Annals of Internal Medicine REVIEW

Hepatitis C Core Antigen Testing for Diagnosis of Hepatitis C Virus Infection

A Systematic Review and Meta-analysis

J. Morgan Freiman, MD; Trang M. Tran, BA; Samuel G. Schumacher, MSc, PhD; Laura F. White, PhD; Stefano Ongarello, PhD; Jennifer Cohn, MD, MPH; Philippa J. Easterbrook, MD, MPH; Benjamin P. Linas, MD, MPH; and Claudia M. Denkinger, MD, PhD

Background: Diagnosis of chronic hepatitis C virus (HCV) infection requires both a positive HCV antibody screen and confirmatory nucleic acid testing (NAT). Testing for hepatitis C virus core antigen (HCVcAg) is a potential alternative to NAT.

Purpose: To evaluate the accuracy of diagnosis of active HCV infection among adults and children for 5 HCVcAg tests compared with NAT.

Data Sources: EMBASE, PubMed, Web of Science, Scopus, and Cochrane Database of Systematic Reviews from 1990 through 31 March 2016.

Study Selection: Case–control, cross-sectional, cohort, or randomized trials that compared any of 5 HCVcAg tests with an NAT reference standard.

Data Extraction: 2 independent reviewers extracted data and assessed quality using an adapted QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies 2) tool.

Data Synthesis: 44 studies evaluated 5 index tests. Studies for the Abbott ARCHITECT HCV Ag assay had the highest quality, whereas those for the Ortho HCV Ag enzyme-linked immunosorbent assay (ELISA) had the lowest quality. From bivariate analyses, the sensitivity and specificity of the assays were as follows: Abbott ARCHITECT, 93.4% (95% CI, 90.1% to 96.4%) and 98.8% (CI, 97.4% to 99.5%); Ortho ELISA, 93.2% (CI, 81.6% to 97.7%) and 99.2% (CI, 87.9% to 100%); and Hunan Jynda Bioengineering Group HCV Ag ELISA, 59.5% (CI, 46.0% to 71.7%) and 82.9% (CI, 58.6% to 94.3%). Insufficient data were available for a meta-analysis about the Fujirebio Lumipulse Ortho HCV Ag and Eiken Lumispot HCV Ag assays. In 3 quantitative studies using Abbott ARCHITECT, HCVcAg correlated closely with HCV RNA levels greater than 3000 IU/mL.

Limitations: Insufficient data were available on covariates, such as HIV or hepatitis B virus status, for subgroup analyses. Few studies reported genotypes of isolates, and data for genotypes 4, 5, and 6 were scant. Most studies were conducted in high-resource settings and reference laboratories.

Conclusion: The HCVcAg assays with signal amplification have high sensitivity, high specificity, and good correlation with HCV RNA levels greater than 3000 IU/mL and have the potential to replace NAT in settings with high HCV prevalence.

Primary Funding Source: National Institutes of Health.

Ann Intern Med. 2016;165:345-355. doi:10.7326/M16-0065 **www.annals.org**
For author affiliations, see end of text.
This article was published at www.annals.org on 21 June 2016.

Approximately 130 to 150 million persons are infected with chronic hepatitis C virus (HCV), and approximately 75% of all cases occur in low- and middle-income countries (LMICs) (1, 2). Direct-acting antivirals allow safe and effective curative treatment, but treatment is the final step in a long cascade that requires screening, confirmation, notification of results, and linkage to care (3, 4). Diagnosis of HCV is a 2-step process that starts with screening for exposure with an assay that detects antibodies to HCV (anti-HCV), followed by nucleic acid testing (NAT) for persons with reactive anti-HCV to confirm active viremia. Among those who acquire a primary infection, 15% to 50% will spontaneously clear the virus within the first 2 to 6 months and remain positive for anti-HCV, although they are not actively infected and do not require treatment (5). The diagnostic process is designed to be cost-effective, with a low-cost screening test followed by targeted testing with the more expensive NAT. In LMICs, implementation of a complex algorithm is often not feasible and diagnostic capacity is low; as a result, fewer than 1% of patients are aware of their infection (6). In addition, a significant proportion of patients who test positive for anti-HCV do not receive diagnostic NAT and are lost to follow-up (7). The 2-step diagnostic process is a major bottleneck to the HCV cascade of care that needs to be addressed to achieve the ambitious elimination strategy proposed by the World Health Organization (WHO) (8).

Testing for hepatitis C virus core antigen (HCVcAg) is a potential replacement for NAT. The HCVcAg forms the internal capsid, which is highly conserved and antigenic (9, 10). During viral assembly, nucleocapsid peptide 22 is released into the plasma (11) and can be detected earlier than antibodies and throughout the course of infection (12). The following 5 tests for HCVcAg detection are commercially available: Abbott ARCHITECT HCV Ag, which is an automated chemiluminescent microparticle immunoassay; Fujirebio Lumipulse Ortho HCV Ag and Eiken Lumispot HCV Ag, which are similar automated chemiluminescent enzyme immunoassays available in Japan and China; Hunan Jynda Bioengineering Group HCV Ag enzyme-linked

See also:

Web-Only
Supplement

immunosorbent assay (ELISA); and Ortho HCV Ag ELISA.

Although all current HCVcAg tests require laboratory capacity, the development of a highly sensitive point-of-care (POC) platform is feasible and probably possible at a lower cost than NAT POC. Such a test has been defined as the highest-priority target product profile in a global stakeholder consultation process (13). As such, tests targeting HCVcAg could be attractive as a single-step diagnosis for chronic HCV infection in high-prevalence settings, which would streamline the HCV cascade of care and reduce loss to follow-up. This WHO-commissioned systematic review to inform forthcoming WHO guidelines on hepatitis testing evaluated the accuracy of diagnosis of active HCV infection among adults and children for 5 commercially available HCVcAg tests compared with NAT.

METHODS

We performed a systematic review of HCV diagnostics literature, extracted data from selected studies, and conducted a bivariate meta-analysis of the test characteristics of HCVcAg as a diagnostic test for HCV infection. We used standard methods for systematic reviews and meta-analyses of diagnostic tests (14–18), including preparation of an a priori protocol for the literature search, article selection, data extraction, quality assessment, and analysis (see **Supplement**, available at www.annals.org).

Data Sources and Searches

We searched EMBASE, PubMed, Scopus, Web of Science, and Cochrane Database of Systematic Reviews for citations related to HCVcAg screening and diagnosis published until 31 March 2016. We did not restrict the search by language, and terms were selected under the guidance of medical librarians. The search strategies included terms related to HCV, antigen, and nucleic acid amplification. See the **Supplement** for specific search strategies and the number of studies retrieved from each database.

Two authors (J.M.F. and T.M.T.) independently assessed titles and abstracts identified by the literature search to select eligible studies. Citations identified by either reviewer were selected for full-text review. These same 2 authors then independently assessed the full-text articles using predefined inclusion and exclusion criteria. Discrepancies were resolved by discussion between the authors and, when needed, by the decision of a third author (C.M.D.).

Study Selection

Inclusion criteria were as follows: case–control, cross-sectional, cohort, or randomized trials; commercially available HCVcAg tests; commercially available NAT as the reference standard; whole blood, plasma, or serum specimens; and at least 10 independent clinically collected samples. Studies done using commercially prepared reference panel specimens, published in abstract form only, or presented as slides or posters were excluded.

We included articles that reported results from populations with any distribution of patient age, from any country, and in any screening setting (for example, hospital- or community-based). Although we were primarily interested in test performance among persons at risk for HCV and with known infection, we also included studies using specimens from healthy blood donors. Because the performance characteristics of NAT are very similar when HCV RNA levels are greater than 50 IU/mL, we accepted any of the following NAT techniques as the reference standard: polymerase chain reaction, branched-chain DNA, or transcription-mediated amplification. Tests were classified as either qualitative or quantitative.

Data Extraction and Quality Assessment

Two authors (J.M.F. and T.M.T.) independently assessed all studies for inclusion and extracted data on study methods, characteristics, and test accuracy using a standardized extraction form (**Supplement**). Foreign-language studies were translated and extracted by native speakers using the same form. We crosschecked data points for 25% of the included studies. Disagreements between reviewers were resolved by discussion or by a third reviewer (C.M.D.). When elements for extraction were missing, we contacted the authors to request further data. We also requested individual specimen data to allow for a quantitative assessment of HCVcAg against HCV RNA. Studies without extractable sensitivity and specificity data were excluded if no further information was acquired after 3 attempts to contact the study authors.

Methodological quality of the included studies was assessed using a validated QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool (19). Details of the QUADAS-2 questions and interpretation are reported in the **Supplement**.

Data Synthesis and Analysis

We defined HCVcAg sensitivity as the proportion of samples with a positive NAT result that was also positive for HCVcAg. We defined HCVcAg specificity as the proportion of samples with a negative NAT result that was also negative for HCVcAg. Sensitivity and specificity were the primary outcome measures. Positive and negative likelihood ratios were calculated when pooled sensitivity and specificity data were available from meta-analysis. Indeterminate test results accounted for fewer than 1% for all index tests and were excluded from further analyses.

We constructed forest plots for each HCVcAg index test to visually assess heterogeneity by examining the CIs of individual studies. We then used summary plots to examine the width of the prediction region, with a wider prediction region suggesting more heterogeneity. When at least 4 studies with limited heterogeneity were available, we used a bivariate random-effects model and carried out meta-analyses using the metandi command in STATA, version 14 (StataCorp) (20, 21).

When at least 4 studies provided sensitivity data only, we did a univariate random-effects meta-analysis

on the sensitivities to use all available data. Results from the univariate analyses (including all studies) were compared with the pooled estimates from the bivariate analyses where possible. Descriptive analyses were done for index tests with fewer than 4 studies and when substantial heterogeneity was evident from inspection of the forest and summary plots.

When quantitative data were available, a locally weighted regression smoother was used to visually assess the linearity of quantitative HCVcAg (measured in fmol/L) to HCV RNA (measured in IU/mL) (22). We identified outliers and recorded descriptive statistics of these points. Quantitative data were insufficient to assess any test other than Abbott ARCHITECT.

We assessed for publication bias when more than 10 studies were available for an index test. We generated funnel plots displaying the log diagnostic odds ratio versus the SE for each study (18). We also did the trim-and-fill statistical assessment in STATA using the metatrim command (23). Unpublished data were not included. All statistical analyses were done using STATA and R, version 3.2.5 (R Foundation for Statistical Computing).

Role of the Funding Source

This systematic review was supported by the National Institutes of Health, which had no direct involvement in the study design, collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication.

RESULTS

Study Selection and Characteristics

The systematic review identified 8508 citations, from which we reviewed 299 full-text articles and identified 44 that met the a priori–defined inclusion criteria (**Appendix Figure 1**, available at www.annals.org). Of the included studies, 44 used the 5 HCVcAg assays; 1 of these studies directly compared 3 antigen tests. Four studies were translated from Mandarin (24–27), 1 from German (28), and 2 from Japanese (29, 30). Characteristics for each study are presented in **Table 1**.

Risk-of-Bias Assessment

The overall risk-of-bias assessment for all included studies across each QUADAS-2 domain is summarized in **Appendix Figure 2** and presented for each study by index test in **Appendix Figure 3** (both figures available at www.annals.org). Studies using Abbott ARCHITECT had the highest quality. However, 15 of the 33 studies did not report on whether patients were recruited consecutively and 1 included only healthy blood donors (31). The 6 studies using the Ortho ELISA had the lowest quality; 2 did not report patient selection methods (32, 33), and only 1 included healthy blood donors (34). In addition, 2 studies did convenience sampling (29, 30) and it was unclear in both studies whether the index test and reference tests were performed on the same sample or within 30 days in the same participant and whether the index test was performed in accordance with manufacturer recommendations. For the Hunan Jynda ELISA, patient selection was unclear in 1 study (25), timing of the index and reference tests was unclear in 1 study (26), and only healthy blood donors were enrolled in 1 study (35). For Eiken Lumispot, both studies had unclear participant selection (36, 37), as did the study that assessed Fujirebio Lumipulse (37).

HCVcAg Assays for Diagnosis of Active HCV Infection

Abbott ARCHITECT

We found 33 studies assessing Abbott ARCHITECT (11, 24, 28, 31, 37–65). All were either cross-sectional or cohort designs with a broad study population (they included patients with HCV infection and those susceptible to HCV infection), except for 1 study that evaluated only healthy blood donors (31). Demographic data were available in 21 studies; the remainder used anonymous specimens, and the authors could not provide further information. HIV status was known in 16 studies, with 3 including only persons with HIV co-infection (59, 61, 64). Hepatitis B virus (HBV) status was known in 13 studies, and all but 4 excluded patients with HBV co-infection. The highest prevalence of HBV co-infection (defined as hepatitis B surface Ag positivity) was 50.5% (53). Only 1 study included children (43).

The bivariate analysis consisted of 23 studies. The 10 remaining studies (11, 37, 40–42, 49, 52, 56, 60, 62) did not have data to calculate specificity and were only included in the pooled sensitivity estimate from the univariate analysis. For the bivariate analysis, there were 12 670 total samples. The pooled sensitivity and specificity, regardless of anti-HCV status, were 93.4% (95% CI, 90.1% to 96.4%) and 98.8% (CI, 97.4% to 99.5%), respectively. The positive and negative likelihood ratios were 80.6 (CI, 36.4 to 178.8) and 0.06 (CI, 0.04 to 0.1), respectively (**Table 2**; **Figure 1**; and **Appendix Figure 4**, available at www.annals.org). The pooled sensitivity estimate from the univariate analysis, including the 10 additional studies (total of 13 638 samples), was similar to that of the bivariate analysis—94.3% (CI, 92.8% to 95.9%) (**Table 2** and **Appendix Figure 5**, available at www.annals.org)—although the estimate was higher among the 10 studies when evaluated alone (99% [CI, 97.8% to 100%]). Among 16 studies with known anti-HCV–positive samples, sensitivity was 92.5% (CI, 86.9% to 95.8%) and specificity was 97.8% (CI, 94.7% to 99.1%) (**Table 2** and **Appendix Figure 6**, available at www.annals.org). In the 5 studies that analyzed anti-HCV–negative samples in the acute or preseroconversion phase, the pooled sensitivity was lower, with a wide CI (92.3% [CI, 3.7% to 99.9%]). Specificity among anti-HCV–negative samples remained high at 98.8% (CI, 97.3% to 99.4%) (**Table 2** and **Appendix Figure 7**, available at www.annals.org).

Heterogeneity was visually assessed in **Figure 1** and **Appendix Figures 4** to **7**. The studies seem to be homogeneous in the overall bivariate analysis, except for 1 outlier study (24) that had no demographic information to perform further analysis. Overall, genotype distribution was reported for 18 studies (**Appendix Ta-**

Table 1. Characteristics of Included Studies Grouped Alphabetically, by Index Test Type

Author, Year (Reference)	Country	Income Category*	Study Design	Study Population	Age Group
Abbott ARCHITECT HCV Ag					
Buket et al, 2014 (38)	Kazakhstan	B	Cohort	Broad	Adults
Chevaliez et al, 2014 (39)	France	A	Cross-sectional	Broad	Adults
Cresswell et al, 2015 (64)	United Kingdom	A	Cohort	Broad	Adults
Descamps et al, 2012 (40)	France	A	Cross-sectional	Broad	Adults
Durante-Mangoni et al, 2013 (41)	Italy	A	Cohort	Broad	Adults
Ergünay et al, 2011 (43)	Turkey	A	Cohort	Broad	Mixed
Florea et al, 2014 (44)	Romania	B	Cross-sectional	Broad	Adults
Garbuglia et al, 2014 (45)	Italy	A	Cohort	Broad	Adults
Gu et al, 2014 (24)	China	B	Cross-sectional	Broad	Unknown
Hadziyannis et al, 2013 (46)	Greece	A	Cross-sectional	Broad	Unknown
Heidrich et al, 2014 (47)	Germany	A	Cohort	Broad	Adults
Kadkhoda et al, 2014 (48)	Canada	A	Cross-sectional	Broad	Adults
Kesli et al, 2011 (31)	Turkey	A	Cohort	Healthy	Adults
Köroğlu et al, 2012 (49)	Turkey	A	Cohort	Broad	Unknown
Kuo et al, 2012 (50)	Taiwan	A	Cohort	Broad	Adults
Li Cavoli et al, 2012 (51)	Italy	A	Cohort	Broad	Adults
Mederacke et al, 2009 (52)	Germany	A	Cohort	Broad	Unknown
Mederacke et al, 2012 (53)	Germany	A	Cross-sectional	Broad	Unknown
Medici et al, 2011 (54)	Italy and Spain	A	Cross-sectional	Broad	Unknown
Medici et al, 2016 (63)	Italy	A	Cross-sectional	Broad	Adults
Miedouge et al, 2010 (55)	France	A	Cohort	Broad	Unknown
Mixson-Hayden et al, 2015 (65)	United States	A	Cohort	Broad	Unknown
Murayama et al, 2012 (37)	Japan	A	Cross-sectional	Broad	Unknown
Ottiger et al, 2013 (56)	Switzerland	A	Cross-sectional	Broad	Adults
Park et al, 2010 (57)	South Korea	A	Cohort	Broad	Adults
Reyes-Méndez et al, 2014 (58)	Mexico	B	Cross-sectional	Broad	Unknown
Rouet et al, 2015 (59)	Gabon	B	Cross-sectional	Broad	Adults
Russi et al, 2014 (60)	Italy	A	Cohort	Broad	Adults
Tedder et al, 2013 (11)	United Kingdom	A	Cohort	Broad	Unknown
Thong et al, 2015 (42)	Thailand	B	Cohort	Broad	Adults
van Helden and Weiskirchen, 2014 (28)	Germany	A	Cross-sectional	Broad	Unknown
Vanhommerig et al, 2015 (61)	The Netherlands	A	Cohort	Broad	Unknown
Vermehren et al, 2012 (62)	Germany	A	Cohort	Broad	Adults
Eiken Lumispot HCV Ag					
Saito et al, 2003 (36)	Japan	A	Cross-sectional	Broad	Unknown
Murayama et al, 2012 (37)	Japan	A	Cross-sectional	Broad	Unknown
Fujirebio Lumipulse Ortho HCV Ag					
Murayama et al, 2012 (37)	Japan	A	Cross-sectional	Broad	Unknown
Hunan Jynda Bioengineering Group HCV Ag ELISA					
Lu et al, 2007 (25)	China	B	Cohort	Broad	Unknown
Ouyang et al, 2006 (26)	China	B	Cross-sectional	Broad	Unknown
Zhang et al, 2007 (35)	China	B	Cohort	Healthy	Unknown
Zhu et al, 2010 (27)	China	B	Cross-sectional	Broad	Mixed
Ortho HCV Ag ELISA					
Agha et al, 2004 (66)	Egypt, Japan, and Uzbekistan	AB	Cohort	Broad	Unknown
el-Sayed et al, 2004 (32)	Egypt	B	Cross-sectional	Broad	Unknown
Letowska et al, 2004 (34)	Poland	A	Cohort	Healthy	Unknown
Nübling et al, 2002 (33)	United States	A	Cohort	Broad	Unknown
Ohta et al, 2004 (39)	Japan	A	Cross-sectional	Broad	Unknown
Okazaki et al, 2008 (30)	Japan	A	Cohort	Broad	Unknown

A = high-income countries; Ag = antigen; B = middle-income countries; ELISA = enzyme-linked immunosorbent assay; HBV = hepatitis B virus; HCV = hepatitis C virus.
* According to the World Bank List of Economies (July 2015).

ble, available at www.annals.org), with genotype 1b being the most prevalent and genotypes 5 and 6 only minimally studied. In the univariate analysis, there were 3 outlier studies (24, 43, 44). In the study by Ergünay and colleagues (43) from Turkey, the status of HIV and HBV co-infection was unknown and genotype distribution was similar overall to other studies that reported data: 60.2% of participants had HCV genotype 1b infection, 2.2% had genotype 1a, 0.8% had genotypes 3 and 4, and 35.8% were unknown (Appendix Table). In the study by Florea and colleagues (44) from Romania, no patients had HIV or HBV infection and the HCV genotype was unknown. For specificity, the results were even more homogeneous with only 1 outlier–the study by Medici and colleagues (54) from Italy and Spain that reported 63 false-positive test results. This study had no

Table 1–Continued

Participants, *n*	Proportion With HIV Infection, %	Proportion With HBV Infection, %	Proportion Female, %	Sample Type	Sample Condition
115	Unknown	Unknown	56.5	Serum	Unknown
514	Unknown	Unknown	36.6	Serum	Unknown
111	100	Unknown	5.4	Serum	Unknown
22	Unknown	Unknown	40.1	Serum	Frozen
114	0	0	43	Serum	Frozen
272	Unknown	Unknown	Unknown	Serum	Frozen
76	0	0	75	Serum	Frozen
292	100	3.8	25.9	Serum	Frozen
304	Unknown	Unknown	Unknown	Whole	Unknown
105	Unknown	Unknown	Unknown	Serum	Frozen
596	Unknown	Unknown	43	Serum	Unknown
154	1.3	0	50	Serum	Unknown
212	Unknown	Unknown	57.5	Serum	Unknown
32	Unknown	Unknown	45.5	Serum	Unknown
405	Unknown	Unknown	52.6	Serum	Unknown
92	1.1	2.2	41.3	Serum	Unknown
118	0	0	Unknown	Serum	Unknown
237	49.5	50.5	Unknown	Serum	Unknown
1480	Unknown	Unknown	52.6	Serum	Frozen
188	Unknown	Unknown	44.25	Serum	Unknown
2850	Unknown	Unknown	Unknown	Serum	Frozen
551	Unknown	Unknown	Unknown	Serum	Frozen
80	Unknown	Unknown	Unknown	Plasma	Frozen
97	6	0	38.1	Plasma	Frozen
282	Unknown	Unknown	49.3	Serum	Unknown
211	Unknown	Unknown	Unknown	Serum	Unknown
54	100	Unknown	70.1	Plasma	Frozen
102	0	0	78.4	Serum	Frozen
54	0	0	Unknown	Plasma	Frozen
189	44.9	0	28.6	Serum	Frozen
3558	4.4	6.6	Unknown	Serum	Unknown
93	100	Unknown	0	Serum	Unknown
160	0	0	54	Serum	Frozen
155	Unknown	Unknown	Unknown	Serum	Frozen
80	Unknown	Unknown	Unknown	Plasma	Frozen
80	Unknown	Unknown	Unknown	Plasma	Frozen
191	Unknown	Unknown	Unknown	Serum	Unknown
149	Unknown	Unknown	Unknown	Serum	Unknown
11	Unknown	Unknown	Unknown	Serum	Frozen
173	Unknown	Unknown	Unknown	Serum	Unknown
246	Unknown	Unknown	Unknown	Serum	Unknown
50	Unknown	Unknown	Unknown	Serum	Frozen
124	Unknown	Unknown	Unknown	Serum	Unknown
52	Unknown	Unknown	Unknown	Plasma	Frozen
225	Unknown	Unknown	Unknown	Serum	Unknown
300	Unknown	Unknown	50.3	Serum	Unknown

demographic data because it was performed on anonymous samples.

The funnel plot of the log diagnostic odds ratio versus SE for all 33 included studies was symmetrical (Appendix Figure 8, available at www.annals.org), which suggested low publication bias. This was further supported by the trim-and-fill statistical test, which found no change in heterogeneity between the random-effects model and a filled model (data not shown).

The Ortho ELISA

Six studies used the Ortho ELISA (29, 30, 32–34, 66). All were either cross-sectional or cohort designs in general study populations, except for 1 study done in healthy blood donors (34). All had unknown demographic information.

Five studies were included in the bivariate analysis with 1177 total samples. Pooled sensitivity and specificity, regardless of anti-HCV status, were 93.2% (CI,

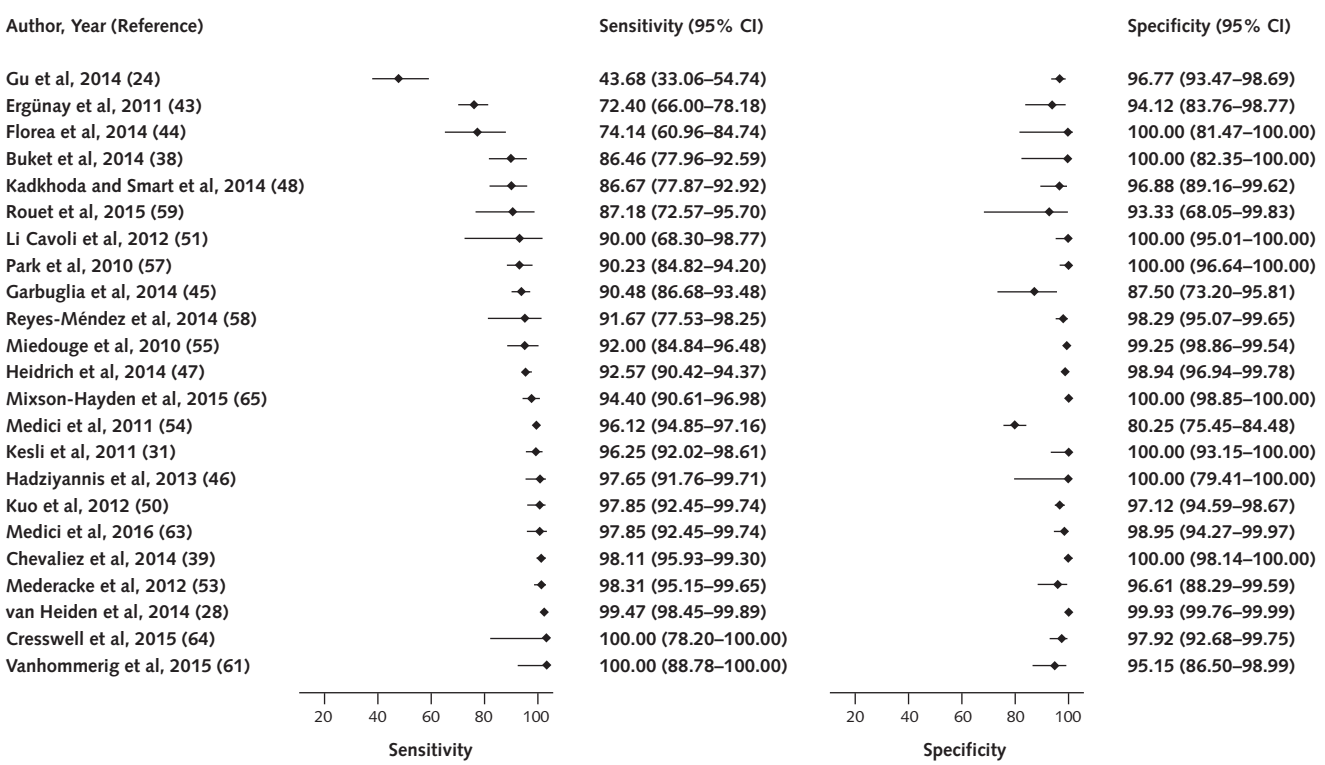
Table 2. Accuracy by HCV cAg Index Test Type for Diagnosis of Active HCV Infection Compared With NAT as the Reference Standard*

Index Test	HCV Antibody Status	Studies, *n*	Samples, *n*	Sensitivity (95% CI), %	Specificity (95% CI), %	Likelihood Ratio (95% CI)†	
						Positive	Negative
Abbott ARCHITECT HCV Ag							
By bivariate meta-analysis	All	23	12 670	93.4 (90.1–96.5)	98.8 (97.4–99.5)	80.6 (36.4–178.8)	0.06 (0.04–0.10)
By univariate meta-analysis	All	33	13 638	94.3 (92.8–95.9)	ND	NA	NA
By bivariate meta-analysis	Known positive	16	5246	92.5 (86.9–95.8)	97.8 (94.7–99.1)	42.0 (16.4–106.4)	0.05 (0.03–0.08)
By univariate meta-analysis	Known positive	27	6189	93.4 (91.4–95.4)	ND	NA	NA
By bivariate meta-analysis	Known negative	5	3415	92.3 (3.7–99.9)	98.8 (97.3–99.4)	73.9 (32.6–167.9)	0.080 (0.004–15.700)
Ortho HCV Ag ELISA							
By bivariate meta-analysis	All	5	1177	93.2 (81.6–97.7)	99.2 (87.9–100.0)	116.5 (6.7–977.0)	0.06 (0.02–0.07)
By univariate meta-analysis	All	6	1423	90.8 (83.5–98.2)	ND	NA	NA
Eiken Lumispot HCV Ag‡	All	2	235	97.5–98.1	ND	NA	NA
Fujirebio Lumipulse Ortho HCV Ag	All	1	80	95.0 (90.2–99.8)	ND	NA	NA
Hunan Jynda Bioengineering Group HCV Ag ELISA, by bivariate meta-analysis	All	4	562	59.5 (46.0–71.7)	82.9 (58.6–94.3)	3.5 (1.1–12.6)	0.28 (0.20–0.30)

Ag = antigen; ELISA = enzyme-linked immunosorbent assay; HCV = hepatitis C virus; HCVcAg = hepatitis C virus core antigen; NA = not applicable; NAT = nucleic acid testing; ND = no data.
* Results from bivariate and univariate analyses, range of studies, and single studies are reported. Bivariate and univariate meta-analyses were done using the "metandi" and "metan" commands in Stata, respectively.
† If sensitivity and specificity results were NA from meta-analysis, likelihood ratios were not calculated.
‡ Meta-analysis was not possible; the range of results seen across studies was reported.

81.6% to 97.7%) and 99.2% (CI, 87.9% to 100%), respectively. Positive and negative likelihood ratios were 116.5 (CI, 6.7 to 977) and 0.06 (CI, 0.02 to 0.07), respectively (Table 2). In the summary plot, the summary point was approaching the upper-left corner, which suggests good accuracy of the Ortho ELISA for diagnosis of active HCV infection. However, these data exhibited some heterogeneity given the wide CIs (Appendix

Figure 1. Forest plot of Abbott ARCHITECT's sensitivity and specificity for the diagnosis of active HCV infection compared with NAT for all samples, regardless of anti-HCV status.



Abbott ARCHITECT = Abbott ARCHITECT HCV Ag assay; Ag = antigen; anti-HCV = antibody to hepatitis C virus; HCV = hepatitis C virus; NAT = nucleic acid testing.

Figures 9 and 10, available at annals.org). The only outlier study (33) found 91 false-negative HCVcAg test results but did not report antibody status and HIV or HBV co-infection information, although the genotype distribution was similar to other studies in which it was reported (11.5% genotype 1 not subtyped, 42.3% genotype 1a, 19.2% genotype 1b, 11.5% genotype 2, and 15.4% genotype 3) (Appendix Table). Further, this study was performed in 494 plasma samples collected from only 52 donors at various time points during HCV infection, and thus the samples did not provide independent data points. Raw quantitative data were not available.

Eiken Lumispot

The Eiken Lumispot was used in 1 cross-sectional study of a general study population (36). Further demographic information was unavailable. Eiken Lumispot, Fujirebio Lumipulse, and Abbott ARCHITECT were compared in 1 cross-sectional study (37) with unknown demographic information.

The first study included 155 samples, and the sensitivity reported was 98.1% (CI, 95.9% to 100%) (Table 2) (36). Most samples were HCV genotype 1 (65.2%), and the remaining were HCV genotype 2. The second study comparing 3 assays (37) included 80 participants and reported a sensitivity of 97.5% (CI, 94.1% to 100%) for Eiken Lumispot. Data were insufficient to determine specificity in either study.

Fujirebio Lumipulse

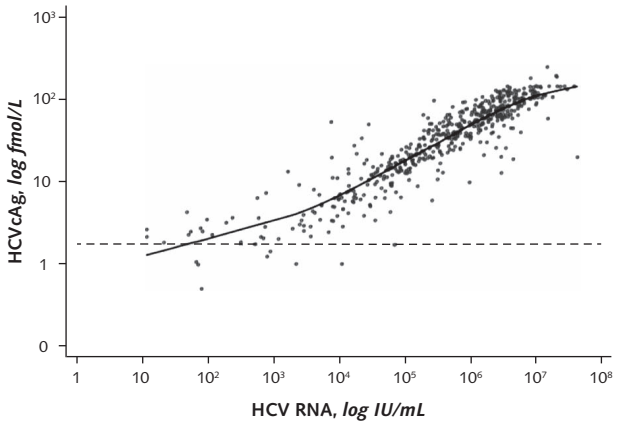
One study was done using Fujirebio Lumipulse with 80 participants (37). Sensitivity for this test was reported as 95.0% (CI, 90.2% to 99.8%) (Table 2). Data were insufficient to determine specificity.

Hunan Jynda ELISA

Four studies assessed the Hunan Jynda ELISA (25–27, 35). Two studies had a cohort or cross-sectional design. One assessed a population of healthy blood donors (35), whereas the others included broad study populations. HIV and HBV co-infection status were unknown in all studies. One study included children (27), and the age groups for the remaining studies were unknown.

All 4 studies were included in the bivariate analysis with 562 total samples. Pooled sensitivity and specificity were 59.5% (CI, 46.0% to 71.7%) and 82.9% (CI, 58.6% to 94.3%), respectively. Positive and negative likelihood ratios were 3.5 (CI, 1.1 to 12.6) and 0.28 (CI, 0.2 to 0.3), respectively (Table 2). Both the forest plot (Appendix Figure 11, available at www.annals.org) and bivariate analysis (Appendix Figure 12, available at www.annals.org) showed heterogeneity among the 4 studies, which limited confidence in the pooled estimate. No covariate assessment was done because HIV status, HBV status, and genotype distribution were unknown for all studies.

Figure 2. Nonparametric regression smoother of pooled quantitative data assessing the correlation between Abbott ARCHITECT and HCV RNA.



The dashed line indicates the positivity threshold of the HCVcAg index test, corresponding to 3 fmol/L. Abbott ARCHITECT = Abbott ARCHITECT HCV Ag assay; Ag = antigen; HCVcAg = hepatitis C virus core antigen; HCV = hepatitis C virus.

Quantitative Data

Three studies provided quantitative data for analysis (48, 54, 57). All used Abbott ARCHITECT in comparison with NAT. Of note, there were 90 anti-HCV–positive specimens in the study by Kadkhoda and Smart (48), 205 anti-HCV–positive and 77 anti-HCV–negative specimens in the study by Park and coworkers (57), and 1152 anti-HCV–positive specimens in the study by Medici and colleagues (54). The HCVcAg was shown to correlate well with RNA, except when levels were less than 3000 IU/mL, which yielded negative HCVcAg test results (Figure 2). In the study by Kadkhoda and Smart, among the 8 specimens with HCV RNA levels greater than 3000 IU/mL and negative HCVcAg test results, the genotype distribution among participants was similar to the cohort as a whole (12.5% unspecified, 37.5% genotype 1, 25% genotype 2, and 25% genotype 3). No genotype or co-infection information was available for the specimens in the studies by Park and coworkers or Medici and colleagues to further characterize outlier points.

DISCUSSION

This systematic review concludes that a well-performing HCVcAg test can achieve similar diagnostic accuracy to NAT for identification of active HCV infection when the viral load exceeds 3000 IU/mL. Both Abbott ARCHITECT and the Ortho ELISA perform similarly with regard to sensitivity (93.4% [CI, 90.1% to 96.4%] vs. 93.2% [CI, 81.6% to 97.7%]) and specificity (98.8% [CI, 97.4% to 99.5%] vs. 99.2% (CI, 87.9% to 100%]). However, the large amount of consistent, homogenous data on Abbott ARCHITECT (33 studies vs. 6 on the Ortho ELISA) allows for greater precision and more confidence in these estimates. The likelihood ratios for both

tests are also very favorable and allow for clinical decision making based on test results.

The Eiken Lumispot and Fujirebio Lumipulse were designed with the same principle technology as Abbott ARCHITECT and have similar sensitivity and specificity, although assessment was limited to 1 and 2 studies, respectively. However, our systematic review included Chinese and Japanese literature. Such tests as the Hunan Jynda ELISA have the lowest sensitivity (59.5% [CI, 46.0% to 71.7%]), which supports the notion that an ELISA is insufficient for detection and that signal amplification (as with chemiluminescence) is necessary to achieve adequate detection limits.

We searched PubMed on 31 March 2016 for recent reviews of HCVcAg and retrieved 1 systematic review done in 2012 (67). Our study adds to this review because we included many studies published since 2012 and eliminated those that used HCVcAg tests that are no longer commercially available. The analysis differs because we evaluated the performance of each commercial test separately, rather than pooling all HCVcAg tests into 1 multivariate random-effects model. Our results allow indirect between-test comparisons and avoid the problematic heterogeneity introduced by pooling performance characteristics of different detection technologies. We also searched ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform, but we did not find any active studies investigating HCVcAg.

Strengths of this review include the development of an a priori protocol and analysis plan. The search was done without language restriction, although 3 articles were excluded because of inability to find translation for Russian, Korean, and Polish. Nevertheless, studies may have been missed and subsequent studies published after the search date could not be included. Article selection and standardized data extraction in accordance with the predefined protocol were ensured by independent reviewers. Authors were contacted to provide missing data and clarifications, and some studies were excluded because of lack of author response. In the analysis, bivariate random-effects modeling was used when appropriate to derive pooled estimates and univariate analyses were done in an effort to use all available data.

The data summarized in this review had limitations. We planned to examine the effects of HBV co-infection, HIV co-infection, and HCV genotype in a meta-regression analysis, but this was not possible because of the limited data on these covariates. Data on HCVcAg test performance in genotypes 4, 5, and 6 are largely lacking, which limits the conclusions. In addition, a sensitivity analysis to examine the effect of the specimen condition (fresh vs. frozen) could not be done because all studies used frozen samples or did not specify the specimen condition. Not enough studies used Eiken Lumispot and Fujirebio Lumipulse to derive pooled estimates, and only descriptive analyses could be completed. Most of the studies were done in high-resource settings and reference laboratories. This might not reflect the population that would be tested if HCVcAg tests were implemented in LMICs, particularly given the limited data for genotypes 4, 5, and 6, which are more prevalent in such countries (68).

The limitations highlight a need for better surveillance data to improve the understanding of how many patients are missed (false-negative test results) by assays that have greater limits of detection (for example, 3000 IU/mL for Abbott ARCHITECT) and whether covariates, such as HIV or HBV co-infection and HCV genotype, affect assay results. More information is needed about the fluctuation of RNA levels and HCVcAg during the preseroconversion phase, as well as for the rare patients with high viral loads who are negative for HCVcAg, to inform the optimization of antigen detection. This study focused only on the use of HCVcAg as a screening and diagnostic test, although NAT is also used in treatment monitoring and to assess sustained virologic response after therapy is completed. The performance of HCVcAg to confirm sustained virologic response at the completion of therapy should be further investigated. However, recent publications (69, 70) suggest that it may no longer be necessary to record viral load measurements of patients receiving direct-acting antivirals.

For both HCVcAg tests and NAT to reach a larger population at risk in LMICs, tests with better POC suitability need to be developed or transport mechanisms with dried blood spots need to be improved to enable better centralized testing depending on local settings. For any HCVcAg POC test, careful sample processing is necessary to lyse viral particles, expose the antigen, and dissociate the antibody from antigen to optimize detection. Signal amplification will be necessary to achieve sufficient sensitivity (as suggested by this review); therefore, an instrument-free assay (for example, a lateral-flow assay) is unlikely to be feasible in the near future. The cost of testing to the patient or health care provider is also a key factor for implementation in LMICs. The cost estimates from LMICs are highly variable and often country-specific, although cost estimates for HCVcAg tests are generally lower (from $10 to $50) than those for HCV RNA tests ($13 to $100) (64, 69, 71, 72) (Foundation for Innovative New Diagnostics; WHO. Diagnostics for active hepatitis C infection: What is needed to support global scale-up of care? In preparation.).

In summary, this systematic review showed that several HCVcAg assays are highly sensitive (>90%) and specific (>98%). Although even tests with the highest performance are not as sensitive as NAT, well-performing HCVcAg tests with an analytic sensitivity reaching into the femtomolar range (equal to 3000 IU/mL) could replace NAT for HCV detection, particularly if a lower cost per test allows more patients to be served. Therefore, HCVcAg should be explored for POC testing to increase the number of patients diagnosed and streamline the HCV cascade of care.

From Boston Medical Center, Boston University School of Public Health, and Beth Israel Deaconess Medical Center, Boston, Massachusetts; Médecins Sans Frontières Access

Campaign, World Health Organization, and Foundation for Innovative New Diagnostics, Geneva, Switzerland; and University of Pennsylvania, Philadelphia, Pennsylvania.

Acknowledgment: The authors thank all of the study authors who provided additional data necessary to complete this review and the following persons: Jeanne Chauffor (Médecins Sans Frontières) and Audrey Albertini (Foundation for Innovative New Diagnostics [FIND]) for translation of studies in French, Kuniaki Arai (World Health Organization) for translation of studies in Japanese, Joseph Tucker (University of North Carolina) for translation of author communication in Mandarin Chinese, David Flynn (Boston University School of Medicine Alumni Medical Library) and Genevieve Gore (McGill University) for their help with search terms, and Ranald Sutherland and Martin Brusdeilins from FIND for their input on the technical aspects and commercial availability of HCVcAg tests.

Grant Support: By the National Institutes of Health (grants 5T32AI052074-10, 5R01DA031059-04, 1P30DA040500-01, and 5P30AI042853-18).

Disclosures: Dr. White reports grants from the National Institutes of Health during the conduct of the study. Dr. Ongarello reports personal fees from FIND (salaried employee) during the conduct of the study and outside the submitted work. Dr. Denkinger reports nonfinancial support and other from Cepheid and grants (to FIND) outside the submitted work. Authors not named here have disclosed no conflicts of interest. Disclosures can also be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M16-0065.

Reproducible Research Statement: *Study protocol*: See the Supplement (available at www.annals.org). *Statistical code*: Available from Dr. Freiman (e-mail, J.Morgan.Freiman@bmc.org). *Data set*: Data from extracted studies available upon request (e-mail, J.Morgan.Freiman@bmc.org).

Requests for Single Reprints: J. Morgan Freiman, MD, One Boston Medical Center Place, Dowling 3 North, Boston, MA 02118; e-mail, j.morgan.freiman@bmc.org.

Current author addresses and author contributions are available at www.annals.org.

References

1. **Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H.** Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol. 2014;61:S45-57. [PMID: 25086286] doi:10.1016/j.jhep.2014.07.027
2. **World Health Organization.** Hepatitis C. 2015. Accessed at www.who.int/mediacentre/factsheets/fs164/en/ on 24 August 2015.
3. **Ward JW, Mermin JH.** Simple, effective, but out of reach? Public health implications of HCV drugs [Editorial]. N Engl J Med. 2015;373:2678-80. [PMID: 26575359] doi:10.1056/NEJMe1513245
4. **Linas BP, Barter DM, Leff JA, Assoumou SA, Salomon JA, Weinstein MC, et al.** The hepatitis C cascade of care: identifying priorities to improve clinical outcomes. PLoS One. 2014;9:e97317. [PMID: 24842841] doi:10.1371/journal.pone.0097317
5. **Kamal SM.** Acute hepatitis C: a systematic review. Am J Gastroenterol. 2008;103:1283-97. [PMID: 18477352] doi:10.1111/j.1572-0241.2008.01825.x
6. **Global Burden of Disease Study 2013 Collaborators.** Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386:743-800. [PMID: 26063472] doi:10.1016/S0140-6736(15)60692-4
7. **Rongey CA, Kanwal F, Hoang T, Gifford AL, Asch SM.** Viral RNA testing in hepatitis C antibody-positive veterans. Am J Prev Med. 2009;36:235-8. [PMID: 19162434] doi:10.1016/j.amepre.2008.10.013
8. **World Health Organization.** Glasgow Declaration on Hepatitis. 2015. Accessed at www.who.int/hepatitis/glasgow-declaration-on-viral-hepatitis/en on 19 November 2015.
9. **Reed KE, Rice CM.** Overview of hepatitis C virus genome structure, polyprotein processing, and protein properties. Curr Top Microbiol Immunol. 2000;242:55-84. [PMID: 10592656]
10. **Dubuisson J.** Hepatitis C virus proteins. World J Gastroenterol. 2007;13:2406-15. [PMID: 17552023]
11. **Tedder RS, Tuke P, Wallis N, Wright M, Nicholson L, Grant PR.** Therapy-induced clearance of HCV core antigen from plasma predicts an end of treatment viral response. J Viral Hepat. 2013;20:65-71. [PMID: 23231086] doi:10.1111/j.1365-2893.2012.01630.x
12. **Tanaka E, Kiyosawa K, Matsumoto A, Kashiwakuma T, Hasegawa A, Mori H, et al.** Serum levels of hepatitis C virus core protein in patients with chronic hepatitis C treated with interferon alfa. Hepatology. 1996;23:1330-3. [PMID: 8675147]
13. **Forum for Collaborative HIV Research, Foundation for Innovative New Diagnostics.** High-priority target product profile for hepatitis C diagnosis in decentralized settings. 2015. Accessed at www.finddx.org/wp-content/uploads/2016/01/HCV-TPP-Report_17July2015_final.pdf on 8 December 2015.
14. **Devillé WL, Buntinx F, Bouter LM, Montori VM, de Vet HC, van der Windt DA, et al.** Conducting systematic reviews of diagnostic studies: didactic guidelines. BMC Med Res Methodol. 2002;2:9. [PMID: 12097142]
15. **Pai M, McCulloch M, Gorman JD, Pai N, Enanoria W, Kennedy G, et al.** Systematic reviews and meta-analyses: an illustrated, step-by-step guide. Natl Med J India. 2004;17:86-95. [PMID: 15141602]
16. **Eden J, Levit L, Berg A, Morton S, eds; Institute of Medicine Committee on Standards for Systematic Reviews of Comparative Effectiveness.** Finding What Works in Health Care: Standards for Systematic Reviews. Washington, DC: National Academies Pr; 2011.
17. **Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al.** The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Ann Intern Med. 2009;151:W65-94. [PMID: 19622512]
18. **Bürkner PC, Doebler P.** Testing for publication bias in diagnostic meta-analysis: a simulation study. Stat Med. 2014;33:3061-77. [PMID: 24753050] doi:10.1002/sim.6177
19. **Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al; QUADAS-2 Group.** QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155:529-36. [PMID: 22007046] doi:10.7326/0003-4819-155-8-201110180-00009
20. **Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH.** Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol. 2005;58:982-90. [PMID: 16168343]
21. **Harbord R.** metandi: meta-analysis of diagnostic accuracy using hierarchical logistic regression. Stata J. 2009;9:211-29.
22. **Cleveland WS.** Robust locally weighted regression and smoothing scatterplots. J Am Stat Assoc. 1979;74:829-36.
23. **Palmer T, Peters J, Sutton A, Moreno S.** Contour-enhanced funnel plots for meta-analysis. Stata J. 2008;8:242-54.

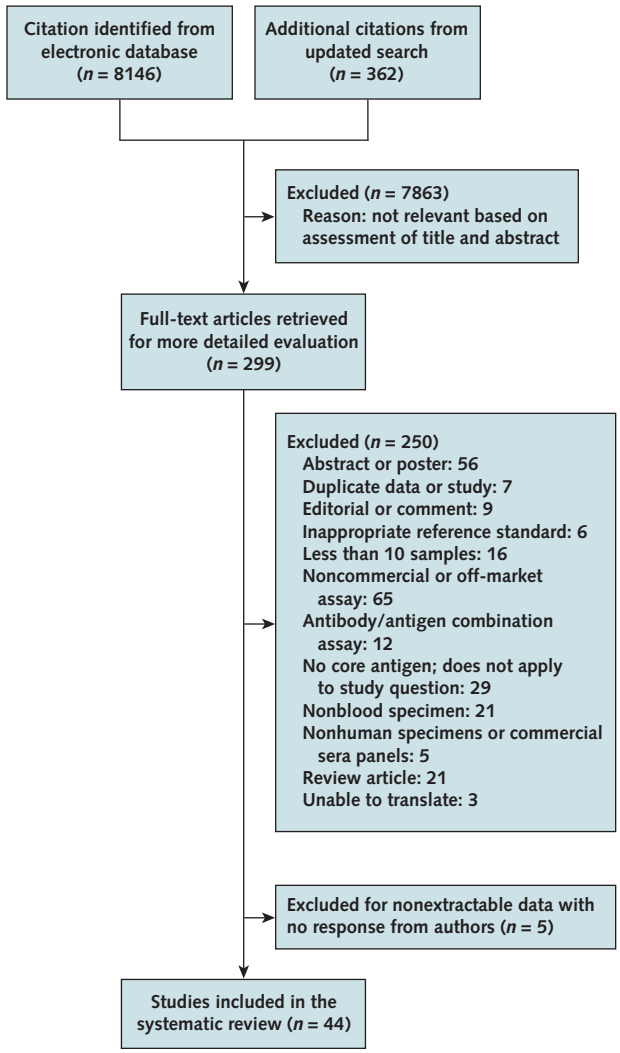
24. **Gu JL, Yu Y, Liang ZL.** Performances of HCV Ag or HCV RNA kits for screening of HCV-infected samples. Chinese Journal of Biologicals. 2014;27:1181-4.
25. **Lu YC, Jiang ZY, Kuang YL, Chen WS, Tan YZ, Li DR.** [Clinical value of the detection of hepatitis C virus core antigen]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2007;23:635-7. [PMID: 17618587]
26. **Ouyang Y, Tan DM, Li TG, Zhou H, Tan C.** [Qualitative detection of hepatitis C virus core antigen in the serum in patients with chronic hepatitis C]. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2006;31:894-6. [PMID: 17213591]
27. **Zhu H, Zhang T, Fan CL, Xiao XG, Zhang FH.** Clinical significance of testing hepatitis C virus core antigen. Journal of Dalian Medical University. 2010;32:211-8.
28. **van Helden J, Weiskirchen R.** [Hepatitis C diagnostics: clinical evaluation of the HCV-core antigen determination]. Z Gastroenterol. 2014;52:1164-70. [PMID: 25313628] doi:10.1055/s-0034-1366618
29. **Ohta H, Takemura M, Furuta N, Akiyama M, Katagiri Y, Ohashi H, et al.** [Clinical significance and problems in HCV measurement–comparison of CLEIA method with PCR method]. Rinsho Byori. 2004; 52:813-8. [PMID: 15624496]
30. **Okazaki K, Nishiyama Y, Saitou T, Shibata N, Yamamoto C, Oosaga J, et al.** [Fundamental evaluation of HCV core antigen method comparison with Cobas Amplicor HCV monitor v2.0 (high range method)]. Rinsho Byori. 2008;56:95-100. [PMID: 18402050]
31. **Kesli R, Polat H, Terzi Y, Kurtoglu MG, Uyar Y.** Comparison of a newly developed automated and quantitative hepatitis C virus (HCV) core antigen test with the HCV RNA assay for clinical usefulness in confirming anti-HCV results. J Clin Microbiol. 2011;49:4089-93. [PMID: 21940466] doi:10.1128/JCM.05292-11
32. **el-Sayed Zaki M, el-Adrosy H.** Recent approach for diagnosis of early HCV infection. Egypt J Immunol. 2004;11:123-9. [PMID: 15724394]
33. **Nübling CM, Unger G, Chudy M, Raia S, Löwer J.** Sensitivity of HCV core antigen and HCV RNA detection in the early infection phase. Transfusion. 2002;42:1037-45. [PMID: 12385416]
34. **Letowska M, Brojer E, Mikulska M, Gronowska A, Rosiek A.** Hepatitis C core antigen in Polish blood donors. Transfusion. 2004;44: 1067-71. [PMID: 15225249]
35. **Zhang HQ, Li SB, Wang GH, Chen K, Song XG, Feng XY.** Detection of hepatitis C virus core antigen for early diagnosis of hepatitis C virus infection in plasma donor in China. World J Gastroenterol. 2007;13:2738-42. [PMID: 17569145]
36. **Saito R, Yokota H, Takahashi E, Mashige F, Yoneyama A, Nakahara K, et al.** Performance of an automated system for quantitation of hepatitis C virus core antigen. J Virol Methods. 2003;112:93-7. [PMID: 12951216]
37. **Murayama A, Sugiyama N, Watashi K, Masaki T, Suzuki R, Aizaki H, et al.** Japanese reference panel of blood specimens for evaluation of hepatitis C virus RNA and core antigen quantitative assays. J Clin Microbiol. 2012;50:1943-9. [PMID: 22495557] doi:10.1128/JCM .00487-12
38. **Buket CA, Ayse A, Selçuk K, Süleyman Ö, Emel SÇ.** Comparison of HCV core antigen and anti-HCV with HCV RNA results. Afr Health Sci. 2014;14:816-20. [PMID: 25834488] doi:10.4314/ahs.v14i4.7
39. **Chevaliez S, Soulier A, Poiteau L, Bouvier-Alias M, Pawlotsky JM.** Clinical utility of hepatitis C virus core antigen quantification in patients with chronic hepatitis C. J Clin Virol. 2014;61:145-8. [PMID: 24973282] doi:10.1016/j.jcv.2014.05.014
40. **Descamps V, Op de Beeck A, Plassart C, Brochot E, François C, Helle F, et al.** Strong correlation between liver and serum levels of hepatitis C virus core antigen and RNA in chronically infected patients. J Clin Microbiol. 2012;50:465-8. [PMID: 22162563] doi:10 .1128/JCM.06503-11
41. **Durante-Mangoni E, Vallefuoco L, Sorrentino R, Iossa D, Perna E, Molaro R, et al.** Clinico-pathological significance of hepatitis C virus core antigen levels in chronic infection. J Med Virol. 2013;85:1913-8. [PMID: 23897630] doi:10.1002/jmv.23672
42. **Thong VD, Akkarathamrongsin S, Avihingsanon A, Theamboonlers A, Poovorawan Y, Tangkijvanich P.** The correlation between hepatitis C core antigen and hepatitis C virus RNA levels with respect to human immunodeficiency virus status, hepatitis C virus genotype and interferon-lambda-4 polymorphism. Intervirology. 2015;58:73-9. [PMID: 25677196] doi:10.1159/000370070
43. **Ergünay K, Sener B, Alp A, Karakaya J, Hasçelik G.** Utility of a commercial quantitative hepatitis C virus core antigen assay in a diagnostic laboratory setting. Diagn Microbiol Infect Dis. 2011;70:486-91. [PMID: 21767705] doi:10.1016/j.diagmicrobio.2011.04.011
44. **Florea D, Neaga E, Nicolae I, Maxim D, Popa M, Otelea D.** Clinical usefulness of HCV core antigen assay for the management of patients with chronic hepatitis C. J Gastrointestin Liver Dis. 2014;23: 393-6. [PMID: 25531997] doi:10.15403/jgld.2014.1121.234.chcv
45. **Garbuglia AR, Monachetti A, Galli C, Sabatini R, Ferreri ML, Capobianchi MR, et al.** HCV core antigen and HCV-RNA in HIV/HCV co-infected patients with different HCV genotypes. BMC Infect Dis. 2014;14:222. [PMID: 24758157] doi:10.1186/1471-2334-14-222
46. **Hadziyannis E, Minopetrou M, Georgiou A, Spanou F, Koskinas J.** Is HCV core antigen a reliable marker of viral load? An evaluation of HCV core antigen automated immunoassay. Ann Gastroenterol. 2013;26:146-149. [PMID: 24714621]
47. **Heidrich B, Pischke S, Helfritz FA, Mederacke I, Kirschner J, Schneider J, et al.** Hepatitis C virus core antigen testing in liver and kidney transplant recipients. J Viral Hepat. 2014;21:769-79. [PMID: 24251818] doi:10.1111/jvh.12204
48. **Kadkhoda K, Smart G.** HCV antigen testing for the diagnosis of hepatitis C infection: a cost-efficient algorithm. Clin Lab. 2014;60: 677-80. [PMID: 24779304]
49. **Kooglu M, Ak S, Ak M, Yakupogullari Y, Ozer A.** Evaluation of diagnostic performance of new antigen-based enzyme immune assay for diagnosis of hepatitis C virus (HCV) infections. Afr J Microbiol Res. 2012;6:809-12.
50. **Kuo YH, Chang KC, Wang JH, Tsai PS, Hung SF, Hung CH, et al.** Is hepatitis C virus core antigen an adequate marker for community screening? J Clin Microbiol. 2012;50:1989-93. [PMID: 22461676] doi: 10.1128/JCM.05175-11
51. **Li Cavoli G, Zagarrigo C, Schillaci O, Servillo F, Tralongo A, Coglitore M, et al.** Hepatitis C virus core antigen test in monitoring of dialysis patients. Hepat Res Treat. 2012;2012:832021. [PMID: 23304475] doi:10.1155/2012/832021
52. **Mederacke I, Ciesek S, Raupach R, Wursthorn K, Deterding K, Steinmann E, et al.** Kinetics of HCV core antigen during antiviral treatment of acute and chronic hepatitis C as determined by a novel chemiluminescent microparticle immunoassay. Journal of Hepatology. 2009;50:S129.
53. **Mederacke I, Potthoff A, Meyer-Olson D, Meier M, Raupach R, Manns MP, et al.** HCV core antigen testing in HIV- and HBV-coinfected patients, and in HCV-infected patients on hemodialysis. J Clin Virol. 2012;53:110-5. [PMID: 22177274] doi:10.1016/j.jcv.2011 .11.009
54. **Medici MC, Furlini G, Rodella A, Fuertes A, Monachetti A, Calderaro A, et al.** Hepatitis C virus core antigen: analytical performances, correlation with viremia and potential applications of a quantitative, automated immunoassay. J Clin Virol. 2011;51:264-9. [PMID: 21621454] doi:10.1016/j.jcv.2011.05.003
55. **Miedouge M, Saune K, Kamar N, Rieu M, Rostaing L, Izopet J.** Analytical evaluation of HCV core antigen and interest for HCV screening in haemodialysis patients. J Clin Virol. 2010;48:18-21. [PMID: 20233674] doi:10.1016/j.jcv.2010.02.012
56. **Ottiger C, Gygli N, Huber AR.** Detection limit of ARCHITECH hepatitis C core antigen assay in correlation with HCV RNA, and renewed confirmation algorithm for reactive anti-HCV samples. J Clin Virol. 2013;58:535-40. [PMID: 24041472] doi:10.1016/j.jcv.2013.08 .028
57. **Park Y, Lee JH, Kim BS, Kim do Y, Han KH, Kim HS.** New automated hepatitis C virus (HCV) core antigen assay as an alternative to real-time PCR for HCV RNA quantification. J Clin Microbiol. 2010;48: 2253-6. [PMID: 20351215] doi:10.1128/JCM.01856-09
58. **Reyes-Méndez MÁ, Juárez-Figueroa L, Iracheta-Hernández P, Medina-Islas Y, Ruiz-González V.** Comparison of two diagnostic algorithms for the identification of patients with HCV viremia using a

new HCV antigen test. Ann Hepatol. 2014;13:337-42. [PMID: 24756008]
59. **Rouet F, Deleplancque L, Mboumba BB, Sica J, Mouinga-Ondémé A, Liégeois F, et al.** Usefulness of a fourth generation ELISA assay for the reliable identification of HCV infection in HIV-positive adults from Gabon (Central Africa). PLoS One. 2015;10:e0116975. [PMID: 25617896] doi:10.1371/journal.pone.0116975
60. **Russi S, Sansonno D, Mariggiò MA, Vinella A, Pavone F, Lauletta G, et al.** Assessment of total hepatitis C virus (HCV) core protein in HCV-related mixed cryoglobulinemia. Arthritis Res Ther. 2014;16: R73. [PMID: 24636026] doi:10.1186/ar4513
61. **Vanhommerig JW, van de Laar TJ, Koot M, van Rooijen MS, Schinkel J, Speksnijder AG, et al.** Evaluation of a hepatitis C virus (HCV) antigen assay for routine HCV screening among men who have sex with men infected with HIV. J Virol Methods. 2015;213:147-50. [PMID: 25528203] doi:10.1016/j.jviromet.2014.11.026
62. **Vermehren J, Susser S, Berger A, Perner D, Peiffer KH, Zeuzem S, et al.** Clinical utility of the ARCHITECT HCV Ag assay for early treatment response monitoring in patients with chronic hepatitis C genotype 1 infection. Journal of Hepatology. 2012;56:S461.
63. **Medici MC, Chezzi C, Conto FD, Ferraglia F, Pinardi F, Arcangeletti MC, et al.** Evolving strategy for HCV testing in an Italian tertiary care hospital. J Clin Virol. 2016;77:92-8. [PMID: 26921741] doi: 10.1016/j.jcv.2016.02.017
64. **Cresswell FV, Fisher M, Hughes DJ, Shaw SG, Homer G, Hassan-Ibrahim MO.** Hepatitis C core antigen testing: a reliable, quick, and potentially cost-effective alternative to hepatitis C polymerase chain reaction in diagnosing acute hepatitis C virus infection. Clin Infect Dis. 2015;60:263-6. [PMID: 25301216] doi:10.1093/cid/ciu782
65. **Mixson-Hayden T, Dawson GJ, Teshale E, Le T, Cheng K, Drobeniuc J, et al.** Performance of ARCHITECT HCV core antigen test with specimens from US plasma donors and injecting drug users. J Clin Virol. 2015;66:15-8. [PMID: 25866329] doi:10.1016/j.jcv.2015.02.015
66. **Agha S, Tanaka Y, Saudy N, Kurbanov F, Abo-Zeid M, El-Malky M, et al.** Reliability of hepatitis C virus core antigen assay for detection of viremia in HCV genotypes 1, 2, 3, and 4 infected blood donors: a collaborative study between Japan, Egypt, and Uzbekistan. J Med Virol. 2004;73:216-22. [PMID: 15122795]
67. **Gu S, Liu J, Zhang H, Gu B, Lai H, Zhou H, et al.** Core antigen tests for hepatitis C virus: a meta-analysis. Mol Biol Rep. 2012;39: 8197-208. [PMID: 22544611] doi:10.1007/s11033-012-1667-z
68. **Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al.** Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;61:77-87. [PMID: 25069599] doi:10.1002/hep.27259
69. **Cohn J, Roberts T, Amorosa V, Lemoine M, Hill A.** Simplified diagnostic monitoring for hepatitis C, in the new era of direct-acting antiviral treatment. Curr Opin HIV AIDS. 2015;10:369-73. [PMID: 26185920] doi:10.1097/COH.0000000000000180
70. **Sidharthan S, Kohli A, Sims Z, Nelson A, Osinusi A, Masur H, et al.** Utility of hepatitis C viral load monitoring on direct-acting antiviral therapy. Clin Infect Dis. 2015;60:1743-51. [PMID: 25733369] doi:10.1093/cid/civ170
71. **Kamal SM, Kassim S, El Gohary E, Fouad A, Nabegh L, Hafez T, et al.** The accuracy and cost-effectiveness of hepatitis C core antigen assay in the monitoring of anti-viral therapy in patients with chronic hepatitis C genotype 4. Aliment Pharmacol Ther. 2015;42:307-18. [PMID: 26018116] doi:10.1111/apt.13261
72. **Médecins Sans Frontières.** Putting HIV and HCV to the Test: A Product Guide for Point-of-Care CD4 and Laboratory-Based and Point-of-Care Virological HIV and HCV Tests. Geneva: Médecins Sans Frontières; 2015.

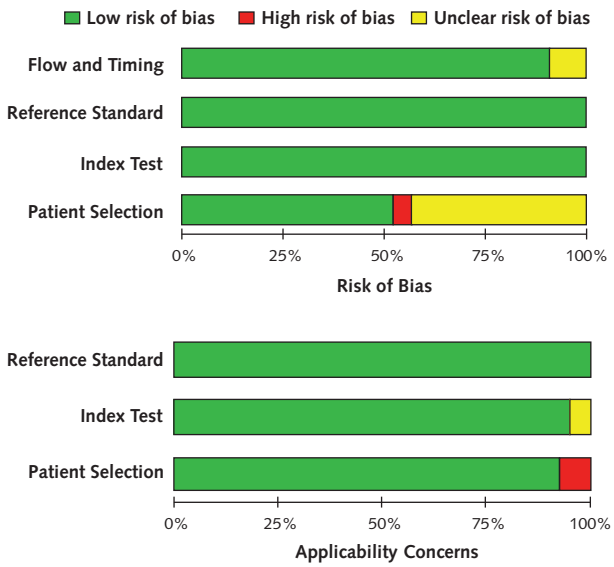
Current Author Addresses: Dr. Freiman: One Boston Medical Center Place, Dowling 3 North, Boston, MA 02118.
Dr. Cohn and Ms. Tran: Médecins Sans Frontières Access Campaign, Rue de Lausanne 78, PO Box 116, CH-1211 Geneva 21, Switzerland.
Drs. Schumacher, Ongarello, and Denkinger: Campus Biotech, Chemin des Mines 9, 1202 Geneva, Switzerland.
Dr. White: Department of Biostatistics, Boston University School of Public Health, Crosstown Building, 801 Massachusetts Avenue, 3rd Floor, Boston, MA 02118.
Dr. Easterbrook: HIV/AIDS Department, World Health Organization, Department 20, Avenue Appia, CH-1211 Geneva 27, Switzerland.
Dr. Linas: Section of Infectious Diseases, Boston University School of Medicine, Crosstown Building, 801 Massachusetts Avenue, 2nd Floor, Boston, MA 02118.

Author Contributions: Conception and design: J.M. Freiman, S.G. Schumacher, J. Cohn, P.J. Easterbrook, C.M. Denkinger.
Analysis and interpretation of the data: J.M. Freiman, S.G. Schumacher, S. Ongarello, J. Cohn, P.J. Easterbrook, C.M. Denkinger.
Drafting of the article: J.M. Freiman, T.M. Tran, S.G. Schumacher, C.M. Denkinger.
Critical revision of the article for important intellectual content: J.M. Freiman, T.M. Tran, S.G. Schumacher, S. Ongarello, J. Cohn, P.J. Easterbrook, C.M. Denkinger.
Final approval of the article: J.M. Freiman, T.M. Tran, S.G. Schumacher, S. Ongarello, J. Cohn, P.J. Easterbrook, C.M. Denkinger.
Provision of study materials or patients: C.M. Denkinger.
Statistical expertise: S.G. Schumacher, L.F. White, S. Ongarello, C.M. Denkinger.
Administrative, technical, or logistic support: T.M. Tran, C.M. Denkinger.
Collection and assembly of data: J.M. Freiman, T.M. Tran, P.J. Easterbrook, C.M. Denkinger.

Appendix Figure 1. Flow diagram of included studies.

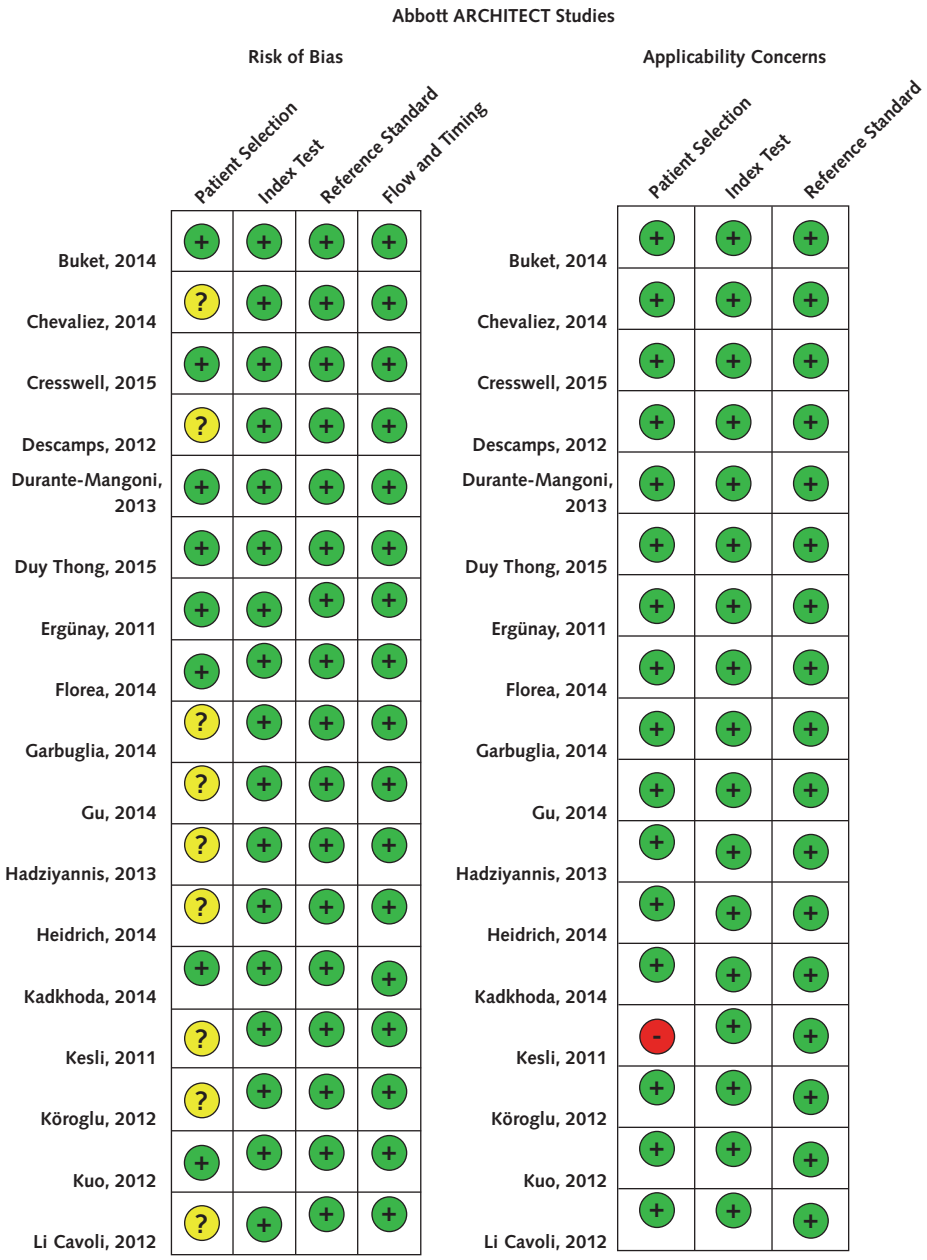


Appendix Figure 2. Risk of bias and applicability summary as judged by reviewers about each QUADAS-2 domain.



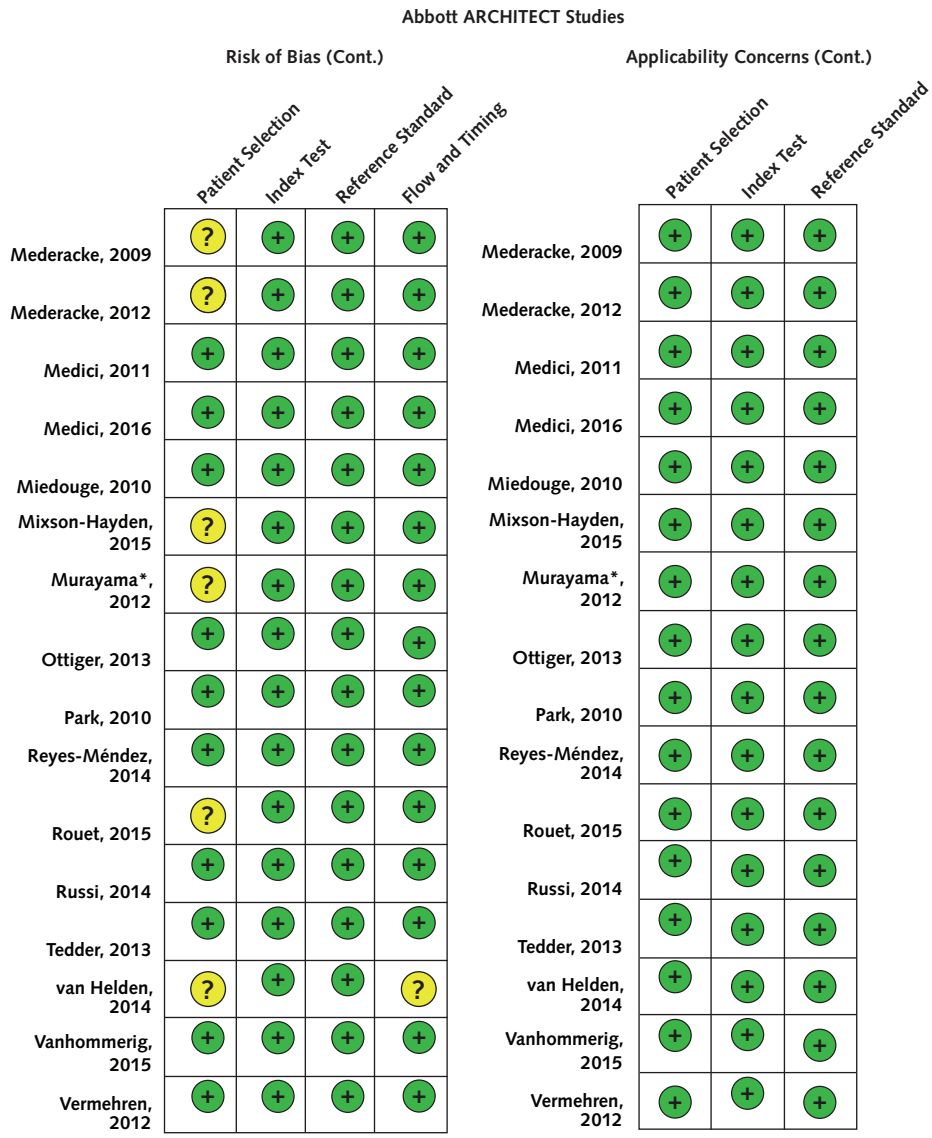
QUADAS-2 = Quality Assessment of Diagnostic Accuracy Studies 2.

Appendix Figure 3. Risk of bias and applicability summary as judged by reviewers about each QUADAS-2 domain, by individual study.



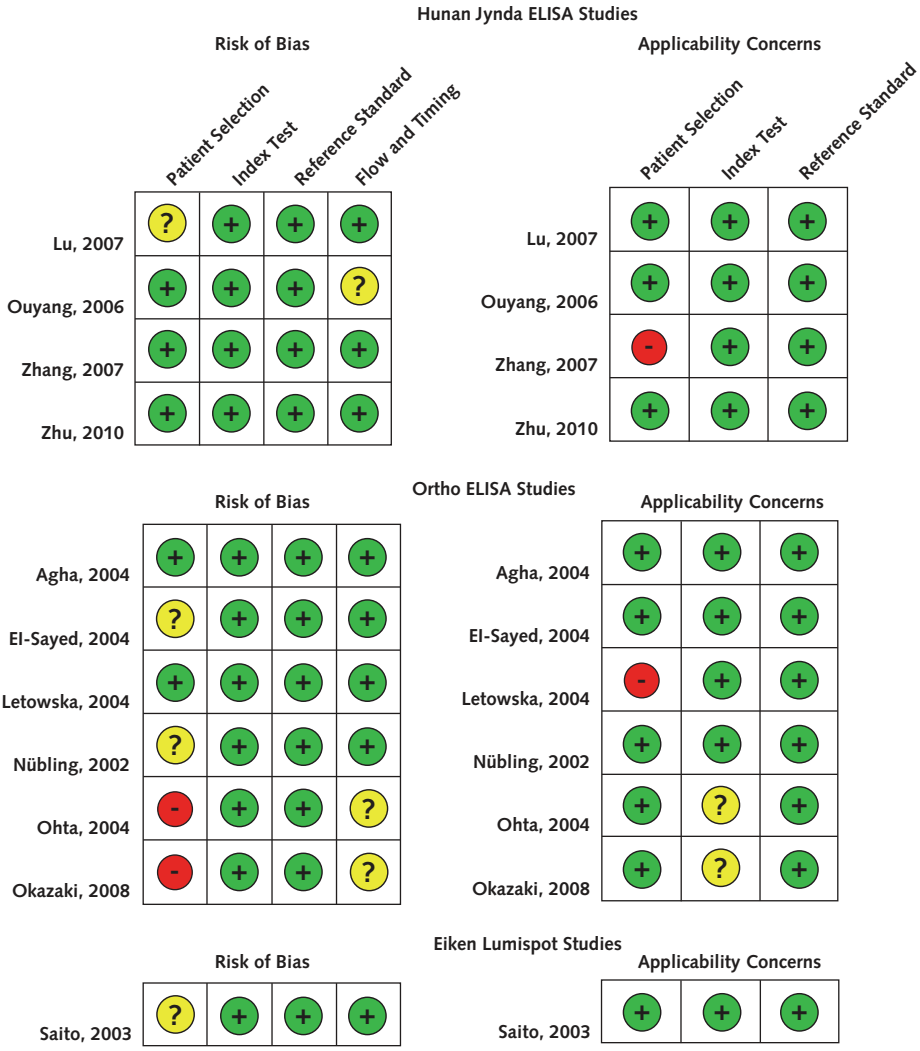
Continued on following page

Appendix Figure 3–Continued



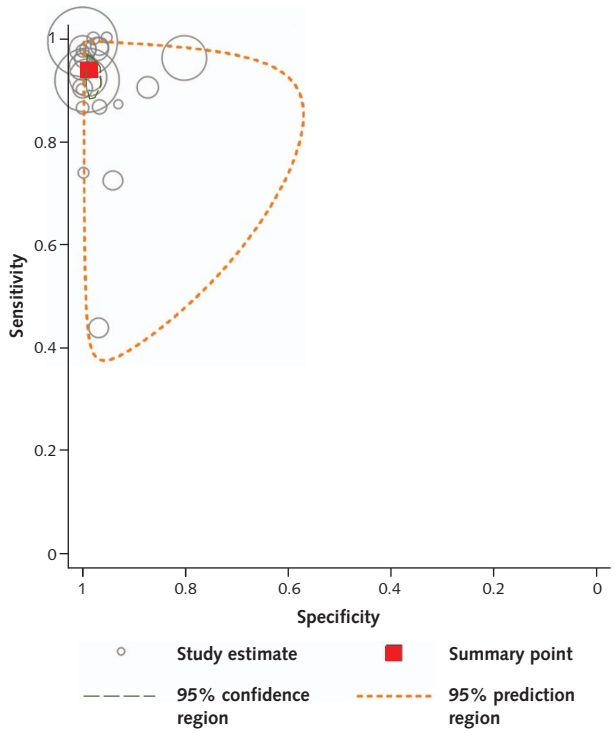
Continued on following page

Appendix Figure 3–Continued



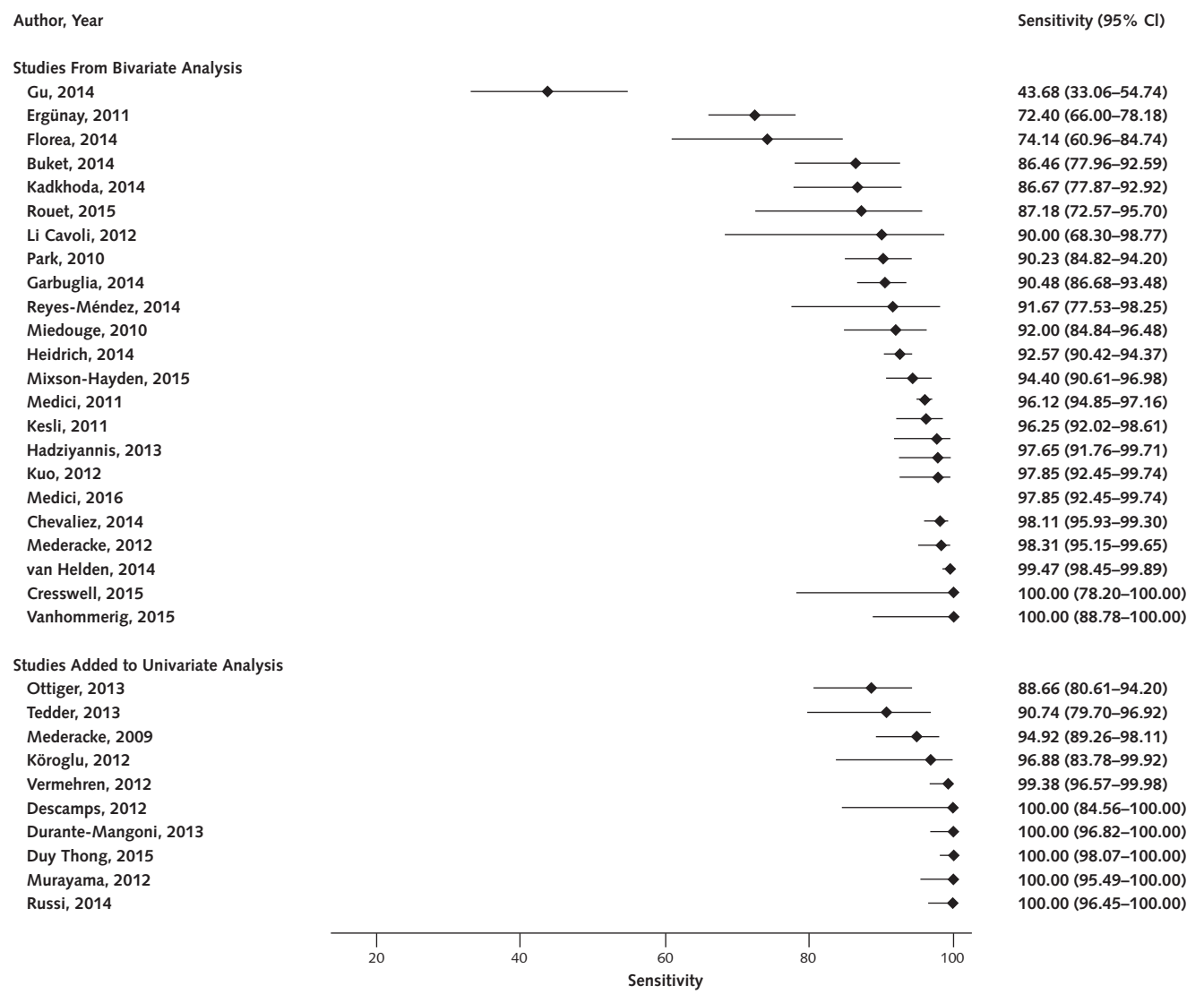
Green circles with a plus sign indicate low risk of bias. Yellow circles with a question mark indicate unclear risk of bias. Red circles with a minus sign indicate high risk of bias. Abbott ARCHITECT = Abbott ARCHITECT HCV Ag assay; Ag = antigen; Eiken Lumispot = Eiken Lumispot HCV Ag assay; HCV = hepatitis C virus; Hunan Jynda ELISA = Hunan Jynda Bioengineering Group HCV Ag ELISA; Ortho ELISA = Ortho HCV Ag ELISA; QUADAS-2 = Quality Assessment of Diagnostic Accuracy Studies 2.
* Same data for Fujirebio Lumipulse Ortho HCV Ag assay and Eiken Lumispot.

Appendix Figure 4. Bivariate analysis of Abbott ARCHITECT's sensitivity and specificity for diagnosis of active HCV infection compared with NAT.



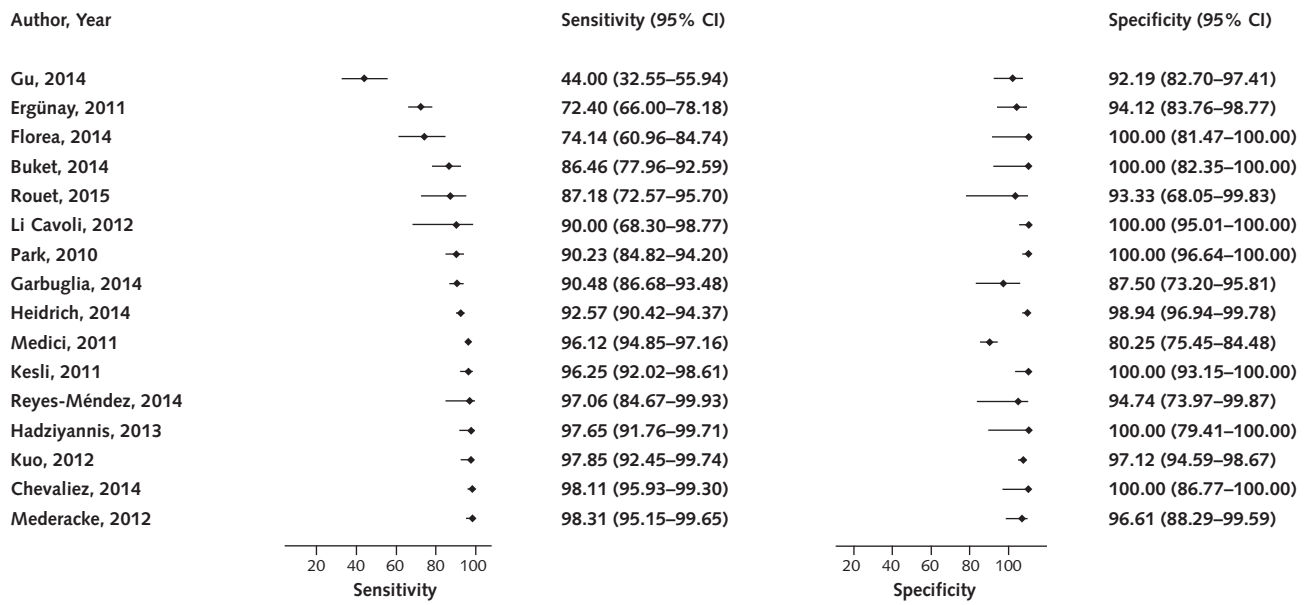
This plot shows the pooled summary estimate (*red square*). The dotted orange line represents the 95% confidence region, and the dashed green line represents the 95% prediction region. The individual circles represent each study, and the size of the circle is proportional to the total sample size. Abbott ARCHITECT = Abbott ARCHITECT HCV Ag assay; Ag = antigen; HCV = hepatitis C virus; NAT = nucleic acid testing.

Appendix Figure 5. Univariate analysis of Abbott ARCHITECT's sensitivity for the diagnosis of active HCV infection compared with NAT for all studies with sensitivity data, regardless of anti-HCV status.



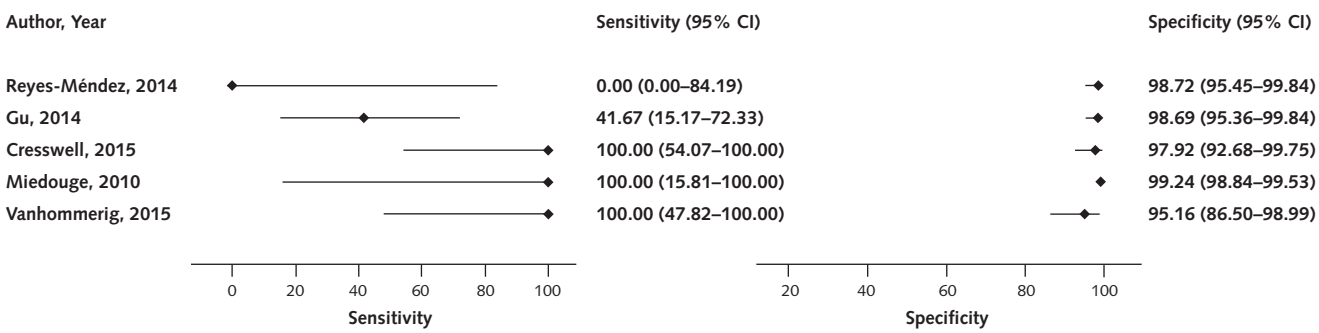
Abbott ARCHITECT = Abbott ARCHITECT HCV Ag assay; Ag = antigen; anti-HCV = antibody to hepatitis C virus; HCV = hepatitis C virus; NAT = nucleic acid testing.

Appendix Figure 6. Forest plot of Abbott ARCHITECT's sensitivity and specificity for the diagnosis of active HCV infection compared with NAT for samples that are positive for anti-HCV.



Abbott ARCHITECT = Abbott ARCHITECT HCV Ag assay; Ag = antigen; anti-HCV = antibody to hepatitis C virus; HCV = hepatitis C virus; NAT = nucleic acid testing.

Appendix Figure 7. Forest plot of Abbott ARCHITECT's sensitivity and specificity for the diagnosis of active HCV infection compared with NAT for samples that are negative for anti-HCV.



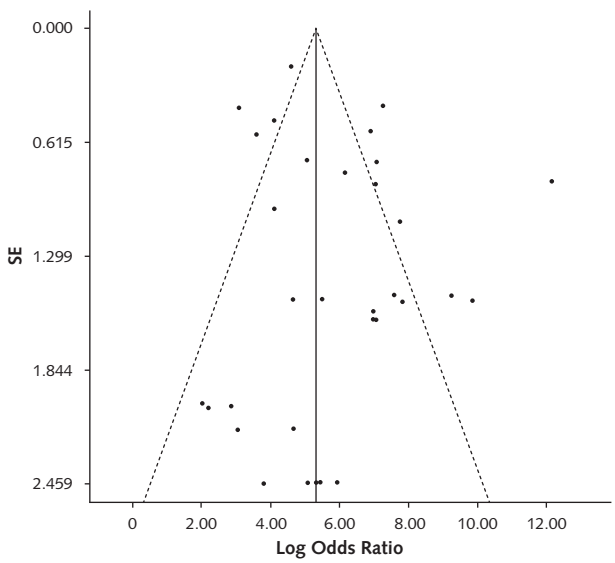
Abbott ARCHITECT = Abbott ARCHITECT HCV Ag assay; Ag = antigen; anti-HCV = antibody to hepatitis C virus; HCV = hepatitis C virus; NAT = nucleic acid testing.

Appendix Table. Available Genotype Information for Included Studies Grouped Alphabetically, by Index Test Type*

Author, Year (Reference)	Participants, *n*	G1	G1a	G1b	G2	G3	G4	G5	G6	Other or Unknown
Abbott ARCHITECT HCV Ag										
Chevaliez et al, 2014 (39)	514	59.3	–	–	5.0	12.3	19.2	1.0	1.9	1.9
Cresswell et al, 2015 (64)	111	–	73.0	7.0	–	13.0	7.0	–	–	–
Descamps et al, 2012 (40)	22	68.2	–	–	–	–	–	–	–	31.8
Durante-Mangoni et al, 2013 (41)	114	49.0	–	–	31.0	20.0	–	–	–	–
Ergünay et al, 2011 (43)	272	0.8	2.2	60.2	–	0.4	0.4	–	–	35.8
Garbuglia et al, 2014 (45)	292	17.1	14.5	9.4	1.0	27.6	15.4	–	–	15.0
Hadziyannis et al, 2013 (46)	105	36.0	–	–	6.0	37.0	21.0	–	–	–
Kadkhoda et al, 2014 (48)	154	30.0	–	–	30.0	30.0	10.0	–	–	–
Kesli et al, 2011 (31)	212	–	–	100.0	–	–	–	–	–	–
Li Cavoli et al, 2012 (51)	92	95.0	–	–	5.0	–	–	–	–	–
Mederacke et al, 2009 (52)	118	45.8	–	–	10.0	19.0	–	–	–	24.6
Miedouge et al, 2010 (55)	2850	2.0	8.2	17.3	15.3	17.3	11.2	8.2	3.1	17.3
Ottiger et al, 2013 (56)	97	30.9	19.5	10.3	23.7	15.5	–	–	–	30.9
Russi et al, 2014 (60)	102	50.0	–	–	48.1	1.9	–	–	–	–
Thong et al, 2015 (42)	189	35.4	–	–	0	44.9	–	–	19.6	–
Tedder et al, 2013 (11)	54	–	40.7	22.2	20.4	16.7	–	–	–	–
Vermehren et al, 2012 (62)	160	19.0	29.0	51.0	–	–	–	–	–	–
Eiken Lumispot HCV Ag										
Saito et al, 2003 (36)	155	65.2	–	–	35.80	–	–	–	–	–
Ortho HCV ELISA										
Agha et al, 2004 (66)	246	37.0	–	–	9.8	5.8	47.3	–	–	–
Nübling et al, 2002 (33)	52	11.5	42.3	19.2	11.5	15.4	–	–	–	–

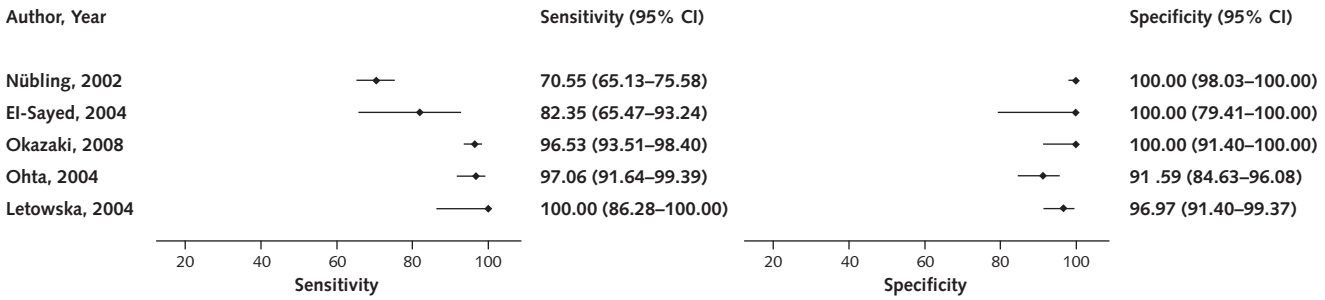
Ag = antigen; ELISA = enzyme-linked immunosorbent assay; G = genotype; HCV = hepatitis C virus.
* Values are percentages unless otherwise indicated.

Appendix Figure 8. Funnel plot of published studies that used Abbott ARCHITECT.



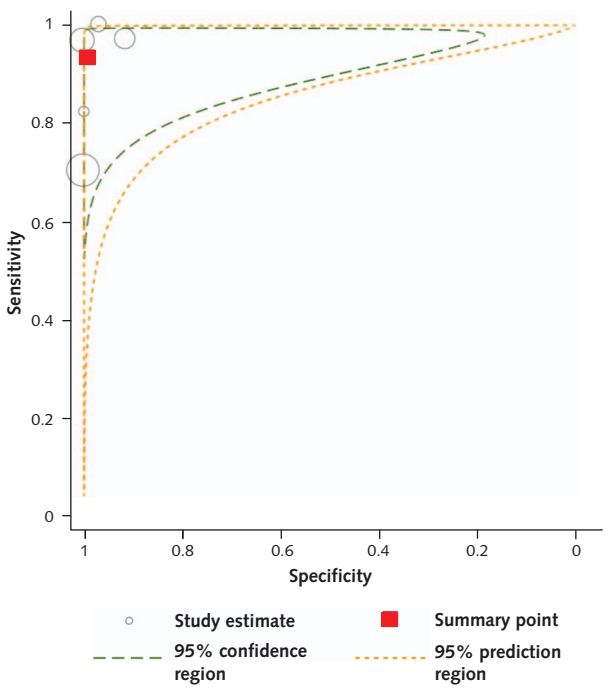
Abbott ARCHITECT = Abbott ARCHITECT HCV Ag assay; Ag = antigen; HCV = hepatitis C virus.

Appendix Figure 9. Forest plot of the Ortho ELISA's sensitivity and specificity for diagnosis of active HCV infection compared with NAT for all samples, regardless of anti-HCV status.



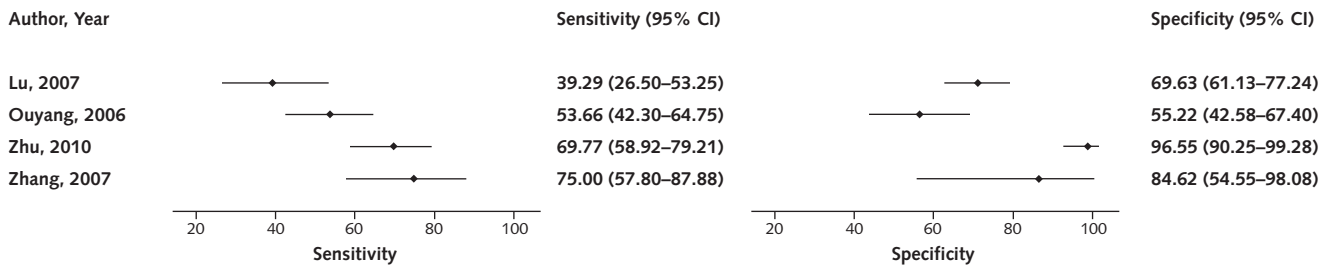
Ag = antigen; anti-HCV = antibody to hepatitis C virus; ELISA = enzyme-linked immunosorbent assay; HCV = hepatitis C virus; NAT = nucleic acid testing; Ortho ELISA = Ortho HCV Ag ELISA.

Appendix Figure 10. Bivariate analysis of the Ortho ELISA's sensitivity and specificity for diagnosis of active HCV infection compared with NAT.



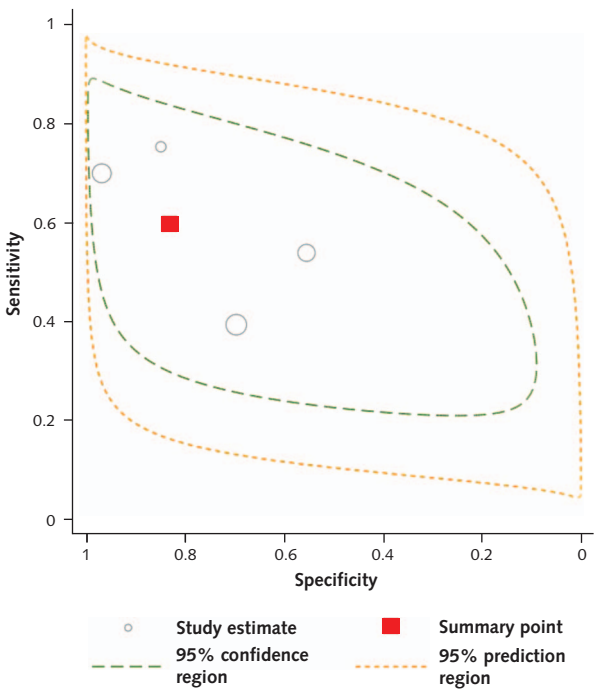
This plot shows the pooled summary estimate (*red square*). The dotted orange line represents the 95% confidence region, and the dashed green line represents the 95% prediction region. The individual circles represent each study, and the size of the circle is proportional to the total sample size. Ag = antigen; ELISA = enzyme-linked immunosorbent assay; HCV = hepatitis C virus; NAT = nucleic acid testing; Ortho ELISA = Ortho HCV Ag ELISA.

Appendix Figure 11. Forest plot of the Hunan Jynda ELISA's sensitivity and specificity for diagnosis of active HCV infection compared with NAT for all samples, regardless of anti-HCV status.



Ag = antigen; anti-HCV = antibody to hepatitis C virus; ELISA = enzyme-linked immunosorbent assay; HCV = hepatitis C virus; Hunan Jynda ELISA = Hunan Jynda Bioengineering Group HCV Ag ELISA; NAT = nucleic acid testing.

Appendix Figure 12. Bivariate analysis of the Hunan Jynda ELISA's sensitivity and specificity for diagnosis of active HCV infection compared with NAT.



This plot shows the pooled summary estimate (*red square*). The dotted orange line represents the 95% confidence region, and the dashed green line represents the 95% prediction region. The individual circles represent each study, and the size of the circle is proportional to the total sample size. Ag = antigen; ELISA = enzyme-linked immunosorbent assay; HCV = hepatitis C virus; Hunan Jynda ELISA = Hunan Jynda Bioengineering Group HCV Ag ELISA; NAT = nucleic acid testing.