.00]	
HCV prevalence	Yes: >75%	4	0.85 [0.75-0.95]	0.35	0.98 [0.95-1.00]	<**0.001**
	No: ≤75%	3	0.85 [0.73-0.98]		1.00 [0.99-1.00]	
COBAS Ampliprep/COBAS TaqMan HCV Real-time PCR	Yes	5	0.80 [0.72-0.89]	<**0.001**	0.99 [0.97-1.00]	<**0.001**
	No	2	0.94 [0.88-1.00]		1.00 [1.00-1.00]	
QUADAS-2 low/unclear overall risk	Yes: Low/unclear	5	0.88 [0.80-0.95]	0.98	1.00 [0.98-1.00]	<**0.001**
	No: high	2	0.78 [0.60-0.96]		1.00 [1.00-1.00]	
Low/unclear risk of bias QUADAS-2	Yes: Low/unclear	6	0.87 [0.82-0.92]	0.31	1.00 [0.98-1.00]	<**0.001**
	No: high	1	0.61 [0.42-0.79]		1.00 [1.00-1.00]	
Low/unclear applicability concerns QUADAS-2	Yes: Low/unclear	6	0.87 [0.82-0.92]	0.31	1.00 [0.98-1.00]	<**0.001**
	No: high	1	0.61 [0.42-0.79]		1.00 [1.00-1.00]	

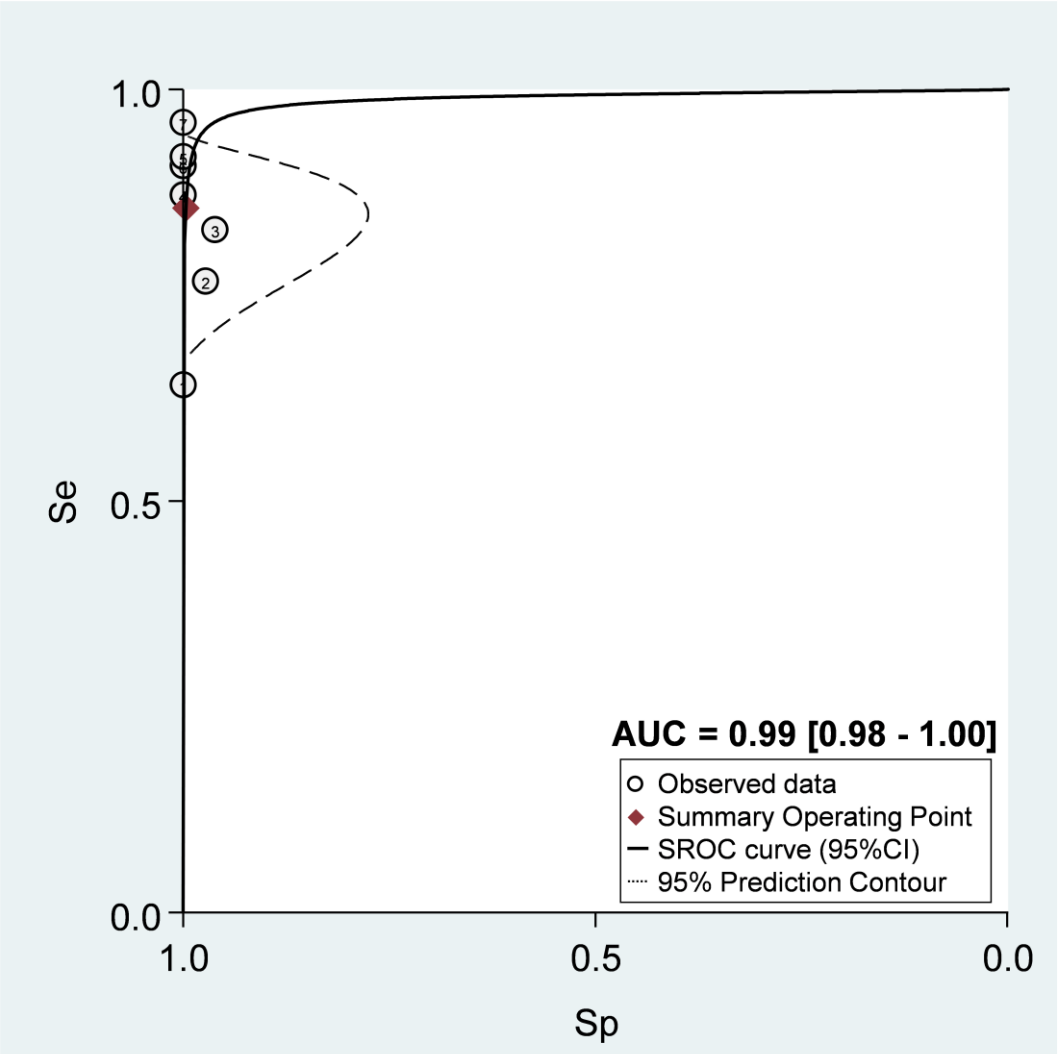
Abbreviations: 95%CI = 95% confidence interval; cAg = core antigen; HCV= hepatitis C virus; LMIC= low- or middle-income country; No.= number of articles; QUADAS-2 = Quality Assessment of Diagnostic Accuracy Studies-2; Se= sensitivity; Sp= specificity.

Supplementary Figures

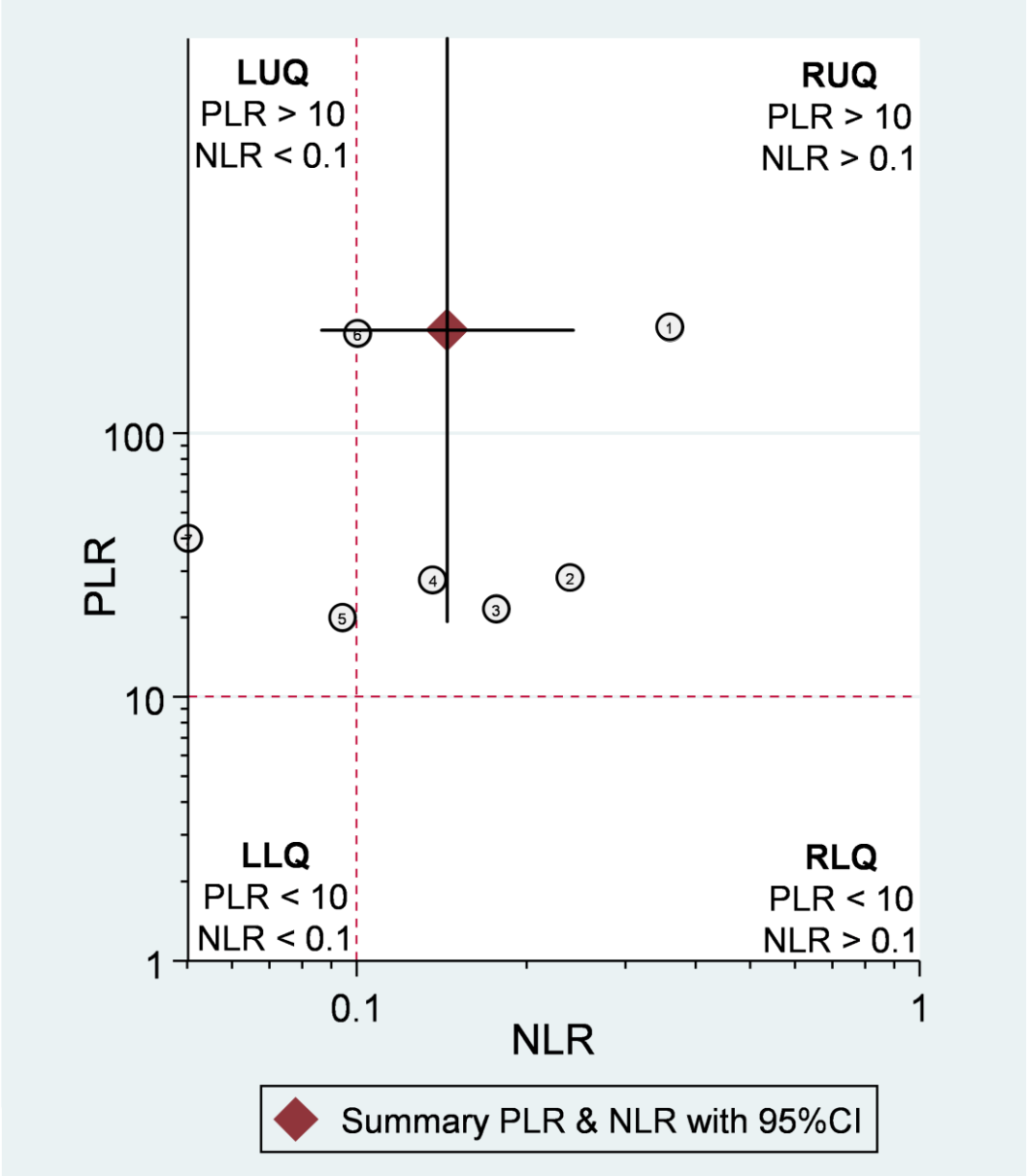
Supplementary Figure 1. Forest plots of pooled sensitivity for all studies included in the univariate analysis in detecting active HCV infection in DBS using Abbott ARCHITECT HCV Ag assay compared to a confirmatory nucleic acid test. **Abbreviations**: 95% CI = 95% confidence interval; df = degrees of freedom; HCV = hepatitis C virus; I^2 = inconsistency index; Q = Cochran's Q test; Se = sensitivity; Sp = specificity.



Supplementary Figure 2. SROC curve plot in detecting active HCV infection in DBS using Abbott ARCHITECT HCV Ag assay compared to a confirmatory nucleic acid test. **Abbreviations**: 95%CI = 95% confidence interval; AUC = area under the curve; HCV = hepatitis C virus; Se = sensitivity; Sp = specificity; SROC = summary receiver operating characteristic.



Supplementary Figure 3. Scatter plot of likelihood ratios used to identify active HCV infection in DBS with the Abbott ARCHITECT HCV Ag assay compared to a confirmatory nucleic acid test. **Abbreviations**: 95% CI = 95% confidence interval; LLQ = left lower quadrant (exclusion only: PLR <10, NLR <0.1); LUQ = left upper quadrant (Confirmation and exclusion: PLR >10, NLR <0.1); NLR = negative likelihood ratio; PLR = positive likelihood ratio; RLQ = right lower quadrant (no confirmation or exclusion: PLR <10, NLR >0.1); RUQ = right upper quadrant (confirmation only: PLR >10, NLR >0.1).



Supplementary Figure 4. Deeks's funnel plot asymmetry test examines publication bias when detecting active HCV infection in DBS using the Abbott ARCHITECT HCV Ag assay compared to a confirmatory nucleic acid test. **Abbreviations**: DOR = diagnostic odds ratio; ESS = single effective size; HCV = hepatitis C virus.