Title page

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Title: Diagnostic accuracy of hepatitis C core antigen detection during therapy with direct-acting antivirals: a meta-analysis

Running head: Diagnostic accuracy of HCVcAg detection

Authors: Daniel SEPÚLVEDA-CRESPO [1]; Ana TREVIÑO-NAKOURA [2]; José M BELLON [3]; Beatriz ARDIZONE JIMÉNEZ [1]; María A. JIMÉNEZ-SOUSA [1,4]; Amanda FERNÁNDEZ-RODRÍGUEZ [1,4]; Isidoro MARTÍNEZ [1,4,(¥)]; Salvador RESINO [1,4,(*,¥)]

(¥), Both authors contributed equally to this study; (*), Corresponding author

Current affiliations:

(1) Unidad de Infección Viral e Inmunidad. Centro Nacional de Microbiología - Instituto de Salud Carlos III, Majadahonda, Spain.

(2) Servicio de Medicina Preventiva y Salud Pública, Hospital Universitario Nuestra Señora de la Candelaria, Santa Cruz de Tenerife, Spain.

(3) Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Madrid, Spain.

(4) Centro de Investigación Biomédica en Red en Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain.

Corresponding author: Salvador Resino, Centro Nacional de Microbiología, Instituto de Salud Carlos III (Campus Majadahonda); Carretera Majadahonda-Pozuelo, Km 2.2; 28220 Majadahonda (Madrid), Spain. Tel: +34 918 223 266; Fax: +34 915 097 946; e-mail: sresino@isciii.es

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Abstract

Background: Hepatitis C virus (HCV) treatment with direct-acting antivirals (DAAs) is monitored by assessing plasma HCV-RNA load, but detection of HCV core antigen (HCVcAg) may be an alternative.

Aim: To evaluate the diagnostic performance of the HCVcAg assay to monitor the efficacy of DAAs treatment in HCV-infected patients.

Methods: We performed searches in multiple electronic databases until Jul 6, 2022, of studies evaluating the HCVcAg detection in plasma/serum compared with the HCV-RNA test (gold standard). We calculated pooled measurement at 2 and 4 weeks of treatment, end-of-treatment (EOT), and sustained virological response (SVR; 12 weeks after EOT).

Results: We selected 16 studies from 2016 to 2022, with 3,237 patients and 8,958 samples. The HCVcAg assay evaluated was the Abbott ARCHITECT HCV Ag assay, and HCV-RNA assays were COBAS Ampliprep/COBAS TaqMan HCV and Abbott RealTime HCV Assay. Overall, the diagnostic performance and clinical utility of the HCVcAg assay were poor at week 2 (Sensitivity=0.40, specificity=0.96, PLR=9.16, NLR=0.63, and AUC=0.57), fair at week 4 (Sensitivity=0.30, specificity=0.90, PLR=3.18, NLR=0.77, and AUC=0.79), acceptable at the EOT (Sensitivity=0.40, specificity =0.98, PLR=16.54, NLR=0.62, and AUC=0.97), and excellent at the SVR (Sensitivity=0.94, specificity=0.99, PLR=107.54, NLR=0.06, and AUC=0.99).

Conclusions: The HCVcAg assay may be a helpful tool for monitoring the efficacy of HCV treatment with DAAs in HCV-infected patients at EOT and SVR, but not during HCV treatment with DAAs (week 2 and week 4) due to poor diagnostic performance.

Keywords: HCV; direct-acting antivirals; HCV core antigen; diagnostic performance; therapy monitoring

Introduction

Hepatitis C virus (HCV) causes both acute and chronic infections. Acute infections are usually mild and resolve without treatment. However, approximately 70% of people infected with HCV develop chronic hepatitis C, resulting in cirrhosis in about 15-30% of patients after 20 years of HCV infection. When cirrhosis is established, hepatitis C can progress to end-stage liver disease and hepatocellular carcinoma in 1-3% of cases [1]. The World Health Organization (WHO) estimates that 58 million people have chronic hepatitis C worldwide and that 1.5 million new infections occur each year [2]. In 2019, an estimated 290,000 people died from hepatitis C, mostly from cirrhosis and liver carcinoma.

HCV treatment with direct-acting antivirals (DAAs) is currently the standard therapy, achieving more than 95% sustained virological response (SVR) [3]. Measurement of HCV-RNA viral load has been the gold standard in monitoring the efficacy of HCV treatment at different points before, during treatment, end-of-treatment (EOT), and at 12 or 24 weeks after treatment completion [3]. The Abbott RealTime HCV and COBAS TaqMan HCV assays are fully automated nucleic acid tests (NAT) that assess HCV RNA viral. Both NAT assays are widely used in routine clinical practice with a lower limit of quantification (LLOQ) of 12 international units (IU)/mL and 15 IU/mL, respectively [4]. However, quantitation of HCV viral load is expensive, time-consuming, and complex. Therefore, other strategies for HCV monitoring are desirable [5].

HCV has a high genetic diversity with seven main genotypes and 67 subtypes [6]. However, the HCV core antigen (HCVcAg) is a 191-amino acid protein highly conserved among the different HCV genotypes [7]. Like the HCV-RNA, the HCVcAg is detected in the serum or plasma of patients two to three weeks after infection [8] and is more stable than HCV-RNA [9]. HCVcAg testing has been suggested as an alternative to NAT to confirm active HCV infection [3], but there is no global data on diagnostic performance, such as those of a meta-analysis, during the monitoring of HCV treatment with DAAs. HCVcAg detection has lower analytical sensitivity than NAT to detect low HCV viremia levels [9]. However, HCVcAg levels highly correlate with HCV-RNA viral load when using the HCV Ag assays developed in the last decade, such as the Abbott ARCHITECT HCV Ag Assay [10]. Besides, the HCVcAg tests are cheaper, faster, and easier to use than HCV RNA assays.

Objective

Through a systematic review and meta-analysis of eligible studies published to date (6 Jul 2022), we aimed to assess the diagnostic performance of HCVcAg testing to monitor the efficacy of DAAs treatment in HCV-infected patients.

Material and Methods

We systematically reviewed the literature reporting plasma/serum HCVcAg determination during follow-up in HCV-infected patients on DAAs therapy. Data from selected studies were extracted, and the accuracy of the HCVcAg test to assess active HCV infection was analyzed. This meta-analysis was performed according to the Cochrane Handbook for Diagnostic Test Accuracy Reviews [11], following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses of Diagnostic Test Accuracy (PRISMA-DTA) guideline [12] (see **Supplementary File 1**).

Search strategy

We used an a priori protocol for the bibliographic search in PubMed, EMBASE, Scopus, Web of Science, and Cochrane Library until Jul 6, 2022. The specific search strategy and the number of studies retrieved from each database are detailed in Supplementary File 2 and Figure 1 and was registered with the International Prospective Register of Systematic Reviews (PROSPERO; CRD42022332178). Several previously published systematic reviews on HCVcAg detection in plasma or serum were reviewed. The search was performed under the guidance of medical librarians, was not restricted by language, and unpublished data were not included.

Study selection

Two investigators (A.T.N. and D.S.C.) independently evaluated the title and abstract of studies found by the bibliographic search. Suitable studies for meta-analysis were finally selected for full-text review. These two investigators independently evaluated the full-text articles according to predefined inclusion and exclusion criteria, and discrepancies were solved by discussion between the authors and a third investigator (S.R.).

The inclusion criteria were as follows: 1) Clinical trials and observational studies evaluating the detection of HCVcAg in plasma or serum, compared with a reference method for detection of HCV-RNA (Abbott RealTime HCV and COBAS TaqMan HCV assays are eligible gold standard), in HCV-infected patients on HCV therapy with DAAs (IFN-free and DAAs with IFN/ribavirin); 2) Studies with available data of true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN), data necessary to construct a 2x2 table and calculate the diagnostic performance measures.

The exclusion criteria were as follows: 1) Studies with insufficient data to estimate sensitivity and/or specificity, which were also not provided by the authors upon request; 2) Data published in abstract form only or presented as slides or posters were excluded; 3) Studies with small sample sizes (less than 10) to avoid bias in the random-effects model.

Data extraction

The main epidemiological characteristics and test accuracy data (TP, TN, FP, and FN) corresponding to each study were extracted independently by two investigators (A.T.N. and S.R.) and then cross-checked. When data were unclear or in doubt, other researchers (B.A.J. and J.M.B.) were consulted to reach a consensus. Authors were contacted for data that were not explicit or were missing. Studies without extractable data were excluded when no further information was obtained after three attempts to contact study authors.

Risk of bias assessment

Two investigators (A.T.N. and D.S.C.) independently assessed the quality of each study and the risk of bias according to the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool [13]. Any disagreement was resolved by a third reviewer (B.A.J.). QUADAS-2 was designed to evaluate the quality of diagnostic accuracy studies through 4 key domains: patient selection, index test, reference standard, and flow and timing. The risk of bias was evaluated for each domain, and applicability concerns were also considered for the first three domains. The risk of bias was described as 'low risk', 'high risk', or 'unclear risk', while applicability concerns were rated as 'low concern', 'high concern' or 'unclear concern'.

Statistical analysis

Accuracy data (TP, FP, FN, and TN) obtained from each selected study were analyzed using STATA 15 (STATA Corp., College Station, TX, USA). The outcome measures were sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and area under the curve of the summary receiver operating characteristic (SROC) curve. We performed univariate meta-analyses including all available data for calculating sensitivity and specificity separately and bivariate meta-analysis only with the articles in which the sum of TP+FN and FP+TN were greater than 0. We evaluated the diagnostic performance of the determination of HCVcAg in plasma/serum at 2 and 4 weeks of treatment, EOT, and SVR (12 weeks after EOT) compared to the gold standard. A random-effects model with the MIDAS module was performed to investigate the overall diagnostic performance [14,15]. Univariate meta-analysis was performed with the "uforest" command and bivariate meta-analysis with the "bforest" command.

The forest plot shows the pooled results and heterogeneity measured using the inconsistency index (I^2) and Cochran's Q test. A *p*-value (Cochran's Q test) ≤0.10 [16] and an I^2 ≥50% indicated substantial heterogeneity [17,18]. The bagplot was also used to evaluate the observed data spread and identify outliers [19]. We assessed publication bias using Deeks' funnel plot [20], indicating publication bias when the *p*-value was <0.10 [16].

The SROC curves shows the accuracy level using the following criteria: AUC >0.90-1: excellent; AUC >0.80-0.90: good; AUC >0.70-0.80: fair; and AUC >0.60-0.70: poor [21].

We examined the clinical utility using the likelihood ratio matrix, which shows the diagnostic evidence, with quadrants of informativeness according to established evidence-based thresholds: i) Left Upper Quadrant (LUQ, PLR >10, NLR <0.1) indicates confirmation and exclusion; ii) Right Upper Quadrant (RUQ, PLR >10, NLR >0.1) indicates only confirmation; iii) Left Lower Quadrant (LLQ, PLR <10, NLR <0.1) indicates only exclusion; iv) Right Lower Quadrant (RLQ, PLR <10, NLR >0.1) indicates the absence of confirmation or exclusion.

Results
Search results
The systematic review identified 851 citations, of which 51 full-text articles were reviewed, identifying 16 studies that meet the inclusion and exclusion criteria (**Figure 1A**) [5,22-36].

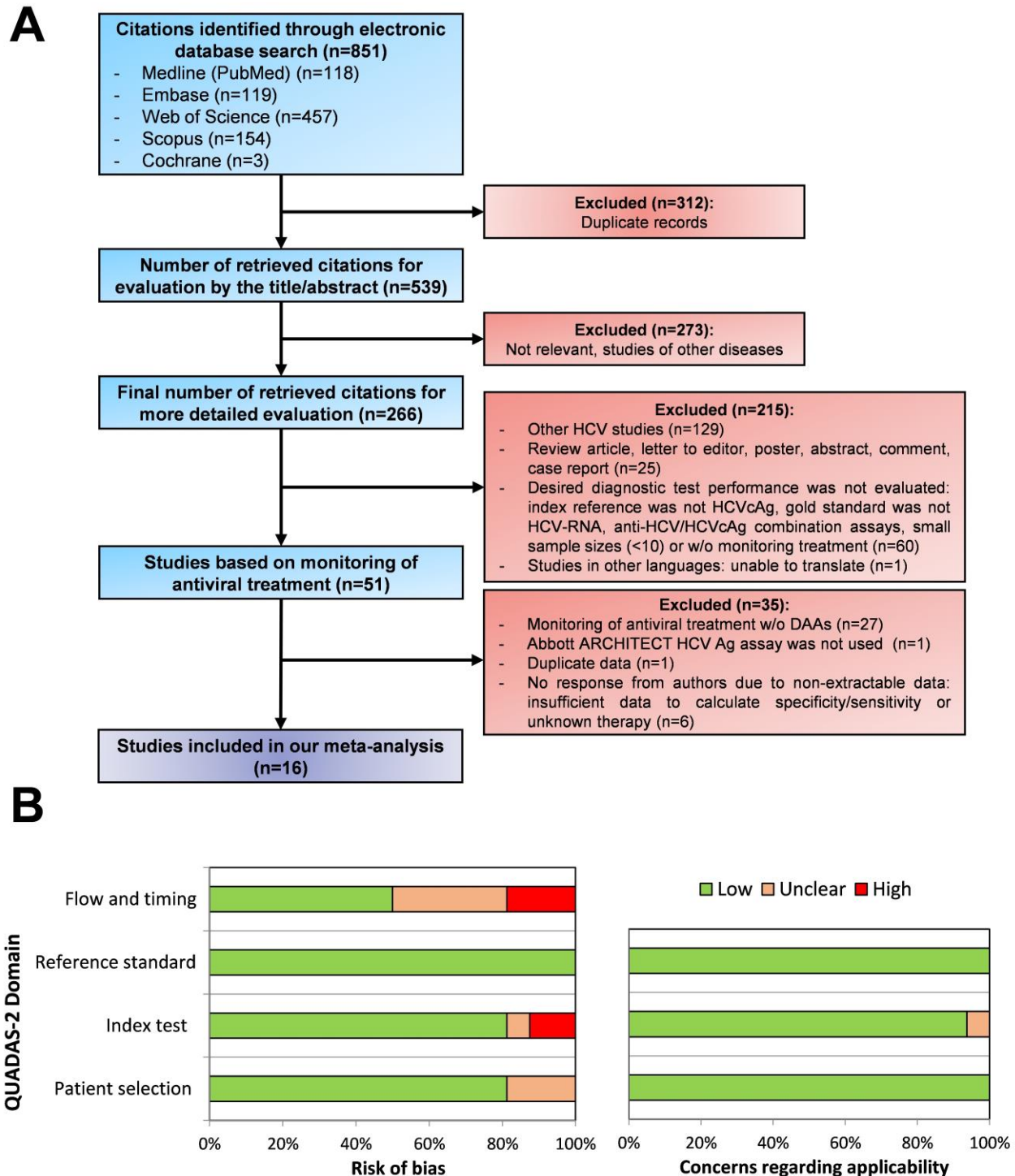**Figure 1.** Flow diagram of included studies **(A)** and risk of bias and applicability concerns graphs using the QUADAS-2 tool **(B)**. In figure (B), green indicates low risk, orange means unclear risk, and red represents a high risk. **Abbreviations**: cAg = core antigen; DAAs = direct-acting antivirals; HCV = hepatitis C virus; QUADAS = quality assessment of diagnostic accuracy study; w/o = without.

Table 1. Summary of studies detecting HCV core antigen in serum and/or plasma samples included in the meta-analysis.

Author, year (reference)	Country	No.	Age (yrs.)	Males (%)	HCV genotype	HIV (%)	HBV (%)	Antiviral therapy	Sample type	Sample Condition
Aghemo et al., 2016 [22]	Italy	58	59	64	1, 2, 3, 4, 5	0	0	DAAs	Serum	N/D
Nguyen et al., 2016 [34]	Ireland	152	47	73	1	N/D	N/D	DAAs/IFN/RBV	Serum/Plasma	N/D
Alonso et al., 2017 [23]	Spain	28	53.1	67.9	1, 2, 3, 4	35.7	28.6	DAAs	Serum	N/D
Arboledas et al., 2017 [24]	Spain	262	53	80.5	1, 2, 3, 4	31.3	N/D	DAAs/IFN/RBV	Plasma	Frozen
Loggi et al., 2017 [27]	Italy	96	60.5	64	1, 2, 3, 4	N/D	36.0	DAAs/IFN/RBV	Serum	N/D
Rockstroh et al., 2017 [36]	Germany	411	50.1	53	1	N/D	N/D	DAAs	Plasma	Frozen
Chevaliez et al., 2018 [25]	France	631	49.8	54.5	1	0	0	DAAs	Plasma	Frozen
Łucejko et al., 2018 [37]	Poland	33	49.4	59	1, 3, 4	0	0	DAAs	Serum/Plasma	Frozen
van Tilborg et al., 2018 [31]	Canada, Germany, and USA	219	54.8	61	1, 2, 3, 4, 5, 6	0	1.4	DAAs/IFN/RBV	Serum	Frozen
Łucejko et al., 2019 [28]	Poland	514	N/D	53	N/D	0	0	DAAs/IFN/RBV	Plasma	Frozen
Pérez-García et al., 2019 [29]	Spain	70	51.2	71.4	1, 2, 3, 4	30	N/D	DAAs	Serum/Plasma	N/D
Chayanupatkul et al., 2020 [33]	Thailand	101	47.1	78.2	1	35.6	0	DAAs	Serum	Frozen
Lin et al., 2020 [5]	Taiwan	110	63.6	57.3	1	N/D	7.0	DAAs	Serum	N/D
Mancebo et al., 2021 [32]	Spain	274	54.8	61.3	1, 2, 3, 4, 5	N/D	N/D	DAAs	Plasma	N/D
Rossetti et al., 2021 [30]	Italy	180	59	60	1, 2, 3, 4	11	0	DAAs	Serum/Plasma	Frozen
Ko et al., 2022 [35]	Taiwan	98	61.7	36.7	1, 2, 6	N/D	0	DAAs	Plasma	Frozen

Abbreviations: No. = sample size; DAAs = direct-acting antivirals; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IFN = interferon; N/D = no data; RBV = ribavirin; USA = United States of America; yrs = years.

Article characteristics

Table 1 summarizes the main characteristics of the 16 studies included in the meta-analysis for HCVcAg detection during the HCV therapy with DAAs [5,22-36]. All studies were published in English and included only adults. The publication year ranged from 2016 to 2022, all studies had a longitudinal design, except for two cross-sectional studies [35,36]. Only one study was carried out in a low- and middle-income country (LMIC) [33]. A total of 3,237 chronically HCV-infected patients, with 8,958 samples, were included for HCVcAg screening during the monitoring of DAAs therapy. The average age was 54 years, and the percentage of men was 62.2%. HCV genotypes analyzed ranged from 1 to 6, and only one study did not provide HCV genotypes [28]. HIV status was known in 10 studies (prevalence between 0 and 35.7%), and five of those excluded patients with HIV/HCV coinfection. HBV status was known in 11 studies (prevalence between 0 and 28.6%), and seven of those excluded patients with HBV/HCV coinfection.

The gold standard for diagnosing HCV viremic patients was the COBAS Ampliprep/COBAS TaqMan HCV (Roche Diagnostics), except for three studies that used the Abbott RealTime HCV Assay (Abbott Diagnostics) [22,33,34] and two other studies that used both NAT assays [5,30]. The HCVcAg was evaluated by chemiluminescence immunoassay with the ARCHITECT HCV Ag assay (Abbott Diagnostics). A negative sample was the one that had values below the LLOQ for COBAS Ampliprep/COBAS TaqMan HCV (<15IU/mL), Abbott RealTime HCV Assay (<12 IU/mL), or ARCHITECT HCVcAg assay (<3 fmol/L). For the HCVcAg assay, values ≥10 fmol/L were considered positive, but values ≥3 fmol/L and <10 fmol/L were considered indeterminate and retested, considering TP if confirmed reactive. In some articles, samples were not retested, but indeterminate results were classified as positive.

Risk of bias assessment

The quality assessment is summarized in **Figure 1B** (full description in **Supplementary File 3**). Only four studies (25%) were at low risk of bias in all four domains of the QUADAS-2 tool. The risk of bias in the patient selection domain was unclear in three studies (18.8%). It was unclear whether consecutive or random samples were enrolled in the study or if an inadequate exclusion of patients arose. For the index test domain, only two studies (12.5%) did not hide the results of the HCV-RNA test. All analyses were judged to have a low risk of bias for the reference standard. The nucleic acid amplification tests are highly sensitive, and their variability is minimal, even with insufficient reporting of blinding. For the flow and timing domain, half of the studies were at a high (n=3; 18.8%) or unclear (n=5; 31.3%) risk of bias because there was missing data. Overall, concerns about the applicability of the studies were low in all studies except one that was rated unclear due to insufficient reporting in the index test domain.

Diagnostic performance for monitoring HCV treatment

After 2 weeks of treatment (**Supplementary Figure 1**), we found 6 studies with available data [5,22,27,30,32,34], all valid for the bivariate meta-analysis (n = 563 samples). The pooled values found were Se = 0.40, Sp = 0.96, PLR = 9.16, and NLR = 0.63.

After 4 weeks of treatment (**Supplementary Figure 2**), we found 11 studies [5,22,24,25,27-32,34], all with available data for bivariate meta-analysis (n = 1,253 samples). We found pooled diagnostic performance values similar to 2 weeks on treatment: Se = 0.30, Sp = 0.90, PLR = 3.18, and NLR = 0.77.

At the EOT (**Figure 2**), we only found 8 studies (n = 1,062 samples) valid for bivariate analysis [5,23-25,28,30-32]. We found pooled values for Se = 0.40, Sp = 0.98, PLR = 16.54, and NLR = 0.62. Of the 12 studies with data at EOT [5,22-25,27-32,37], fourth did not contribute to sensitivity (not TP and FN) [22,27,29,37], and a univariate meta-analysis of the specificity was carried out separately (**Supplementary Figure 3A**), including all available data (n = 1,249 samples). The result was an specificity of 0.97 (95%CI = 0.96 – 0.98), a pooled value similar to those of the bivariate analysis.
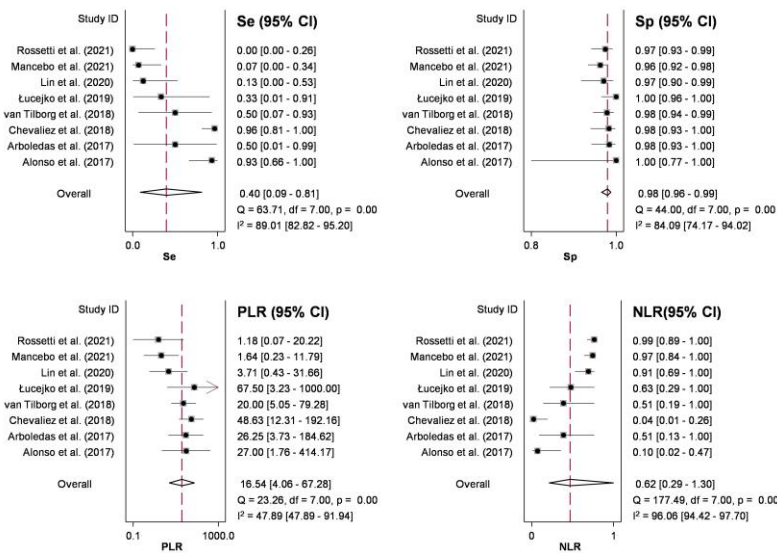
Figure 2. Forest plots at the EOT (bivariate analysis) showing sensitivity, specificity, and positive and negative likelihood ratios for the HCV diagnosis with Abbott ARCHITECT HCV Ag assay compared with a confirmatory nucleic acid test. **Abbreviations**: 95% CI = 95% confidence interval; EOT = end of treatment; HCV = hepatitis C virus; I^2 = inconsistency index; NLR = negative likelihood ratio; PLR = positive likelihood ratio; Q = Cochran's Q test; Se = sensitivity; Sp = specificity.

At the SVR (**Figure 3**), we only found 13 studies (n = 2,360 samples) valid for bivariate analysis [5,22,24,25,27-33,35,36]. We found pooled values of Se = 0.94, Sp = 0.99, PLR = 107.54, and NLR = 0.06. Of the 14 studies with data at SVR [5,22,24,25,27-33,35-37], only one did not contribute to sensitivity [37]. The univariate meta-analysis of the specificity was carried out separately with all available data (n = 2,393 samples) (**Supplementary Figure 3B**), finding similar values of specificity to the bivariate analysis (0.99; 95%CI = 0.99 – 1.00).
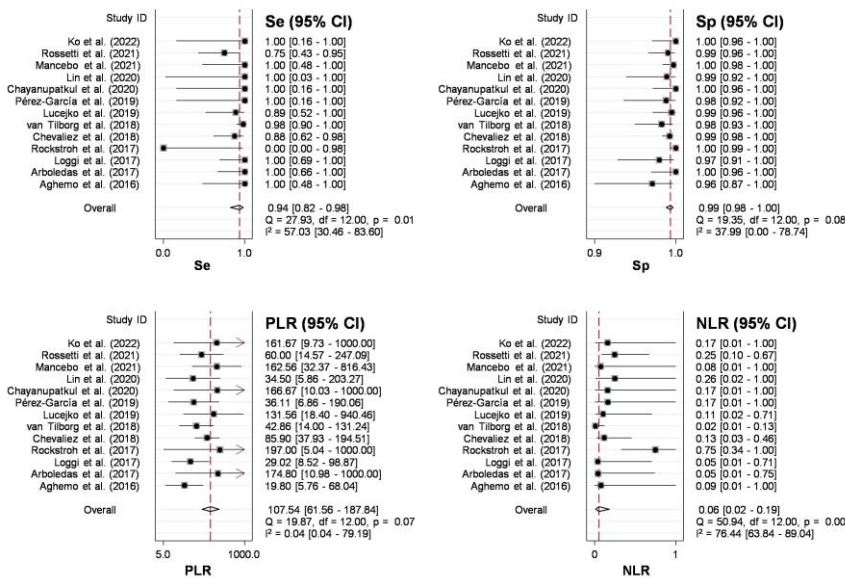
Figure 3. Forest plots at the SVR (bivariate analysis) showing sensitivity, specificity, and positive and negative likelihood ratios for the HCV diagnosis with Abbott ARCHITECT HCV Ag assay compared with a confirmatory nucleic acid test. **Abbreviations**: 95% CI = 95% confidence interval; HCV = hepatitis C virus; I^2 = inconsistency index; NLR = negative likelihood ratio; PLR = positive likelihood ratio; Q = Cochran's Q test; Se = sensitivity; Sp = specificity; SVR = sustained virological response.

The AUC of the SROC curve was 0.57, 0.79, 0.97, and 0.99 for two weeks of treatment, four weeks of treatment, EOT, and SVR, respectively (**Supplementary Figure 4**), indicating excellent diagnostic accuracy (AUC>0.90) at the last two study times.

Other control times were also found in the studies selected (one week and eight weeks of treatment, and 24 weeks post-treatment, among others), but these were discarded because there were five or fewer data for each.

Clinical application

Detection or exclusion of active HCV infection was depicted in likelihood ratio scattergram according to recommended threshold values for clinical use [38]. The likelihood scatter-plots for 2 and 4 weeks of treatment (**Supplementary Figure 5A & 5B**) showed that most studies were in the RLQ, indicating no confirmation or exclusion of HCV infection. For EOT (**Supplementary Figure 5C**), the summary of PLR and NLR was in the RUQ, indicating only confirmation of HCV infection. Finally, the summary of PLR and NLR at SVR (**Supplementary Figure 5D**) was in the LUQ, indicating confirmation and exclusion of HCV infection.

Exploration of heterogeneity

Heterogeneity was visually evaluated in **Figures 2-3** and **Supplementary Figures 1-2**. A significant percentage of diagnostic performance measures showed substantial heterogeneity (Cochran's Q test $p<0.10$ and/or $I^2>50\%$). Specifically, sensitivity and specificity for two weeks of treatment (**Supplementary Figure 1**) and all diagnostic performance measures for four weeks of treatment (**Supplementary Figure 2**) and EOT (**Figure 2**). We also used bagplots to explore the data distribution and identify outliers (**Supplementary Figure 6**). Overall, studies were not clustered together, but two obvious outliers were Łucejko et al. [37] and Rockstroh et al. [36] for SVR. It should be noted that Rockstroh et al. [36] showed a risk of bias in index test and the flow and timing (**Supplementary File 3**). However, these heterogeneity analyses should be interpreted with caution since due to the small number of studies available, it was impossible to explore heterogeneity by subgroup analysis or meta-regression.

Publication bias was discarded by Deeks' funnel plot asymmetry test ($p>0.10$), except for SVR ($p=0.05$) (**Supplementary Figure 7**).

Discussion

DAAs therapy's efficacy is monitored by detecting HCV-RNA levels in serum or plasma during the treatment, EOT, and especially to define SVR. The HCVcAg assay is being used as an alternative to NAT to screen for chronic hepatitis C [3], but there is no consensus on monitoring HCV treatment with DAAs. Our meta-analysis shows that the diagnostic performance (validity and utility) of the HCVcAg assay was poor at 2 and 4 weeks of DAAs treatment, acceptable at EOT, and excellent for confirmation of SVR after DAAs therapy. Therefore, the HCVcAg assay could be a helpful tool for monitoring HCV treatment at EOT and SVR, optimizing available health resources [39].

In a recent meta-analysis, Flores et al. [40] evaluated the diagnostic accuracy of HCVcAg for monitoring HCV treatment. However, they performed the meta-analysis without taking into account stratification at different treatment monitoring points. In our opinion, studying the validity of the HCVcAg test as a clinical tool to assess the efficacy of HCV treatment at different time points is critical and constitutes a strength of our study.

This systematic review was performed according to a priori protocol using several bibliographic sources (PubMed, EMBASE, Scopus, Web of Science, and Cochrane Library). Nevertheless, some studies might not have been included in this meta-analysis. The systematic review was conducted without language restrictions, but we could not translate two articles written in Chinese and Japanese, so all the studies analyzed were English. The article selection, data extraction, and the evaluation of the risk of bias were standardized according to a predefined protocol; two independent reviewers and a third researcher, in case of disagreement, always carried out tasks. Regarding the quality of the studies included, we found that all studies fulfilled the criteria with a low risk of applicability, but eight studies had at least one item related to a high risk of bias. An analysis based only on high-quality studies could not be performed due to the limited number of studies that fulfilled the selection criteria. Remarkably, the funnel plot was symmetrical, and a non-significant value confirmed the absence of publication bias by Deeks' test, except at the SVR.

The test's diagnostic validity is an intrinsic property and is evaluated by sensitivity, specificity, and AUC. Other critical parameters are NLR and PLR, which report the potential usefulness of the HCVcAg test. In our meta-analysis, at the EOT, the diagnostic performance to rule out active HCV infection was low since sensitivity was 0.40 (60% chance of finding FN) and NLR was 0.62. However, the diagnostic validity to confirm active HCV infection at the EOT was excellent because specificity was 0.98 (2% chance of finding FP) and PLR was 16.54, allowing detection of viremic patients with treatment failure. Low amounts of HCV RNA at the end of DAA treatment do not predict SVR at 12 weeks [41-43], so an LLOQ of 1000 IU/ ml could probably be sufficient to rule out viral rebound at the EOT. Therefore, a suboptimal sensitivity of the HCVcAg test at EOT might be acceptable. At the SVR (12 weeks after EOT), 95% sensitivity and 99% specificity were indicators of high diagnostic validity, while PLR >10 and NLR <0.1 provided strong evidence to accept or rule out active HCV infection. Note that our meta-analysis used bivariate random-effects models when appropriate (TP+FN>0 and FP+TN>0) and univariate random-effects models when studies with TP+FN=0 or FP+TN=0 were included, but similar findings were observed with both models. Therefore, the HCVcAg test had an excellent diagnostic performance for evaluating the efficacy of DAAs therapy at the SVR, and consequently, the HCVcAg test constitutes an exciting alternative to NAT assays since it is faster, easier to perform, and cheaper [44].

The cut-off of the reference assay and the test evaluated the impact on diagnostic performance. In this meta-analysis, two commercial assays widely used in the clinic routine and with similar HCV detection limits were used as reference tests, COBAS Ampliprep/COBAS TaqMan HCV (LLOQ <15 IU/mL) and Abbott RealTime HCV Assay (LLOQ <12 IU/mL). Both tests have excellent analytical sensitivity with LLOQ <15 IU/mL [4]. The test evaluated was always the Abbott ARCHITECT HCV Ag assay (LLOQ <3 fmol/L or 3000 IU/mL), with a higher detection limit than the two NAT assays evaluated in this meta-analysis. This difference in the LLOQ is very relevant when HCV-RNA vales

are low due to HCV therapy, which may have contributed to the poor diagnostic performance of the Abbott ARCHITECT HCV Ag assay at 2 and 4 weeks of treatment.

Fluctuations in HCV-RNA and HCVcAg levels during DAAs therapy may also affect the proportion of FN and FP. For example, HCVcAg levels tend to decline more rapidly than HCV-RNA levels in the early stages of HCV treatment, and later, HCVcAg shows lower values than HCV-RNA [5,22,27,34]. Several articles have analyzed different cut-off points to assess their diagnostic performance. On the one hand, the HCV-RNA cut-off of 1,000 IU/mL decreased the number of discordant results of HCVcAg and HCV RNA at week 4 of HCV therapy [25]. However, although HCV-RNA <1000 IU/mL decreases mainly in the number of FN, it also slightly increases the number of FP [25]. Despite this, the HCV-RNA cut-off of 1,000 IU/mL could be an appropriate indicator of adherence during HCV therapy [36]. On the other hand, the Abbott ARCHITECT HCV Ag assay has a detection limit of 3 fmol/L, equivalent to about 3,000 IU/mL, so there may still be samples with detectable HCV-RNA increasing the number of FN [45]. Most included studies in the meta-analysis performed a retest in samples with 3 to 10 fmol/L. However, three studies only reported the number of undetermined (<1%) [5,27,29]. Thus, we gave positive all patients who had values ≥3 fmol/L, although some may be misclassified.

Another factor to consider is the heterogeneity, which can be affected by many factors, such as sample size, the prevalence of an active HCV infection, reference tests, screening tests, HCV viral load and genotype, HIV and HBV coinfection, and sample type and condition, among others. In our meta-analysis, the heterogeneity was high because many diagnostic performance measures had Cochran's Q p-value <0.10 and/or I^2 >50%. Heterogeneity among studies is frequent in meta-analyses. Thus, Cochran's Q test has difficulty detecting true heterogeneity with small numbers of studies, while this test is very sensitive when there are many studies with a large sample size [18]. The I² test is substantially biased when the number of studies is small, and the I² values should be interpreted cautiously. Furthermore, there is no consensus on interpreting heterogeneity in a meta-analysis of diagnostic performance. The meta-analyses often include studies with different objectives, study designs, and outcomes. Therefore, substantial heterogeneity between studies is expected, and a random-effects analysis should be performed [17]. Moreover, some factors could not be analyzed by meta-regression (HCV viral load and genotype, HIV and HBV coinfection, and fresh or frozen sample) due to the lack of data in the articles included or statistical calculation failure when performing the meta-regression due to an unstable or asymmetric model. However, other factors could almost be ruled out early on because they were fairly homogeneous in our meta-analysis.

Limitations

Some limitations to consider for the proper interpretation of this meta-analysis are the following: Firstly, the number of studies was small at some time points, leading to limited representativeness. Secondly, we could not analyze the impact of HCV genotype, HBV coinfection, and HIV coinfection due to limited data access to these covariates. Thirdly, the effect of sample condition (fresh *vs.* frozen) could not be examined because some studies did not specify it. Fourthly, all but one of the studies were conducted in high-income settings; therefore, more studies should be carried out in LMIC with limited access to reference laboratories.

Conclusions

In conclusion, the available evidence shows that the HCVcAg assay may be a helpful tool for monitoring the efficacy of HCV treatment with DAAs in HCV-infected patients at EOT and SVR, but not during HCV treatment with DAAs (week 2 and week 4) due to poor diagnostic performance.

Declarations
Ethics approval and consent to participate

Availability of data

All relevant data are within the paper and its Supporting Information files.

Competing interests

The authors have no competing interests.

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Author contributions:

Daniel Sepúlveda-Crespo: investigation, resources, writing – original draft

Ana Treviño: investigation, resources, writing – review, and editing

José M Bellón: investigation, methodology, formal analysis, writing – review, and editing

Beatriz Ardizone: investigation, methodology, writing – review, and editing

María A Jiménez-Sousa: writing – review, and editing

Amanda Fernandez-Rodriguez: writing – review, and editing

Isidoro Martínez: funding acquisition, writing – original draft

Salvador Resino: funding acquisition, conceptualization, formal analysis, writing – original draft, supervision.

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Supplementary File 1: PRISMA-DTA Checklist and Abstracts Checklist

Section/topic	#	PRISMA-DTA Checklist Item	Reported on page #
TITLE / ABSTRACT			
Title	1	Identify the report as a systematic review (+/- meta-analysis) of diagnostic test accuracy (DTA) studies.	1
Abstract	2	Abstract: See PRISMA-DTA for abstracts at the end.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Clinical role of index test	D1	State the scientific and clinical background, including the intended use and clinical role of the index test, and if applicable, the rationale for minimally acceptable test accuracy (or minimum difference in accuracy for comparative design).	3
Objectives	4	Provide an explicit statement of question(s) being addressed in terms of participants, index test(s), and target condition(s).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	PROSPERO CRD42022332178
Eligibility criteria	6	Specify study characteristics (participants, setting, index test(s), reference standard(s), target condition(s), and study design) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full search strategies for all electronic databases and other sources searched, including any limits used, such that they could be repeated.	Suppl. 5-10
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Fig.1
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Definitions for data extraction	11	Provide definitions used in data extraction and classifications of target condition(s), index test(s), reference standard(s) and other characteristics (e.g. study design, clinical setting).	5
Risk of bias and applicability	12	Describe methods used for assessing risk of bias in individual studies and concerns regarding the applicability to the review question.	6

Diagnostic accuracy measures	13	State the principal diagnostic accuracy measure(s) reported (e.g. sensitivity, specificity) and state the unit of assessment (e.g. per-patient, per-lesion).	6
Synthesis of results	14	Describe methods of handling data, combining results of studies and describing variability between studies. This could include, but is not limited to: a) handling of multiple definitions of target condition. b) handling of multiple thresholds of test positivity, c) handling multiple index test readers, d) handling of indeterminate test results, e) grouping and comparing tests, f) handling of different reference standards	6-7

Section/topic	#	PRISMA-DTA Checklist Item	Reported on page #
Meta-analysis	D2	Report the statistical methods used for meta-analyses, if performed.	6-7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Provide numbers of studies screened, assessed for eligibility, included in the review (and included in meta-analysis, if applicable) with reasons for exclusions at each stage, ideally with a flow diagram.	9
Study characteristics	18	For each included study provide citations and present key characteristics including: a) participant characteristics (presentation, prior testing), b) clinical setting, c) study design, d) target condition definition, e) index test, f) reference standard, g) sample size, h) funding sources	Table 1
Risk of bias and applicability	19	Present evaluation of risk of bias and concerns regarding applicability for each study.	10
Results of individual studies	20	For each analysis in each study (e.g. unique combination of index test, reference standard, and positivity threshold) report 2x2 data (TP, FP, FN, TN) with estimates of diagnostic accuracy and confidence intervals, ideally with a forest or receiver operator characteristic (ROC) plot.	Figs 2, 3 and Suppl. Figs 1 and 2
Synthesis of results	21	Describe test accuracy, including variability; if meta-analysis was done, include results and confidence intervals.	11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression; analysis of index test: failure rates, proportion of inconclusive results, adverse events).	12
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence.	13
Limitations	25	Discuss limitations from included studies (e.g. risk of bias and concerns regarding applicability) and from the review process (e.g. incomplete retrieval of identified research).	15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence. Discuss implications for future research and clinical practice (e.g. the intended use and clinical role of the index test).	16

FUNDING			
Funding	27	For the systematic review, describe the sources of funding and other support and the role of the funders.	2

Section/topic	#	PRISMA-DTA for Abstracts Checklist item	Reported on page #
TITLE and PURPOSE			
Title	1	Identify the report as a systematic review (+/- meta-analysis) of diagnostic test accuracy (DTA) studies.	2
Objectives	2	Indicate the research question, including components such as participants, index test, and target conditions.	2
METHODS			
Eligibility criteria	3	Include study characteristics used as criteria for eligibility.	2
Information sources	4	List the key databases searched and the search dates.	2
Risk of bias & applicability	5	Indicate the methods of assessing risk of bias and applicability.	2
Synthesis of results	A1	Indicate the methods for the data synthesis.	2
RESULTS			
Included studies	6	Indicate the number and type of included studies and the participants and relevant characteristics of the studies (including the reference standard).	2
Synthesis of results	7	Include the results for the analysis of diagnostic accuracy, preferably indicating the number of studies and participants. Describe test accuracy including variability; if meta-analysis was done, include summary results and confidence intervals.	2
DISCUSSION			
Strengths and limitations	9	Provide a brief summary of the strengths and limitations of the evidence	2
Interpretation	10	Provide a general interpretation of the results and the important implications.	2
OTHER			
Funding	11	Indicate the primary source of funding for the review.	2
Registration	12	Provide the registration number and the registry name	PROSPERO CRD42022332178

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 quantification assays"[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 "cleia method"[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 "HCVcAg"[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 ("direct acting antiviral" [Title/Abstract] OR "direct acting antivirals" [Title/Abstract] OR "DAA" [Title/Abstract] OR "DAAs" [Title/Abstract] OR "monitoring" OR "monitor" [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 'HCVcAg':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   'direct acting antiviral':ti,ab,kw OR 'direct acting antivirals':ti,ab,kw OR 'DAA':ti,ab,kw OR 'DAAs':ti,ab,kw OR monitoring:ti,ab,kw OR monitor:ti,ab,kw

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

#7   #6 AND ('Article'/it OR 'Article in Press'/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=(HCVcAg)) 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 ((((((ALL=(direct acting antiviral)) OR ALL=(direct acting antivirals)) OR ALL=(DAA)) OR ALL=(DAAs)) OR ALL=(monitoring)) OR ALL=(monitor)

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

#7 #6 AND ('Article'/it OR 'Article in Press'/it) and Review Articles or Proceedings Papers or Editorial Materials or Meeting Abstracts or Book Chapters or Letters (Exclude – Document Types)

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 ("HCVcAg") 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 ("direct acting antiviral") OR TITLE-ABS-KEY ("direct acting antivirals") OR TITLE-ABS-KEY ("DAA") OR TITLE-ABS-KEY ("DAAs") OR TITLE-ABS-KEY ("monitor*")) AND (EXCLUDE (DOCTYPE,"re") OR EXCLUDE (DOCTYPE,"cp") OR EXCLUDE (DOCTYPE,"le") OR EXCLUDE (DOCTYPE,"sh"))

Search strategy Cochrane

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#3   ("hepatitis c"):ti,ab,kw
#4   ("hepatitis c virus"):ti,ab,kw
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#7   ("h c v"):ti,ab,kw
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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 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 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 HCV-cAg test was reported blinded to the HCV-RNA test or if it was clear that the HCV-cAg test was reported before the results of the HCV-RNA test were available
- No: results of reference standard were unblinded. The results of the HCVcAg test were reported on previous knowledge of the HCV-RNA test
- Unclear: we were unable to identify whether stored 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; 'high risk' if one or two questions were answered with 'no'; and 'unclear risk' if questions were answered with 'yes' and 'unclear'.

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 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? We will score "yes" for all studies
- Yes: The nucleic acid amplification test is the usual standard for HCV-RNA testing. Although the accuracy of this reference standard is not 100%, overall, the tests are highly sensitive, and the variability is minimal. Moreover, given that viral loads measured in this technique correlate well with HCVcAg, we scored 'yes' for all studies using a nucleic acid amplification test as the reference standard.

Signaling question 2: Were the reference standard results interpreted without knowing the index test results?
- Yes: This topic is similar to the signaling question related to the interpretation of the index test. It is unlikely to introduce bias even if the reference standard resulted in knowledge of the index test result. We scored 'yes' for all studies.

We judged risk of bias to be of 'low risk for all studies.

3.2 Applicability: Are there concerns that the target condition as defined by the reference standard does not match the question?
We judged applicability to be of 'low concern' for all studies.

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: samples for HCVcAg and reference standards tests did obtain at the same time
- No: 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 test 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		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Aghemo et al., 2016	Low	Unclear	Low	Low	Low	Low	Low
Alonso et al., 2016	Low	High	Low	Low	Low	Low	Low
Nguyen et al., 2016	Unclear	Low	Low	Low	Low	Low	Low
Arboledas et al., 2017	Low	Low	Low	Unclear	Low	Low	Low
Loggi et al., 2017	Low	Low	Low	Unclear	Low	Low	Low
Rockstroh et al., 2017	Low	High	Low	High	Low	Unclear	Low
Chevaliez et al., 2018	Low	Low	Low	High	Low	Low	Low
Łucejko et al., 2018	Low	Low	Low	Low	Low	Low	Low
van Tilborg et al., 2018	Low	Low	Low	Unclear	Low	Low	Low
Łucejko et al., 2019	Unclear	Low	Low	Low	Low	Low	Low
Pérez-García et al., 2019	Low	Low	Low	Low	Low	Low	Low
Chayanupatkul et al., 2020	Low	Low	Low	Low	Low	Low	Low
Lin et al., 2020	Low	Low	Low	High	Low	Low	Low
Mancebo et al., 2021	Unclear	Low	Low	Unclear	Low	Low	Low
Rossetti et al., 2021	Low	Low	Low	Unclear	Low	Low	Low
Ko et al., 2022	Low	Low	Low	Low	Low	Low	Low

Supplementary Figures
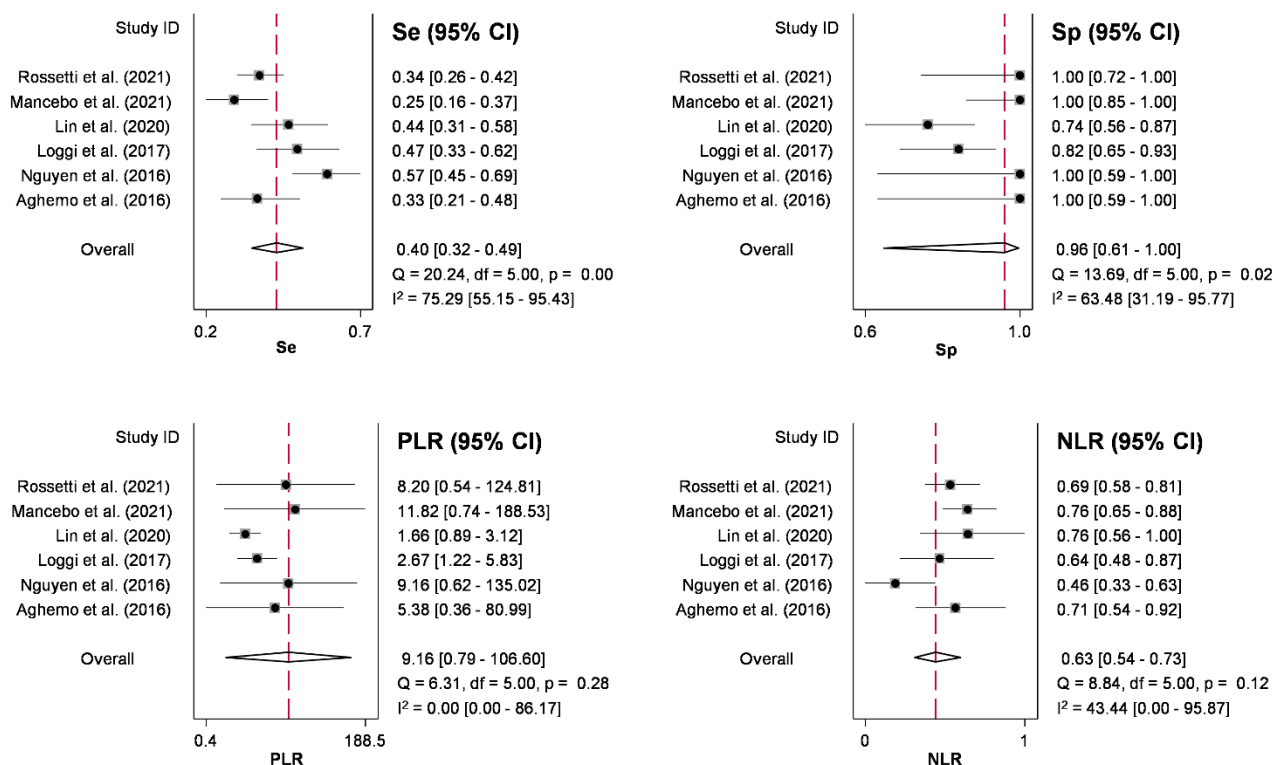
Supplementary Figure 1. Forest plots: bivariate analysis at week 2 of treatment showing sensitivity, specificity, positive and negative likelihood ratios for the HCV diagnosis with Abbott ARCHITECT HCV Ag assay compared with a confirmatory nucleic acid test. **Abbreviations**: 95% CI = 95% confidence interval; HCV = hepatitis C virus; I^2 = inconsistency index; NLR = negative likelihood ratio; PLR = positive likelihood ratio; Q = Cochran's Q test; Se = sensitivity; Sp = specificity.
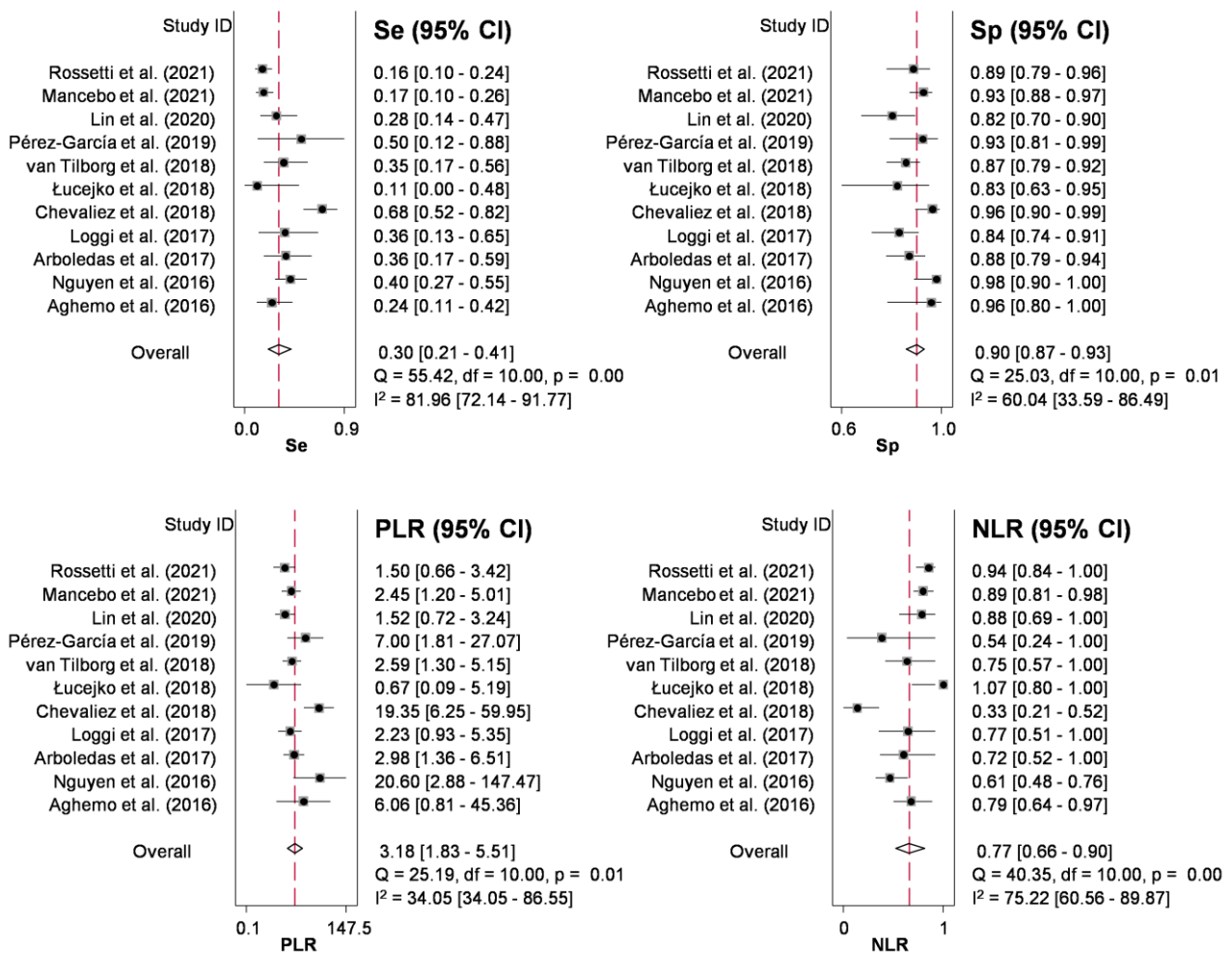
Supplementary Figure 2. Forest plots: bivariate analysis at week 4 of treatment showing sensitivity, specificity, positive and negative likelihood ratios for the HCV diagnosis with Abbott ARCHITECT HCV Ag assay compared with a confirmatory nucleic acid test. **Abbreviations**: 95% CI = 95% confidence interval; HCV = hepatitis C virus; I^2 = inconsistency index; NLR = negative likelihood ratio; PLR = positive likelihood ratio; Q = Cochran's Q test; Se = sensitivity; Sp = specificity.
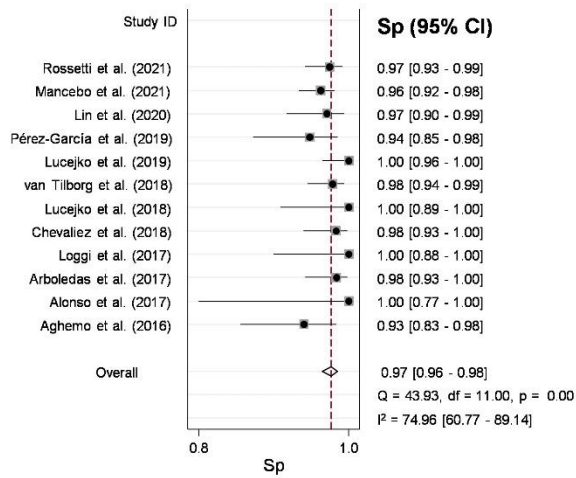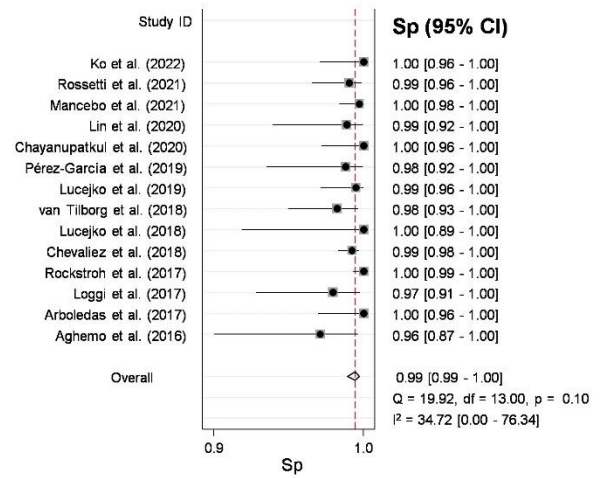
Supplementary Figure 3. Forest plots for specificity: univariate analysis for the detection of active HCV infection with Abbott ARCHITECT HCV Ag assay compared with a confirmatory nucleic acid test at the end of treatment **(A)** and SVR (12 weeks post-treatment) **(B)**. **Abbreviations**: 95% CI = 95% confidence interval; EOT = end of treatment; I^2 = inconsistency index; Q = Cochran's Q test; Sp = specificity; SVR = sustained virological response.
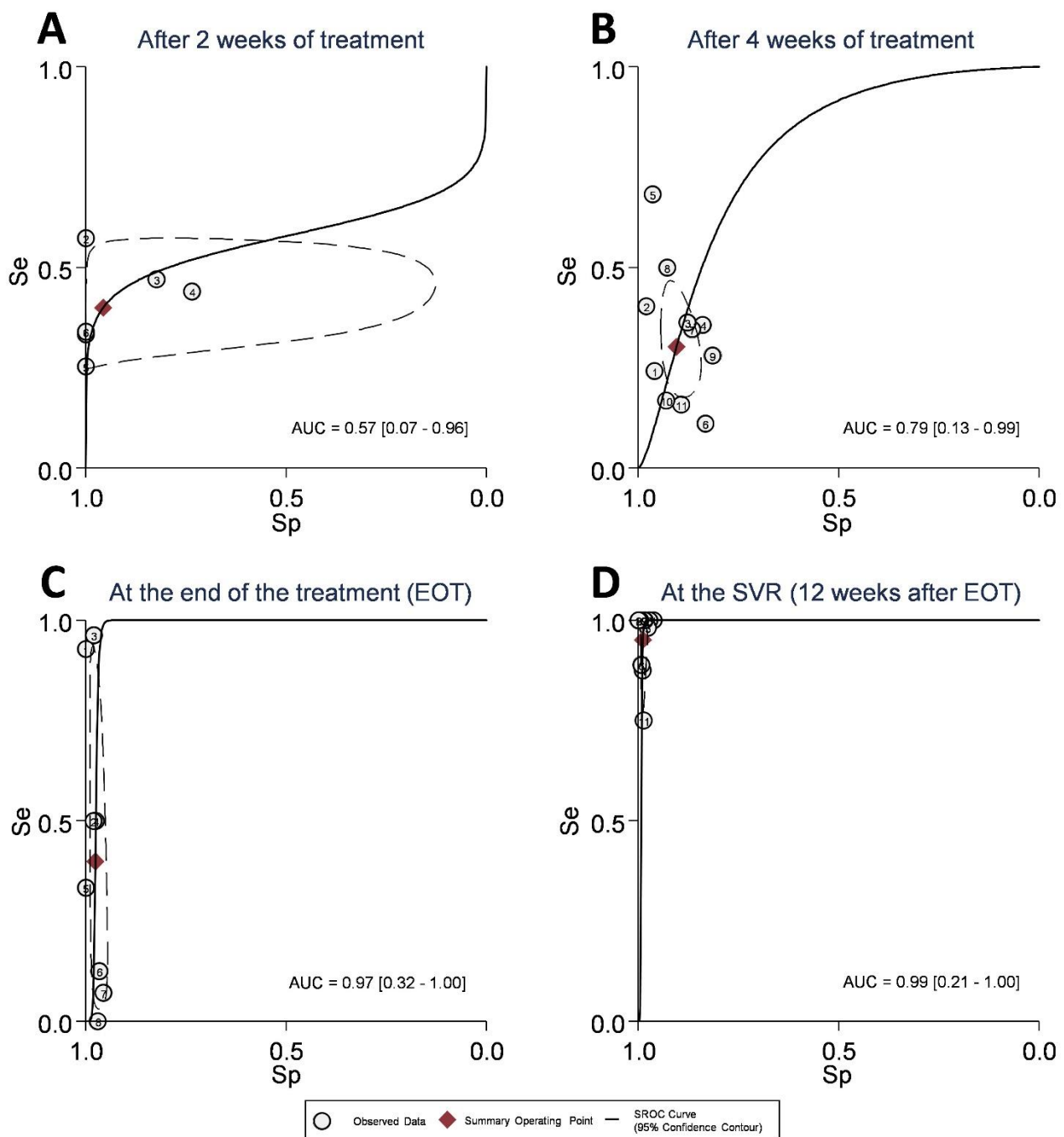
Supplementary Figure 4. SROC plot for the detection of active HCV infection with Abbott ARCHITECT HCV Ag assay compared with a confirmatory nucleic acid test after two **(A)** and four **(B)** weeks of treatment, at the end of treatment **(C)** and at the SVR **(D)**. **Abbreviations**: AUC = area under the curve; EOT = end of treatment; HCV = hepatitis C virus; Se = sensitivity; Sp = specificity; SROC = summary of receiver operating characteristic; SVR = sustained virological response.
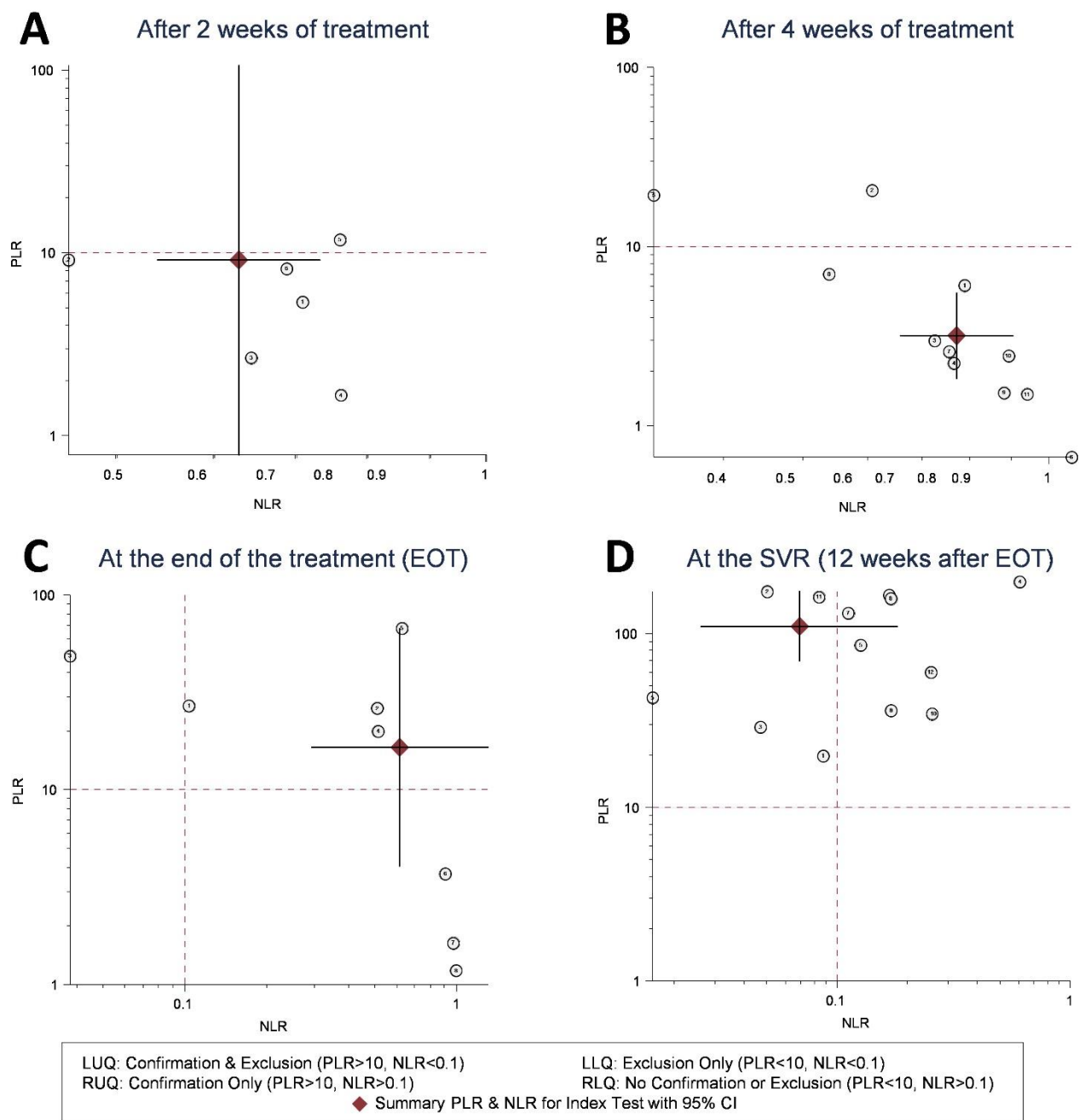
Supplementary Figure 5. Likelihood ratio scattergram for the HCV diagnosis with Abbott ARCHITECT HCV Ag assay at 2 and 4 weeks of treatment, end of treatment (EOT), and SVR (12 weeks after EOT). **Abbreviations:** 95% CI = 95% confidence interval; HCV = hepatitis C virus; LLQ = left lower quadrant; LUQ = left upper quadrant; NLR = negative likelihood ratio; PLR = positive likelihood ratio; RLQ = right lower quadrant; RUQ = right upper quadrant.
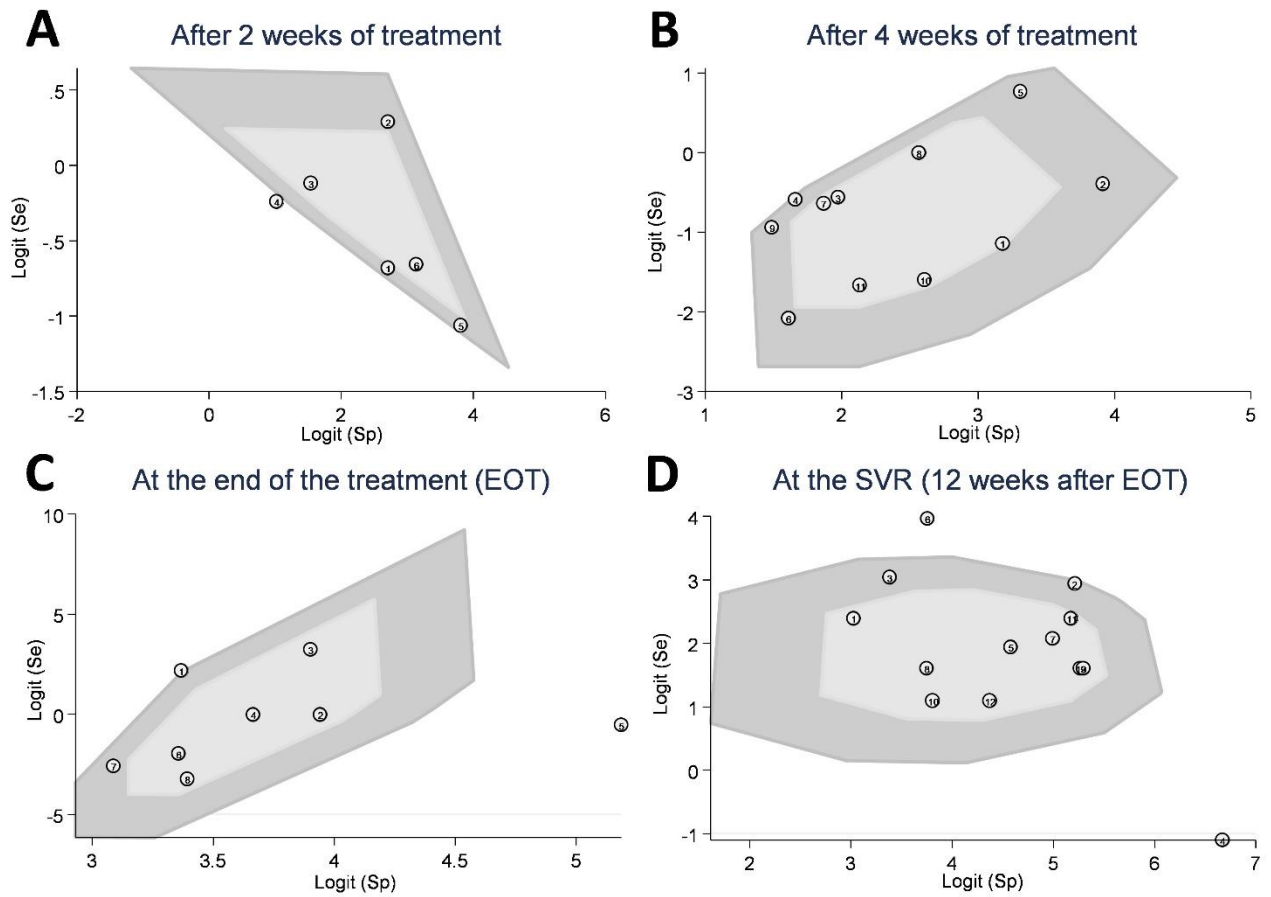
Supplementary Figure 6. Bivariate boxplot (bagplot) of the sensitivities and specificities after two (**A**) and four (**B**) weeks of treatment, at the end of treatment (**C**) and at the SVR (**D**). Abbreviations: EOT = end of treatment; Se = sensitivity; Sp = specificity; SVR = sustained virological response.
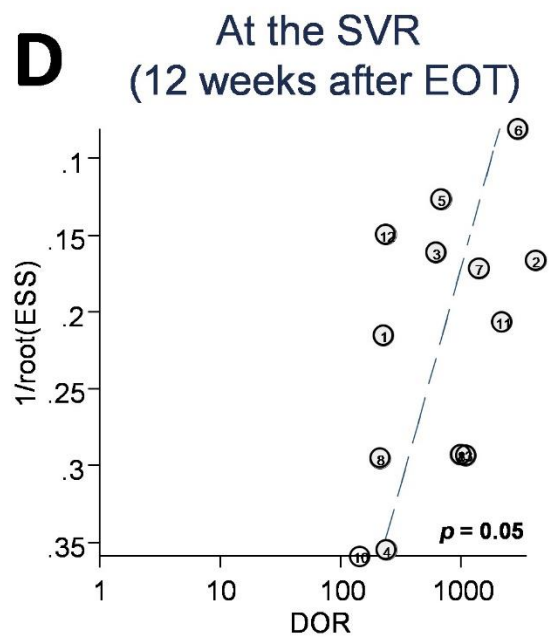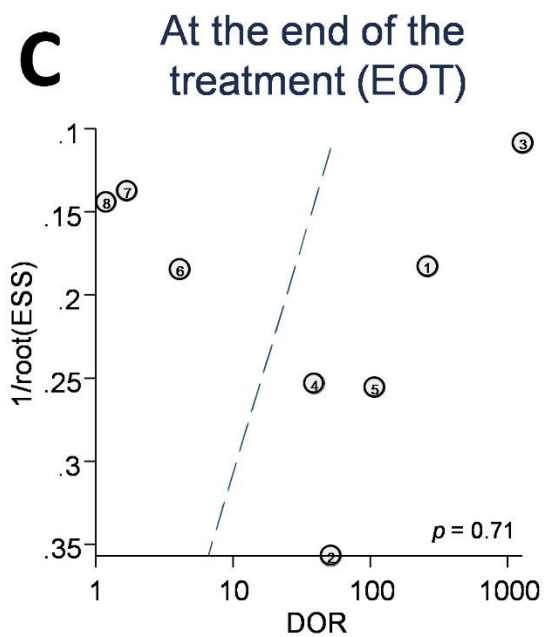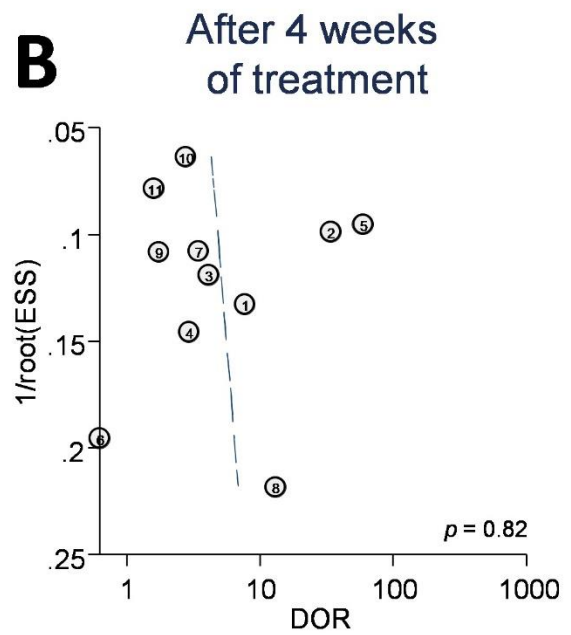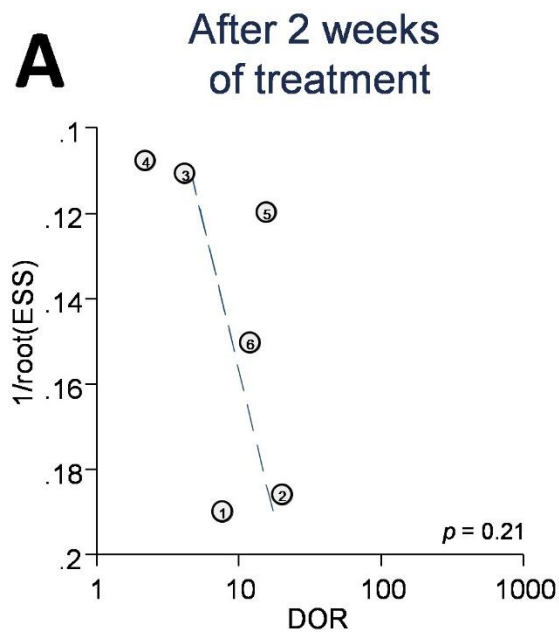
Supplementary Figure 7. Deeks' funnel plot asymmetry test for assessing publication bias after two **(A)** and four **(B)** weeks of treatment, at the end of treatment **(C)** and at the SVR **(D)**. **Abbreviations**: DOR = diagnostic odds ratio; EOT = end of treatment; ESS = effective simple size; SVR = sustained virological response.