Criteria Grid
Best Practices and Interventions for the Diagnosis and Treatment of Hepatitis C

Best Practice/Intervention:	Gu S. et al. (2012) Core antigen tests for hepatitis C virus: a meta-analysis. *Molecular Biology Reports*, 39(8):8197-8208.
Date of Review:	February 15, 2015
Reviewer(s):	Christine Hu

Part A

Category:	**Basic Science** ☐ **Clinical Science** ☐ **Public Health/Epidemiology** ☐ **Social Science** ☐ **Programmatic Review** ☒
Best Practice/Intervention:	**Focus:** **Hepatitis C** ☒ **Hepatitis C/HIV** ☐ **Other:** _____ **Level:** **Group** ☒ **Individual** ☐ **Other:** _____ **Target Population:** **HCV patients** **Setting:** **Health care setting/Clinic** ☒ **Home** ☐ **Other:** _____ **Country of Origin:** **China** **Language:** **English** ☒ **French** ☐ **Other:** _____

Part B

	YES	NO	N/A	COMMENTS
Is the best practice/intervention a meta-analysis or primary research?	☒	☐	☐	meta-analysis; systematic review to analyze accuracy of HCV core antigen (cAg) assay to evaluate its potential clinical use in HCV diagnosis and management
The best practice/intervention has utilized an evidence-based approach to assess:				
Efficacy	☐	☒	☐	
Effectiveness	☒	☐	☐	Analyzed the diagnosis accuracy of HCV-cAg assay
The best practice/intervention has been evaluated in more than one patient setting to assess:				
Efficacy	☐	☒	☐	

Effectiveness	☒	☐	☐	25 included studies

	YES	NO	N/A	COMMENTS
The best practice/intervention has been operationalized at a multi-country level:	☒	☐	☐	Studies originated from 12 different countries
There is evidence of capacity building to engage individuals to accept treatment/diagnosis	☐	☐	☒	
There is evidence of outreach models and case studies to improve access and availability	☐	☐	☒	
Do the methodology/results described allow the reviewer(s) to assess the generalizability of the results?	☒	☐	☐	Methodology clearly stated
Are the best practices/methodology/results described applicable in developed countries?	☐	☒	☐	HCV-cAg detection kit has not been approved by FDA
Are the best practices/methodology/results described applicable in developing countries?	☒	☐	☐	HCV-cAg assay is less expensive then PCR testing
Evidence of manpower requirements is indicated in the best practice/intervention	☐	☒	☐	
Juried journal reports of this treatment, intervention, or diagnostic test have occurred	☒	☐	☐	*Molecular Biology Reports*
International guideline or protocol has been established	☐	☒	☐	
The best practice/intervention is easily accessed/available electronically	☐	☒	☐	Available for download with a cost at http://link.springer.com/
Is there evidence of a cost effective analysis? If so, what does the evidence say? ***Please go to Comments section***	☐	☒	☐	
How is the best practice/intervention funded? ***Please got to Comments section***	☒	☐	☐	The study was supported by Project funded by Science and Technology Bureau of Hangzhou City
Other relevant information: _____	☐	☐	☐	- HCV-cAg detection is a promising method as a confirmatory test for HCV antibody positive, therapy-naive individuals

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Core antigen tests for hepatitis C virus: a meta-analysis

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Abstract Diagnosis and monitoring of hepatitis C virus (HCV) infection relies mainly on the detection of HCV antibodies and HCV RNA. HCV antibody test has a longer window period and is not applicable in the immunosuppressed population. Although HCV RNA test reduces the window period, it is still not widely recommended because of its high cost and requirement of specific equipment. HCV core antigen is another direct virological marker which has been investigated in recent years. HCV core antigen assay is as simple as the HCV antibodies assay and can detect HCV infection only 1 day delay compared to the HCV RNA assay. In order to evaluate the application of HCV core antigen test in HCV diagnosis and management, we performed this meta-analysis. Twenty five articles were finally included in meta-analysis. All statistical analyses were performed with MetaDisc 1.4 and Stata 11.0. The pooled sensitivity of HCV core antigen assay was 0.84 (95 % CI, 0.83–0.85), and the pooled specificity was 0.98 (95 % CI, 0.97–0.98). HCV core antigen assays may not displace HCV RNA assays to be a definitive diagnosis of HCV infection until now. Considering the higher sensitivity (0.926) and specificity (0.991) of subgroup, HCV-cAg detection is a promising method as a confirmatory test for HCV antibody positive, therapy-naive individuals. Explored by meta-regression and subgroup analysis, possible sources of heterogeneity of specificity was found, while the heterogeneity of sensitivity was still significant.

Keywords Hepatitis C virus · Core antigen · Antibody · Diagnosis · Meta-analysis

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Introduction

Hepatitis C virus (HCV) is a parenterally transmitted hepatitis virus infecting approximately 3 % of the world population[1].The severity of disease varies widely from mild illness to cirrhosis and hepatocellular carcinoma. HCV is a single-strand RNA virus belonging to the Flaviviridae family. Its genome is contained in an icosahedral capsid formed by polymerization of the HCV core protein which is antigenic [2]. Anti-HCV (antibodies to HCV), HCV RNA and HCV-cAg (HCV core antigen) are three of the virological markers, which are currently used to diagnose and monitor HCV infection [3]. Serologic assay of anti-HCV has been developed for more than 10 years and become the most widely used diagnostic method because of the simplicity and rapidity. However, it has been found that the excellent specificity and sensitivity of this method were only observed in immunocompetent patients with active viral replication [4, 5]. Anti-HCV can be negative in small proportion of hemodialysis and profoundly immunodeficient patients despite ongoing HCV replication [6]. Furthermore, the serological window, estimated to be approximately 60 days on average, makes it difficult to discover the pre-seroconversion infection [7]. Blood transfusion is considered as an effective route for HCV

transmission, so a more effective method to screen blood donors is crucial to prevent further transmission, especially in high-risk population. The detection of HCV RNA carried out using nucleic acid amplification testing (NAT) is efficient for diagnosis in the early stage of HCV infection and regarded as reference standard, however, other limits such as requirement for specific equipment, a long delay in the time to result, the risk of contaminations, and high cost preclude its use as routine screening tools, especially in developing country [8]. The average price paid by the US Veterans Administration for EIA-OD (enzyme immunoassay-optical density) was $7 per test, while PCR was $65 per test [9]. HCV-cAg is another direct marker of HCV infection. HCV-cAg becomes positive only 1 day later than HCV-RNA, reducing the long period of HCV seronegativity. HCV-cAg levels closely track HCV RNA dynamics, and quantification might be used to follow patients and predict the response to interferon therapy [6]. The use of a HCV core antigen assay for the screening of plasma donors would provide an added level of redundancy to antibody and NAT screening. However, no HCV-cAg detection kit has been approved by FDA. In China, due to logistics and cost NAT screening, only detection of anti-HCV is mandatory in blood screening. The safety of the blood supply would increase in a cost-effective manner if HCV cAg was screened routinely. Thus we systematically reviewed the studies that analyzed the accuracy of HCV cAg assay to evaluate its potential clinical use in HCV diagnosis and management.

Methods

Search methods for identification of studies

Two investigators independently searched medline, embase, cochrane library, China National Knowledge Infrastructure (CNKI), and Wangfang data for the studies in English and Chinese using the search terms HCV, hepatitis C, core antigen, diagnosis, and diagnostic test. The studies were published from 1989 to 2011. Patents, reviews and duplicate reports were eliminated. The text of relevant citations and their bibliographies were reviewed.

Criteria for considering studies for this review

We identified all relevant HCV diagnostic test if the following criteria were met: HCV-RNA detection was used as a "gold standard" no matter of method and commercial brand; HCV cAg detection was used as index reference, any method was acceptable, including chemiluminescence immunoassay(CLIA), enzyme-linked immunosorbent assay (ELISA) and trak-C; the studies should give the absolute data of true positive(TP), false positive (FP), true negative (TN) and false negative (FN), or they can be obtained from the reported data; the patient could be representative for susceptible population including HIV-infected individuals, patients on hemodialysis, intravenous drug users, blood donors, or HCV patients during treatment; criteria for diagnosis were described explicitly; all the involved samples should be diagnosed by both gold standard and index reference.

Data extraction and assessment of study quality

Data were abstracted for study design, study size, information on the original sample source, type and brand name of HCV cAg test, infection phase of patients, numbers of TP, FP, TN and FN. We excluded samples diagnosed by HCV cAg only. We also excluded duplicated data.

We rated quality using QUADAS method. The tool assesses in the following 14 domains: patient representativeness, selection criteria clarity, reference standard, duration between test and reference standard, verification bias, completeness of verification, consistency of verification, completeness of index, completeness of reference test descriptions, blinding of reference, index test results, similarity to practice, uninterpretable tests, and withdrawals. In addition, we rated whether the total number of reference standard results was equal to the number of patients [10].

Statistical analysis and data synthesis

Statistical analysis was performed by MetaDisc 1.4 and Stata 11.0. We pooled sensitivity and specificity with a random-effects model. We explained heterogeneity from threshold effect, different patients spectrum, commercial brand, study quality and application area. The threshold effect was explored by computing Spearman correlation coefficient between the logit of sensitivity and logit of 1-specificity. The meta regression was implemented using Littenberg and Moses Linear model weighted by inverse of the variance [11, 12]. Egger's linear regression test was used to assess the possibility of publication bias [13].

Results

Study characteristics

The electronic database searches identified 668 citations. Ninety full articles among these were selected for detailed analysis on the basis of title or abstract. Twenty five articles met the inclusion criteria. Retrieval and inclusion flow is shown in Fig. 1.

The study characteristics of the 25 included studies are listed in Table 1. Patient demographic information

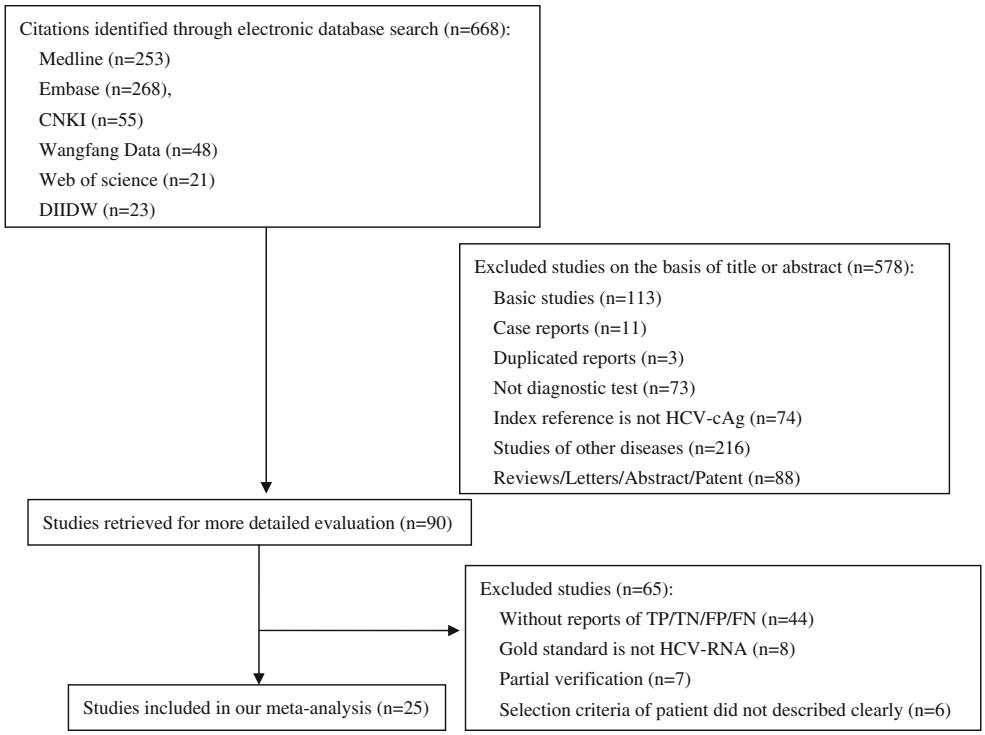

Fig. 1 Flow chart of studies through the retrieval and inclusion process in the meta-analysis

generally was not provided. Only five studies [14–18] reported the age of the subjects, they were aged from age 5 ~ 82, and four studies [14, 15, 17, 18] reported gender of patients. Patients were mainly blood donors, or those on hemodialysis and anti-viral therapy, while two studies [14, 16] chose HCV patients and health individuals as control. Study [19just told the samples were from clinic. Most studies did not provide infection phase, only study [20] and study [21] gave the information that the patients were in preseroconversion window period. In studies [1, 22–29], HCV-cAg was used as a marker of HCV replication in anti-HCV positive, therapy-naive individuals. In studies [30–32], HCV-cAg level was used as a quantitative marker of HCV replication and monitoring of antiviral therapy. And HCV-cAg test was used as HCV screening in other studies [14–21, 33–37Among the 25 included studies, two kinds of methods were used to detect HCV cAg, they were ELISA and CLIA. Most of the HCV cAg test kits were purchased from ortho clinical diagnostics, ABBOTT diagnostics and Hunan JYNDA, while study [23] used the kit made by themselves and study [19] did not mention the brand of kit they used. The HCV RNA diagnostic kits include HCV Monitor Test procedure version 1.2, COBAS AmpliPrep®/COBAS TaqMan® HCV kit and AMPLICOR HCV test version 2.0 from Roche diagnostics, quantitative VERSANT HCV assay from Bayer healthcare and other in-house RT-PCR and realtime-PCR. Studies originated from 12 different countries, China ($n = 8$), France ($n = 3$), Japan ($n = 3$), India ($n = 3$), Italy($n = 2$), Scottish ($n = 1$), Egypt ($n = 1$),Uzbekistan ($n = 1$), Spain ($n = 1$), Tunisia ($n = 1$), Canada ($n = 1$), Thailand ($n = 1$). Study [17] only reported the patients from Asia and Caucasians. Study [15, 20, 22, 31, 32, 37] gave the genotype of HCV.

Methodological quality of included studies

Table 1 shows that the average QUADAS score of the 25 studies was 8.7 (range 3–14) of a maximum score of 14. Ten studies [1, 14–17, 19, 33, 34, 36, 37] did not recruit a represented spectrum. All the studies used the same reference standards. Two studies [14, 16] recruited a group of healthy controls and a group known to have HCV, which were not considered representative by QUADAS [10]. And one study [31] reported the inclusion of a blinded interpretation of the index test. Other quality problems are listed in Table 4 in Appendix.

Overall analysis

The sensitivity and specificity for HCV-cAg test of involved studies were presented as forest plots in Fig 2. The pooled sensitivity of HCV-cAg assay was 0.84 (95 % CI, 0.83–0.85), chi-square = 629.98, $p < 0.001$, $I^2 = 96.2$ %. The pooled estimate for specificity was 0.98 (95 % CI, 0.97–0.98), chi-square = 375.30, $p < 0.001$, $I^2 = 93.6$ %. The chi-square test revealed significant heterogeneity for both diagnosis accuracy parameters.

Table 1 Study characteristics

Study ID	Source of samples/Infection Phase	Application area	Country	Method/commercial brand	QUADAS score
Agha 2004 [22]	Blood donor/anti-HCV-positive	Confirmatory test	Japan (109) Egypt (142) Uzbekistan (15)	ELISA/ortho clinical diagnostics	9
Aoyagi 1999 [23]	Blood donor/anti-HCV-positive	Confirmatory test	Japan	EIA/lab made	10
Bouzgarrou 2005 [15]	Hemodialysis (anti-HCV and/or HCV RNA positive)	Screening	Tunisia	Trak-C ELISA/ortho clinical diagnostics	10
Chen 2010 [33]	HCV inpatient and HCV outpatient	Screening	China	ELISA/Hunan JYNDA	7
Courouce 2000 [20]	Hemodialysis/preseroconversion	Screening	France	ELISA/ortho clinical diagnostics	12
Daniel 2007 [1]	Treatment naive individuals and those on anti-viral therapy	Confirmatory test	India	Trak-C ELISA/ortho clinical diagnostics	6
Fabrizi 2005 [17]	Hemodialysis anti-HCV-positive (167)/anti-HCV-positive -negative (125)	Screening	Asian (7) Caucasians (278)	Trak-C ELISA/ortho clinical diagnostics	8
Krajden 2004 [24]	Treatment-naïve anti-HCV-positive individuals	Confirmatory test	Canada	Trak-C ELISA/ortho clinical diagnostics	10
Kurtz 2001 [30]	Liver disease clinic patients/anti-HCV-positive	Treatment monitoring	Scottish	Prototype 'total' hepatitis C core antigen immunoassay/ortho clinical diagnostics	11
Laperche 2003 [21]	Hemodialysis/preseroconversion	Screening	France	Trak-C ELISA/ortho clinical diagnostics	10
Long 2010 [25]	Clinical sample/anti-HCV-positive	Confirmatory test	China	ELISA/Hunan JYNDA	10
Lorenzo 2004 [31]	Chronic hepatitis C on different antiviral treatment schedules (interferon alpha plus ribavirin)	Treatment monitoring	Spain	Trak-C ELISA/ortho clinical diagnostics	14
Lu 2007 [14]	HCV patients healthy individuals	Screening	China	ELISA/Hunan JYNDA	6
Medhi 2008 [18]	Hemodialysis	Screening	India	Ortho HCV 3.0	8
Miedouge 2010 [34]	Hemodialysis/anti-HCV-negative	Screening	France	CLIA/ABBOTT ARCHITECT® HCV Ag test, ABBOTT diagnostics	10
Netski 2004 [26]	Intravenous drug user/anti-HCV-positive	Confirmatory test	Thailand	Trak-C ELISA/ortho clinical diagnostics	6
Ouyang 2006 [27]	Chronic hepatitis C/anti-HCV-positive	Confirmatory test	China	ELISA/Hunan JYNDA	7
Reddy 2006 [35]	Hemodialysis	Screening	India	ELISA/ortho clinical diagnostics	12
Tanaka 2000 [28]	Acute hepatitis C (17), chronic hepatitis C (75), Asymptomatic individuals (167)/anti-HCV-positive chronic hepatitis B (58), nonviral liver diseases (24), healthy individuals (50)/anti-HCV-negative	Confirmatory test	Japan	CLIA	10
Valcavi 2004 [29]	Serum or plasma from medical departments, outpatient clinics, from surgical wards/anti-HCV-positive	Confirmatory test	Italy	Trak-C ELISA/ortho clinical diagnostics	10

Table 1 continued

Study ID	Source of samples/Infection Phase	Application area	Country	Method/commercial brand	QUADAS score
Xiao 2009 [19]	Clinical sample/both anti-HCV and HCV RNA positive (39)	Screening	China	ELISA/unclear	6
Yu 2009 [36]	HCV patients (214)	Screening	China	ELISA/Hunan JYNDA	11
	HIV patients (78)				
	HBV patients (20)				
	HEV patients (10)				
	Nonviral liver diseases (10)				
	Healthy individuals (60)				
Zanetti 2003 [32]	HCV patients treated with interferon (37.9 % sustained responders, 27.6 % relapsers and 34.5 % nonresponders)/ anti-HCV-positive	Treatment monitoring	Italy	ELISA/ortho clinical diagnostics	12
Zhang 2007 [37]	Regular plasma donor (11, from 5 different plasma station)/anti-HCV-positive	Screening	China	ELISA (Kinda Gene, Changsha, Hunan Province, China)	10
Zhu 2010 [16]	HCV patients/anti-HCV-positive	Screening	China	ELISA/Hunan JYNDA	10
	Health control/anti-HCV-negative				

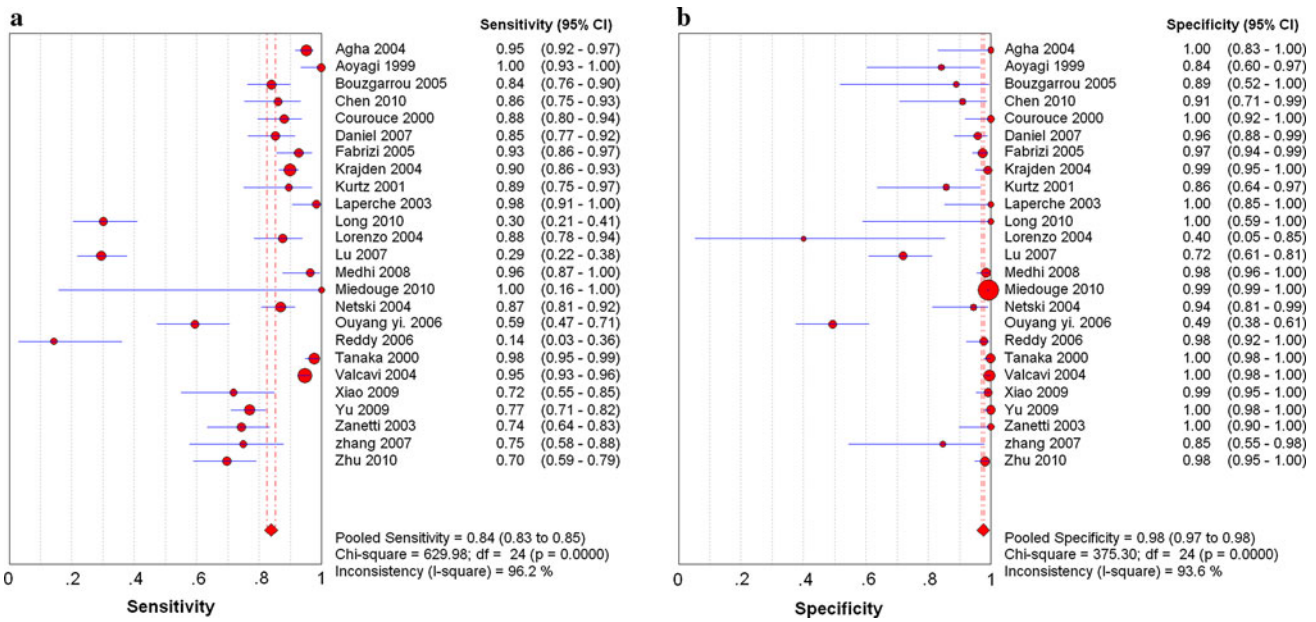

Fig. 2 Forest plot of sensitivities (**a**) and specificities (**b**) of all involved studies

Subgroup analysis

Commercial brand, patient spectrum, quality, and application area were four covariates. Commercial brand meant the merchant of HCV-cAg assay kit (1 = ortho clinical diagnostics, 2 = Hunan JYNDA, 3 = others). Patient spectrum meant whether the spectrum of patients representative of the patients who would receive the test in practice (judged by the first domain "patient representativeness" of QUADAS, 0 = NO, 1 = YES, Table 4). Quality meant quality score of study judged by QUADS (1 = score 1 ~ 5, 2 = score 5 ~ 10, 3 = score 11 ~ 14). Application areas were divided into three, HCV screening = 1, confirmatory test in anti-HCV positive therapy-naive individuals = 2, antiviral therapy treatment monitoring = 3. The data extracted from studies and the results of subgroup analysis were shown in Table 2. We further

Table 2 Pooled sensitivity and pooled specificity for each subgroup

Covariates	No. of study	Pooled sensitivity (95 % CI)	Pooled specificity (95 % CI)
Commercial Brand 1[a]	14	0.901 (0.888 ~ 0.913)	0.980 (0.971 ~ 0.987)
		Chi-squared = 128.03, $p < 0.001$,	Chi-squared = 41.53, $p < 0.001$
		$I^2 = 89.8$ %	$I^2 = 68.7$ %
Commercial Brand 2	7	0.604 (0.567 ~ 0.640)	0.872 (0.840 ~ 0.899)
		Chi-squared = 140.44, $p < 0.001$	Chi-squared = 150.16, $p < 0.001$
		$I^2 = 95.7$ %	$I^2 = 96.0$ %
Commercial Brand 3	4	0.949 (0.919 ~ 0.971)	0.992 (0.988 ~ 0.995)
		Chi-squared = 32.41, $p < 0.001$	Chi-squared = 15.44, $p = 0.001$
		$I^2 = 90.7$ %	$I^2 = 80.6$ %
Spectrum 0	10	0.730 (0.700 ~ 0.759)	0.983 (0.978 ~ 0.987)
		Chi-squared = 162.63, $p < 0.001$	Chi-squared = 130.27, $p < 0.001$
		$I^2 = 94.5$ %	$I^2 = 93.1$ %
Spectrum 1	15	0.883 (0.869 ~ 0.896)	0.955 (0.942 ~ 0.965)
		Chi-squared = 364.25, $p < 0.001$	Chi-squared = 217.17, $p < 0.001$
		$I^2 = 96.2$ %	$I^2 = 93.6$ %
Quality 2	18	0.850 (0.836 ~ 0.864)	0.949 (0.937 ~ 0.959)
		Chi-squared = 561.92, $p < 0.001$	Chi-squared = 257.75, $p < 0.001$
		$I^2 = 97.0$ %	$I^2 = 93.4$ %
Quality 3	6	0.778 (0.739 ~ 0.813)	0.992 (0.988 ~ 0.994)
		Chi-squared = 51.72, $p < 0.001$	Chi-squared = 27.65, $p < 0.001$
		$I^2 = 90.3$ %	$I^2 = 81.9$ %
Application Area 1	13	0.747 (0.719 ~ 0.773)	0.984 (0.979 ~ 0.987)
		Chi-squared = 246.95, $p < 0.001$	Chi-squared = 130.70, $p < 0.001$
		$I^2 = 95.1$ %	$I^2 = 90.8$ %
Application 2	9	0.890 (0.875 ~ 0.904)	0.947 (0.930 ~ 0.960)
		Chi-squared = 278.29, $p < 0.001$	Chi-squared = 184.73, $p < 0.001$
		$I^2 = 97.1$ %	$I^2 = 95.7$ %
Application 3	3	0.826 (0.766 ~ 0.876)	0.902 (0.798 ~ 0.963)
		Chi-squared = 6.45, $p = 0.040$	Chi-squared = 15.26, $p < 0.001$
		$I^2 = 69.0$ %	$I^2 = 86.9$ %
Spectrum 1	4	0.858 (0.806 ~ 0.901)	0.986 (0.967 ~ 0.995)
Commercial brand 1		Chi-squared = 72.48, $p < 0.001$	Chi-squared = 2.26, $p = 0.520$
Application area 1		$I^2 = 95.9$ %	$I^2 = 0.0$ %
Spectrum 1	4	0.926 (0.911 ~ 0.939)	0.991 (0.980 ~ 0.997)
Commercial brand 1		Chi-squared = 16.93, $p = 0.001$	Chi-squared = 5.26, $p = 0.154$
Application area 2		$I^2 = 82.3$ %	$I^2 = 43.0$ %

[a] Commercial brand meant the merchant of HCV-cAg assay kit: 1 = ortho clinical diagnostics, 2 = Hunan JYNDA, 3 = others. Spectrum: 0 = NO, 1 = YES, judged by the first domain "patient representativeness" of QUADAS, Table 4

Quality meant quality score of study judged by QUADS: 2 = score 5 ~ 10, 3 = score 11 ~ 14

Application areas: HCV screening = 1, confirmatory test in anti-HCV positive therapy-naive individuals = 2, antiviral therapy treatment monitoring = 3

analyzed the diagnosis accuracy in different application areas in studies matched both the conditions of patient spectrum = 1 and commercial brand = 1, showing the results in Fig. 3. As data shown in Table 2, studies used Hunan JYNDA ELISA kit (commercial brand 2) and studies in HCV screening (application area 1) showed obvious lower sensitivity. Studies of higher quality also showed lower sensitivity. Obvious difference was found between different commercial brand and study quality. Studies with "commercial brand 2" and "quality 1" gave the lowest specificity. We continued to compare the diagnosis accuracy in different application area with Spectrum was "1" and commercial brand was "1". The sensitivity was up to 0.926 and the specificity was 0.991. It is seemed HCV-cAg more suitable to be used as a marker of HCV replication in anti-HCV

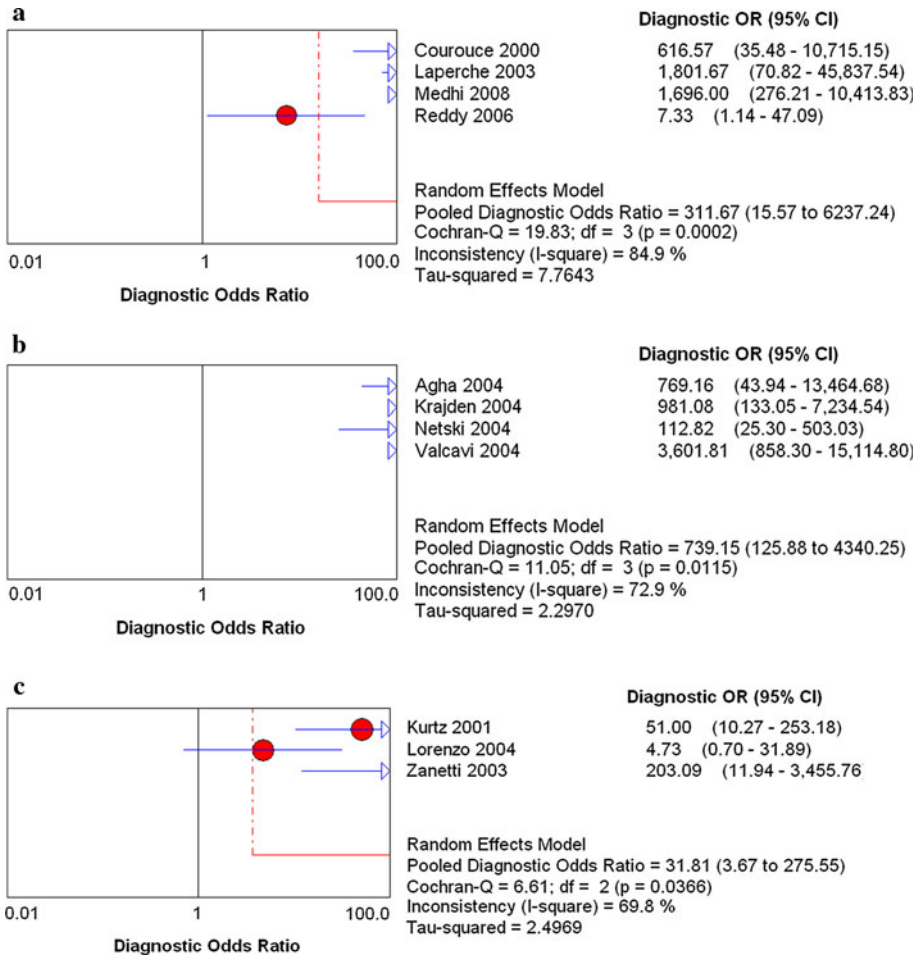

Fig. 3 Forest plots of DOR of different filter condition (**a** spectrum = 1, commercial brand = 1 and application area = 1, **b** spectrum = 1, commercial brand = 1 and application area = 2, **c** spectrum = 1, commercial brand = 1 and application area = 3)

positive, therapy-naive individuals. And the results of diagnostic odds ratio (DOR) value in Fig. 3 also agree with the above conclusion.

Heterogeneity analysis

As shown in receiver operating characteristic (ROC) plane (Fig. 4), no pattern of "shoulder arm" was observed, and spearman correlation coefficient $= -0.18$, $p = 0.389$. Therefore, threshold effect did not exist. In the forest plot of DOR (Fig. 5), the DOR of individual study deviated largely from the line corresponding to the pooled DOR, and Cochran-Q $= 383.78$, $p < 0.001$. The results indicated heterogeneity other than threshold effect.

We used meta-regression to find the possible sources of the above heterogeneity across the studies. The following covariates were used as predictor variables: commercial brand of HCV cAg detection, patients spectrum, study quality and application area. Results were shown in Table 3, which showed that patients spectrum was strongly

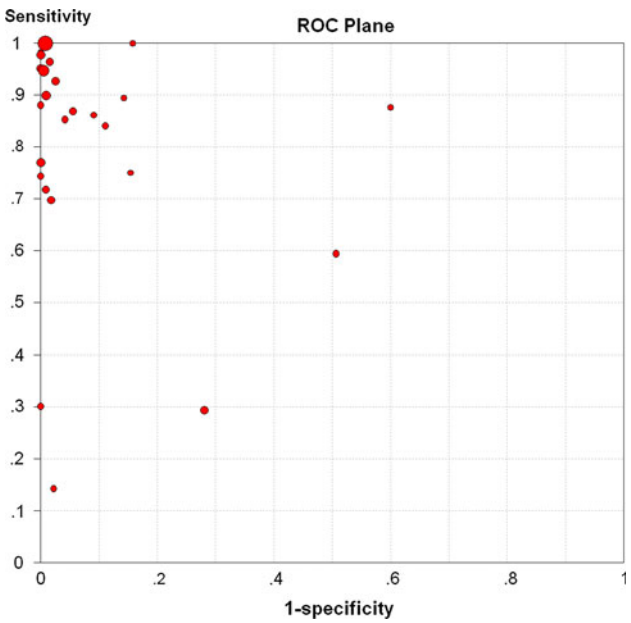

Fig. 4 Representation of sensitivity against (1-specificity) in receiver operating characteristics (ROC) plane for each study

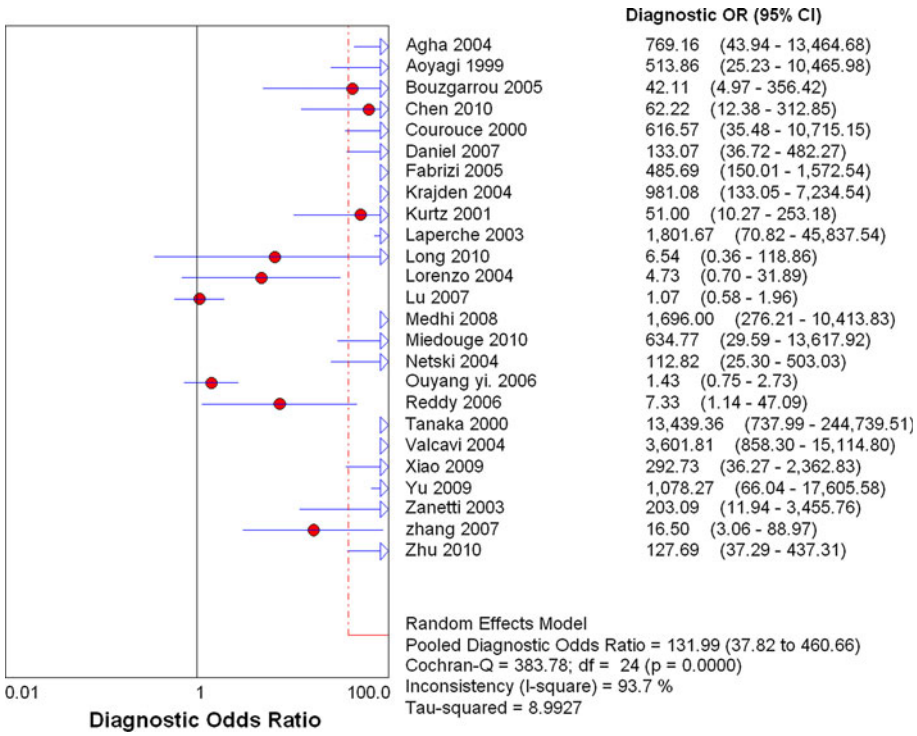

Fig. 5 Forest plot of DOR

Table 3 Results of meta-regression analysis

Var.	Coeff.	Std. err.	*p* value	RDOR	[95 % CI]
Meta-regression (inverse variance weights) (1)					
Cte.	5.917	3.4107	0.0990	–	–
S	−0.238	0.3233	0.4699	–	–
Spectrum	1.410	1.4142	0.3311	4.10	(0.21;79.08)
Quality	−0.761	1.3675	0.5846	0.47	(0.03;8.18)
Commercial brand	0.124	0.7960	0.8775	1.13	(0.21;5.99)
Application	−0.459	1.0883	0.6778	0.63	(0.06;6.16)
Meta-regression (inverse variance weights) (2)					
Cte.	6.192	2.8292	0.0406	–	–
S	−0.242	0.3156	0.4528	–	–
Spectrum	1.356	1.3414	0.3240	3.88	(0.24;63.72)
Quality	−0.786	1.3292	0.5608	0.46	(0.03;7.29)
Application	−0.459	1.0618	0.6701	0.63	(0.07;5.79)
Meta-regression (inverse variance weights) (3)					
Cte.	5.953	2.7176	0.0399	–	–
S	−0.298	0.2829	0.3041	–	–
Spectrum	1.045	1.1051	0.3552	2.84	(0.29;28.30)
Quality	−0.965	1.2402	0.4453	0.38	(0.03;5.02)
Meta-regression (inverse variance weights) (4)					
Cte.	3.976	0.9621	<0.001	–	–
S	−0.241	0.2713	0.3837	–	–
Spectrum	0.837	1.0648	0.4404	2.31	(0.25;21.01)

associated with accuracy. The representative patient spectrum was associated with two-fold higher accuracy compared to the non-representative patient spectrum (RDOR = 2.40, 95 % CI = 0.21–27.83, $p = 0.467$). However, the heterogeneity was still significant when analyzed separately by spectrum = 0 and spectrum = 1,

so as other subgroups (Table 2). We continued to find the cause of heterogeneity from commercial brand and application area. When we filtered studies by spectrum = 1 and commercial brand = 2, there were only two studies left. When filtering the studies by spectrum = 1, commercial brand = 1 and each application area, the heterogeneity of specificity was eliminated (I = 0.0 and 43.0 %). The residue heterogeneity of sensitivity was still significant. We also tried to explain the heterogeneity by sample size, country of origin, publication year, or type of index test (data not shown), but that made no difference to the results.

Publication bias

The *P*-value of the Egger's test was 0.220, suggesting that publication bias did not exist.

Discussion

We analyzed the diagnosis accuracy of HCV-cAg assay. The overall estimates give good sensitivity and excellent specificity. Potential use of HCV-cAg assay in three different areas was also analyzed separately. This assay showed the highest sensitivity when HCV-cAg was used as a marker of HCV replication in anti-HCV positive therapy naïve individuals. This might result from the fact that the relevant clinical population for recruitment was a group of patients already known to be anti-HCV positive. Studies of higher quality showed even lower sensitivity and specificity. This might be because of unavoidable patient selection bias. Such as study [31], items of QUADAS were complied well. The patients were selected randomly, and levels of HCV RNA and HCV-cAg were determined simultaneously and in parallel. However, the number of samples with HCV RNA negative was only five and the specificity was 0.40, which brought statistic error. Ortho clinical diagnostics and Hunan JYNDA are the two main manufacturers of HCV-cAg assay kits. The subgroup analysis showed that the kits of ortho clinical diagnostics have much higher sensitivity and specificity. Hunan JYNDA kits can only detect free HCV core antigen, that is to say, HCV-cAg is only detectable in the anti-HCV negative phase of HCV infection. Some articles studied on Hunan JYNDA kits chose HCV patients as test objects, which may cause lower sensitivity. Therefore, the Hunan JYNDA kit is not appropriate to be applied to as conformation test in anti-HCV positive individuals or treatment monitoring.

We found evidence for heterogeneity of specificity. According to the results of meta-regression, patient spectrum was the source. When diagnosis was performed in represented spectrum, using the kit of ortho clinical diagnostics, and applied to HCV screening or confirmatory test, the heterogeneity of specificity was eliminated. We failed to find an appropriate reason for the heterogeneity of sensitivity from both of subgroup analysis and meta-regression. We also failed to explain the heterogeneity by sample size, country of origin, publication year, or type of index test. When we tried to explore the heterogeneity by more limits, unfortunately, only a little or no information was obtained. However, we still try to consider the heterogeneity from the other aspects. Some methodology or clinical biases were not assessed by QUADAS. In studies [25, 27], the index test method had its intrinsic shortage in testing patient with anti-HCV positive and that was due to low sensitivity. An additional limitation is that we only obtained few studies to perform subgroup analysis such as in Fig. 3. The available data are not enough to give a more valuable conclusion. Further studies are required to better estimate the diagnosis accuracy of HCV-cAg detection. Finally, we limited our search to published English and Chinese languages. The publication bias was not found by Egger's test.

In conclusion, HCV-cAg detection is a promising method as confirmatory test for HCV antibody positive, therapy-naive individuals. Although the diagnostic accuracy in HCV screening and treatment monitoring was not as excellent as confirmatory test, we still think it is a supplementary method to HCV antibody testing, because HCV antigen assay eliminates pre-seroconversion window period, provides low cost, and is easy to operate.

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Appendix

See Table 4.

Table 4 Quality problems of included studies according to QUADAS

Study ID	Represented spectrum	Patients selection	Acceptable reference standard	Acceptable delay between Tests	Partial verification avoided	Differential verification avoided	Incorporation avoided	Detailed described Index	Detailed described Reference	Reference standard results blinded	Index test results blinded	Relevant clinical information	Uninterpretable/ intermediate results reported	Withdrawals explained
Agha 2004 [22]	Yes	No	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Aoyagi 1999 [23]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes
Bouzgarrou 2005 [15]	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Chen 2010 [33]	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Unclear	Yes	Yes	Yes
Courouce 2000 [20]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes
Daniel 2007 [1]	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Unclear	Unclear	Yes	Yes	Yes
Fabrizi 2005 [17]	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes
Krajden 2004 [24]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes
Kurtz 2001 [30]	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Laperche 2003 [21]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Long 2010 [25]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes
Lorenzo 2004 [31]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Lu 2007 [14]	No	No	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	No	Unclear	Yes	Yes	Yes
Medhi 2008 [18]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Unclear	Unclear	No	Yes	Yes
Miedouge 2010 [34]	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes
Netski 2004 [26]	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No
Ouyang yi, 2006 [27]	Yes	No	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	No
Reddy 2006 [35]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes
Tanaka 2000 [28]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	No	Yes	Yes
Valcavi 2004 [29]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes
Xiao 2009 [19]	No	No	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	No	Yes	Unclear	Yes	Yes
Yu 2009 [36]	No	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Zanetti 2003 [32]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 4 continued

Study ID	Represented spectrum	Patients selection	Acceptable reference standard	Acceptable delay between Tests	Partial verification avoided	Differential verification avoided	Incorporation avoided	Detailed described Index	Detailed described Reference	Reference standard results blinded	Index test results blinded	Relevant clinical information	Uninterpretable/ intermediate results reported	Withdrawals explained
Zhang 2007 [37]	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes
Zhu 2010 [16]	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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