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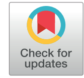
Original Article

Performance of Hepatitis Delta Virus (HDV) RNA Testing for the Diagnosis of Active HDV Infection: Systematic Review and Meta-analysis



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Abstract

Background and Aims: Hepatitis delta virus (HDV) is a defective virus and causes severe liver disease. Several HDV RNA assays have been developed, however the diagnostic efficacy remains unclear.This systematic review and meta-analysis aims to evaluate the diagnostic accuracy of HDV RNA assays to aid in the diagnosis of active hepatitis D. ***Methods:*** The PubMed, Embase, and Cochrane Library databases were systematically searched from the beginning to June 31, 2022. Information on the characteristics of the literature and data on sensitivity, specificity, and area under curve (AUC) of the receiver operating characteristic (ROC) were extracted. Stata 14.0 was used for meta-analysis of the combined sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio. ***Results:*** A total of 10 studies were included in the meta-analysis. The summary sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of HDV RNA assays for HDV diagnosis were 0.92 (95% CI: 0.87–0.95), 0.90 (95% CI: 0.86–0.93), 7.74 (95% CI: 5.31–11.29), 0.10 (95% CI: 0.06–0.18) and 99.90 (95% CI: 47.08–211.99), respectively. The AUC of the pooled ROC curve was 0.95 (95% CI: 0.92–0.96). ***Conclusions:*** The results show that HDV RNA assays had high diagnostic performance. However, that is limited by the number and quality of studies. Standard protocols for the development of assays by manufacturers and larger studies on the use of the assays are needed.

Keywords: Hepatitis viruses; Hepatitis delta virus; Diagnosis; Sensitivity; Specificity.

Abbreviations: AUC, area under curve; FN, false negative; FP, false positive; HBV, hepatitis B virus; HDV, hepatitis delta virus; NLR, negative likelihood ratio; OR, odds ratio; PLR, positive likelihood ratio; ROC, receiver operating characteristic; TN, true negative; TP, true positive.

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Introduction

Hepatitis delta virus (HDV), a worldwide human pathogen, is a defective virus in that its infection, packaging, and release require the assistance of hepatitis B virus surface antigen (HBsAg).[1] Hepatitis D is a viral hepatitis caused by HDV infection. It is infectious and a global public health problem.[2] HDV is transmitted by contact with contaminated blood or blood products through broken skin, and in a few cases by mother-to-child vertical transmission. There is evidence of sexual transmission.[3] HDV/hepatitis B virus (HBV) can be either a chronic superinfection with HBV or a chronic coinfection of a healthy person. HDV currently included eight genotypes based on the genetic sequence.[4] Type I is distributed worldwide and is characterized by a variable disease course. The other genotypes occur mainly in specific geographic regions. Multiple genotype infections can occur repeatedly in high-risk patients, but usually one genotype is the predominant virus strain.[5–8]

It has been suggested that chronic HDV/HBV infections increase the risk of cirrhosis by two or three times and liver cancer by three to six times compared with HBV infection only.[9] HDV infection may increase the rate of disease progression, risk of liver failure, and rate of death from the disease. A 2019 Meta-analysis[10] estimated that 62–72 million people were infected with HDV. Given the significantly increased rate of end-stage liver disease in patients with HBV/HDV coinfection, enhanced screening for HDV in HBsAg-positive patients and healthy populations with high-risk factors is key to improving the prognosis.

HDV induces an innate and adaptive immune response in the infected host, stimulating the production of immunoglobulins IgM and IgG.[11] Diagnostic tests for HDV are of two types, molecular tests to detect viral RNA and serologi-

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cal tests to detect anti-HDV antibodies. Serological antibody testing for hepatitis D is widely used in clinical practice because it is convenient and rapid. However, it has several limitations:[12–15] in acute HDV infection, HDV-antibody (Ab) persists for a very short time and is difficult to detect, so HDV-Ab seronegativity cannot rule out the occurrence of hepatitis D. In chronic HDV infection the HDV-Ab and HDV-antigen (Ag) form a circulating immune complex, so they need to be separated and then detected. Because of its short existence and the low sensitivity of existing IgM assay kits, IgM antibody negativity cannot deny the occurrence of active hepatitis D. In addition, currently no HDV antibody detection kits are available in every country or region, and the results obtained with different commercial kits may not be comparable.

HDV RNA molecular testing is widely used as a gold reference standard for the diagnosis of active HDV infection.[16] Potential target populations for HDV RNA testing are include those who test positive for HDV antibodies, especially HBsAg-positive individuals who are at risk of HDV infection, such as HIV-infected individuals and injection-drug users. The second population is patients with hepatitis D infection who need to be evaluated for prognosis. In patients with hepatitis D infection in the treatment or healing phase, HDV RNA level is the primary indicator to monitor and predict the efficacy of anti-HDV drugs. In addition, HDV RNA testing is the basis for identifying patients indicated for HDV genotyping. However, many existing HDV RNA assays respond well to HDV-1 and have relatively low ability to detect other genotypes. In addition, there are only international standards for the HDV-1 genotype[17] and no standards for the other seven genotypes, making it impossible for the testers to correct the quantitative results for the other genotypes. An increasing number of kits for HDV RNA detection are becoming available, but their diagnostic sensitivity and specificity are not yet clear. The aim of this systematic review and meta-analysis was to evaluate the diagnostic accuracy of HDV RNA assays and to aid in the diagnosis of hepatitis D.

Methods

This meta-analysis conducted following the guidelines of the Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies (PRISMA-DTA Statement) guidelines[18] and it was registered in PROSPERO (CRD42022379115).

Eligibility criteria

The inclusion criteria were: (1) a case group of patients with hepatitis D diagnosed by pathological or clinical examination, and a control group of healthy people or people with other liver diseases; (2) a study objective of evaluating HDV RNA assays for the diagnosis of HDV; and (3) reporting true positive (TP), false positive (FP), true negative (TN), and false negative (FN) diagnosis rates. Studies in which (1) the gold-standard method was not precise or not used; (2) the number of cases was <10; (3) previously published results were reported; and (d) the study results were inconsistent were excluded.

Information sources

We performed a systematic literature search of PubMed, Embase, and the Cochrane Library and retrieved relevant studies published from January 1, 1980, to June 31, 2022. The list of search terms is shown in Supplementary File 1.

Data collection

Two investigators independently extracted data from the enrolled studies (CSS, ZXY). Disagreements were resolved by consulting third-party experts. After data extraction the final results were jointly reviewed and summarized.

Definition of extracted data

The information extracted from the eligible studies included the year of publication, first author's name, the number of samples, detection method, detection limitation, detected genotypes, gold standard, TP, FP, TN, FN, positive likelihood ratio (PLR), negative likelihood ratio (NLR), area under the curve (AUC).

Risk of bias and applicability

The quality of each study was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool, which consists of four parts, patient selection, index test, gold-standard method, and flow and timing.[19] All parts were assessed for risk of bias and the first three were also assessed for clinical applicability. According to answers of "yes," "no," or "uncertain," to the relevant landmark questions included in each part of the tool, the risk of bias was judged as "low," "high," or "uncertain".[20]

Synthesis of results

This meta-analysis was done with Stata 14.0. The combined sensitivity, specificity, PLR, NLR, diagnostic odds ratio (OR) and 95% confidence interval (CI) were calculated as evaluation indicators. Combined receiver operating characteristic (ROC) curves were generated, and the corresponding AUC value was obtained to evaluate the accuracy of the overall detection. The closer the AUC value was to 1, the better the diagnostic performance.[19] Heterogeneity was assessed by calculating the I^2 statistic. The threshold of the heterogeneity test result was 0.05, and that of the level of goodness-of-fit test was 0.05. A $p \geq 0.05$ and an $I^2 \leq 50\%$ it indicated that the statistical heterogeneity of the research results was low, and a fixed-effect model was used. A $p<0.05$ and an $I^2>50\%$ indicated significant heterogeneity of the studies, and a fixed-effect model is used.[21] Random-effects model analysis, sensitivity analysis, meta-regression analysis, and subgroup analyses were performed to explore the source of heterogeneity. A Deek's funnel plot was drawn to detect potential publication bias.[22] The inspection level, $\alpha=0.05$.

Results

Study selection

Following the literature search strategy, a total of 743 relevant articles were retrieved, including 190 from PubMed, 404 from Embase, and 149 from the Cochrane Library. There were 328 duplicates, and 292 titles or abstracts did not meet the inclusion criteria. Fourteen had an inappropriate gold standard, 26 had fewer than 10 study subjects, 51 did not match the research content, and 24 did not report sensitivity and specificity and other outcome indicators. The remaining 10 studies were included in the meta-analysis.[23–32] Figure 1 shows the process of study selection.

Study characteristics

The information reported in the 10 studies is shown in Table 1. The studies were conducted in eight countries, published between 1986 to 2022, included a total of 1,213 patients and had sample sizes ranging from 37 to 611 patients. Of the ten included studies, eight had a case-control design and two were cohort study. Two were from the USA, two from

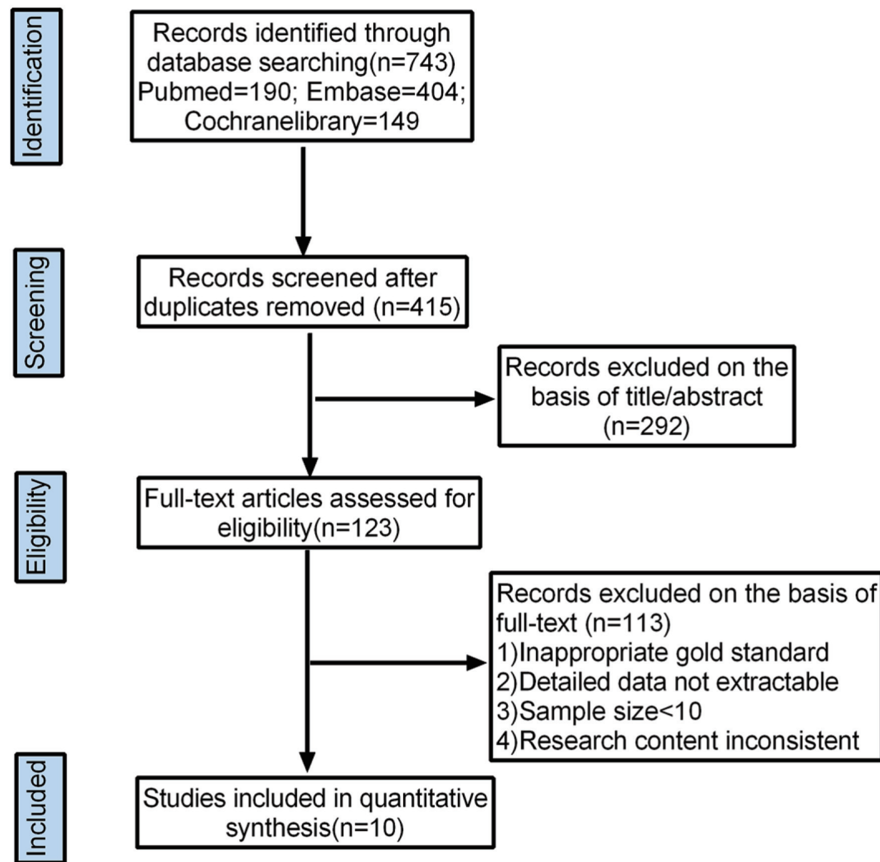


Fig. 1. Flowchart of literature identification.

Germany, and the remaining five from UK, France, Spain, Japan, Italy, and China.

Study quality assessment

Using the QUADAS-2 tool, the methodological quality of nine articles was evaluated independently by two investigators. In terms of patient selection, four studies had a high risk of bias because of their case-control design and lack of consecutive or random sampling. For the index test domain, approximately 33% of the studies had a high risk of bias and 40% of the studies had an unclear risk. The remaining studies had an unclear risk because of their indistinct thresholds. In terms of the reference standard, two studies had an unclear risk and remaining had a low risk. Regarding the flow and timing domain, about 67% of the studies had a low risk of bias. The rest were had an unclear risk because of lack of reporting the time interval between the index test and the gold-standard method. In terms of the applicability of case selection, one study was not clear, and the remaining had low risk. Results of the quality evaluation are shown in Figure 2.

Diagnostic accuracy

Eight studies analyzed the diagnostic value of the HDV RNA assays. According to the heterogeneity test results (I^2>50%, p<0.05), a random effect model was used for meta-analysis. The summary results showed that the sensitivity was 0.92 (95% CI: 0.87–0.95), the specificity was 0.90 (95% CI: 0.86–0.93), and the PLR was 7.74 (95% CI: 5.31–11.29), NLR was 0.10 (95% CI: 0.06–0.18), diagnostic OR was 99.90 (95% CI: 47.08–211.99), diagnostic score was 4.60 (95% CI: 3.85–5.36) and AUC was 0.95 (95% CI: 0.92–0.96), see Figure 3. The results suggest that HDV RNA assays had high diagnostic accuracy.

Subgroup analysis

Subgroup analyses were conducted to explore potential sources of heterogeneity in Table 2. The results indicated that there was overlap of the 95% CIs for the combined effect sizes of the subgroups and that within-group heterogeneity was significant across groups. No heterogeneity was found for any of the parameters included in the analysis (e.g., sample source, sample type, sample size, sample storage conditions, etc.) in terms of covariates.

Sensitivity analysis

Sensitivity analysis results are shown in Figure 4A. After excluding individual studies, no significant factors affecting the results and heterogeneity were found.

Publication bias

Deek's test results for the summary study of diagnostic value of HDV RNA assays were t=−2.76 and p=0.02, indicating significant publication bias. The result is shown in Figure 4B.

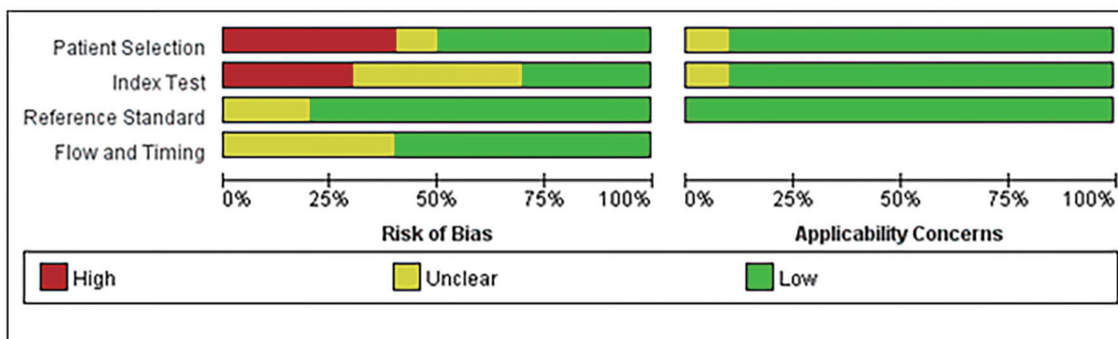
Diagnostic efficacy verification

Post-test probability was calculated by drawing a Fagan diagram. As shown in Supplementary Figure 1, if the HDV nu-

Table 1. Characteristics of included studies for the systematic review on detection of HDV RNA

No.	First author	Year	Country	Study design	Reference standard	Target gene	Genotypes detected	Sample type	Frozen/fresh	Sample size	Limit of detection	TP	FP	TN	FN	Detection method
1	Smedi	1986	America	cohort	spot hybridization	[a]NR	[a]NR	serum	frozen	120	[a]NR	60	5	50	5	hybridization assay
2	Ferns	2012	Britain	cohort	[d]RT-qPCR	HDAg-coding region	[a]NR	plasma	frozen	95	$3.8*10^2$ copies/ml	44	5	40	6	one step [d]RT-qPCR
3	Kodani	2013	America	cross-sectional	[d]RT-qPCR	upstream of the L-HDAg ORF	[a]NR	serum	frozen	132	$7.5*10^2$ copies/ml	51	7	66	8	one step [d]RT-qPCR
4	Mederacke	2010	Germany	cross-sectional	[d]RT-qPCR	HDAg-coding region	[a]NR	serum/plasma	fresh	37	$3*10^2$ copies/ml	14	2	20	1	one step [d]RT-qPCR
5	Hofmann	2010	Germany	cross-sectional	Nested-[b]PCR	HDAg-coding region	I,III	serum	fresh	50	$2*10^3$ copies/ml	30	3	14	3	[c]RT-PCR
6	Schaper	2009	Spain	cross-sectional	[b]PCR	HDAg-coding region	I	serum	frozen	40	$1*10^3$ copies/ml	19	4	14	3	[d]RT-qPCR
7	Yamashiro	2004	Japan	cohort	[b]PCR	HDAg-coding region	I,IIa,IIb	serum	[a]NR	48	$1*10^3$ copies/ml	25	5	15	3	[c]RT-PCR
8	Le Gal	2016	France	cross-sectional	[d]RT-qPCR	Ribozyme region	I-VIII	serum/plasma	frozen	611	$5*10^1$ copies/ml	389	14	198	9	one step [d]RT-qPCR
9	Olivero	2022	Italy	cross-sectional	[d]RT-qPCR	Conserved regions	I-VIII	plasma	frozen	36	$8.9*10^0$ copies/ml	26	0	10	0	droplet digital [b]PCR
10	Xu	2022	China	cross-sectional	[d]RT-qPCR	Conserved regions	I-VIII	serum	frozen	44	$1*10^0$ copy/ml	24	0	14	6	droplet digital [b]PCR

[a]NR, not reported; [b]PCR, polymerase chain reaction; [c]RT-PCR, reverse-transcription polymerase chain reaction; [d]RT-qPCR, reverse-transcription quantitative real time polymerase chain reaction.



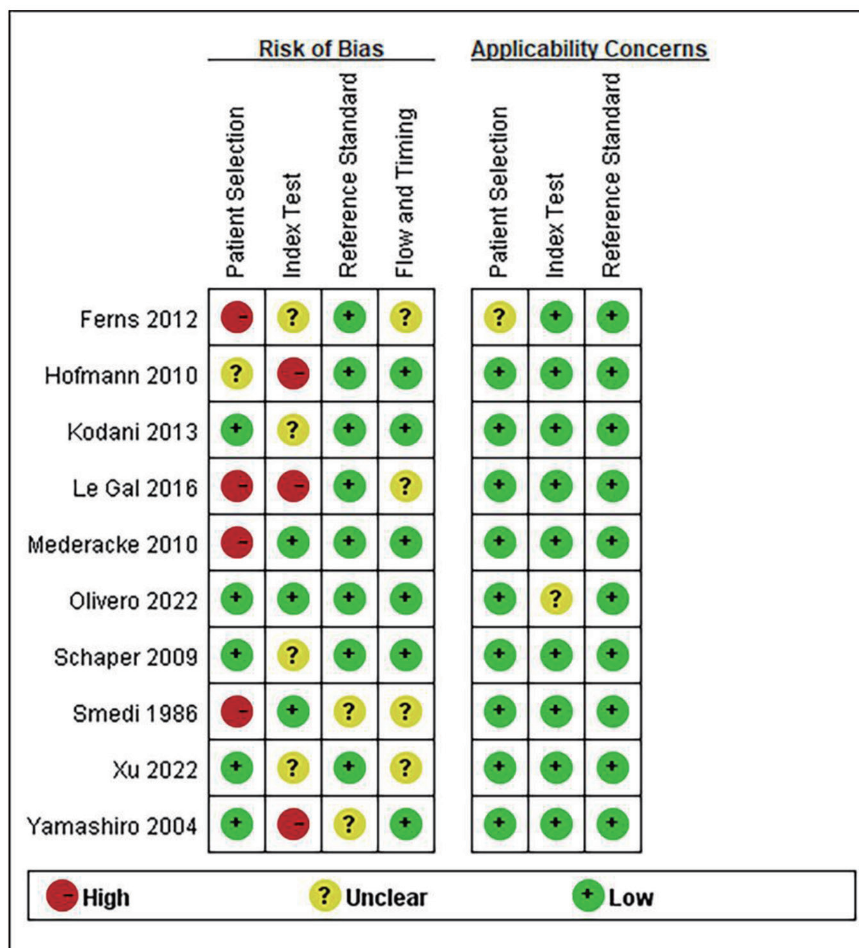


Fig. 2. Quality assessment of the study. (A) Risk of bias and applicability concerns graph. (B) Risk of bias and applicability concerns summary.

cleic acid detection assay diagnosed hepatitis D, the probability of being diagnosed with hepatitis D was 69%; if the result was negative, the probability of having hepatitis D was 2%. The result indicates that the HDV RNA assay had a high accuracy rate.

Discussion

Since the first report of the HDV virus in 1977,[33] it is estimated that 15–20 million people have been infected worldwide.[34] After HDV/HBV coinfection, about 90% of hepatitis patients have chronic progression, and the proportion who develop end-stage liver diseases such as liver failure, liver cirrhosis, and liver cancer increases, and the risk of adverse outcomes increases.[35] Therefore, it is very important to test and monitor HDV infection. However, current global epidemiological data on hepatitis D are limited. A very likely reason for this is that the reported studies used various nonstandardized assays. The diagnosis of hepatitis D generally relies on the use of commercial kits to detect specific anti-HDV antibodies (IgG and IgM), followed by confirmation of active infection by detection of HDV RNA using methods within each clinical institution. However, the sensitivity of serological methods varies, and the reliability of nucleic acid assays testing

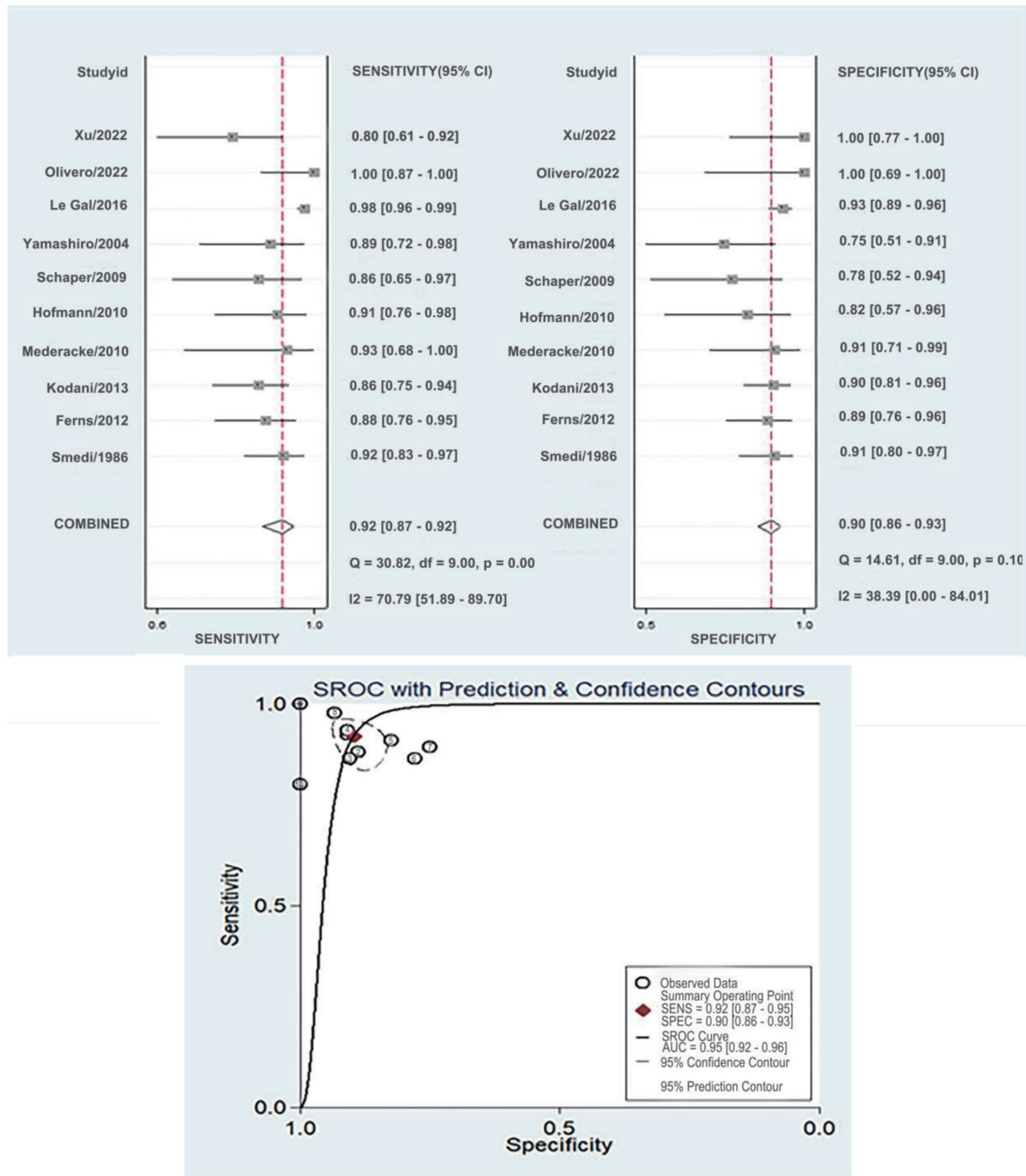


Fig. 3. Diagnostic accuracy of the use of HDV RNA assays for detection of hepatitis D-RNA. (A) Forest plot of sensitivities and specificities of the use of HDV RNA assays for detection of hepatitis D-RNA. (B) SROC curve of HDV RNA assays diagnostic value. HDV, hepatitis delta virus; SROC, summary receiver operating characteristic.

is questionable owing to the lack of internationally accepted HDV RNA standards.[30,36,37] Recently, various methods of HDV nucleic acid detection have emerged, and it is of great public health significance to evaluate the diagnostic efficacy of the available HDV RNA assays.

In this meta-analysis, the sensitivity of HDV RNA assays for the diagnosis of active hepatitis D was 0.92% (95% CI: 0.87–0.95) and the specificity was 0.90% (95% CI: 0.86–0.93), having a satisfactory diagnostic efficacy in patients with active hepatitis D. The meta-analysis also assessed the overall diagnostic accuracy by plotting the summary receiver operating characteristic (SROC) curve and the corresponding AUC, aiming to provide a more meaningful reference for clinical use. The results showed that the AUC value of RNA assays for the diagnosis of HDV was 0.95 (95% CI: 0.92–0.96). At present, different RNA detection sites, techniques, or methods are used by different institutions, resulting in mixed results in terms of sensitivity and specificity. Therefore, the au-

Table 2. Subgroup analysis of quantitative HDV RNA assays diagnostic value for HDV

Subgroup	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)	Diagnostic OR (95%CI)	AUC (95%CI)
Sample type						
Serum	0.88 (0.83–0.92)	0.88 (0.82–0.92)	7.21 (5.04–10.62)	0.13 (0.09–0.19)	54.00 (30.12–96.11)	0.87 (0.85–0.89)
Plasma	0.89 (0.84–0.92)	0.87 (0.83–0.93)	7.92 (3.44–18.21)	0.14 (0.06–0.29)	58.67 (16.61–207.18)	0.94 (0.92–0.96)
Serum/plasma	0.93 (0.90–0.95)	0.89 (0.81–0.92)	8.85 (3.86–20.3)	0.05 (0.02–0.15)	181.22 (40.88–803.35)	0.91 (0.89–0.95)
Sample size						
≥50	0.91 (0.87–0.94)	0.89 (0.84–0.93)	8.19 (5.56–12.06)	0.11 (0.08–0.17)	77.05 (41.98–141.42)	0.96 (0.94–0.97)
<50	0.89 (0.82–0.95)	0.82 (0.78–0.86)	4.36 (2.56–7.42)	0.142 (0.07–0.29)	32.05 (11.40–90.13)	0.84 (0.80–0.89)
Sample storage						
Frozen	0.91 (0.82–0.97)	0.89 (0.79–0.95)	7.59 (5.21–11.08)	0.12 (0.08–0.18)	69.34 (37.84–127.06)	0.90 (0.86–0.92)
Fresh	0.92 (0.83–0.96)	0.87 (0.73–0.93)	6.68 (2.96–15.09)	0.10 (0.04–0.26)	66.50 (16.12–274.31)	0.88 (0.83–0.92)
Continent						
North America	0.89 (0.80–0.94)	0.91 (0.88–0.94)	9.47 (5.51–16.30)	0.12 (0.07–0.21)	79.77 (34.83–182.69)	0.90 (0.87–0.94)
Europe	0.91 (0.85–0.95)	0.87 (0.82–0.92)	6.31 (4.06–9.82)	0.11 (0.07–0.18)	61.12 (28.91–129.21)	0.87 (0.80–0.93)
Asia	0.88 (0.81–0.94)	0.86 (0.81–0.89)	3.57 (1.65–7.71)	0.14 (0.05–0.43)	25.00 (5.21–119.92)	0.83 (0.79–0.87)

AUC, area under curve; HDV, hepatitis delta virus; NLR, negative likelihood ratio; PLR, positive likelihood ratio.

thor emphasizes that quantitative RNA detection techniques should be standardized in the collection, isolation or detection stages, especially in the design of probes and primers. These results suggested that HDV RNA assays meet a high level of diagnostic criteria.

Subgroup analyses were conducted in this meta-analysis to explore potential sources of heterogeneity. No heterogeneity was found for any parameter (e.g., sample source, sample type, sample size, sample storage conditions, etc.) with respect to the covariates included in the analysis. Sensitivity analyses did not identify studies that significantly affected heterogeneity. Therefore, heterogeneity may be due to other causes, which could not be assessed here owing to insufficient data.

In clinical practice, the results of this analysis found that several commonly used HDV RNA detection assays and emerging RNA detection technologies have high sensitivity and specificity, with results that are highly reliable for potential target populations requiring HDV RNA testing. They are useful for screening populations at high risk of hepatitis D, confirming the diagnosis of HDV-positive populations, and determining the efficacy of antiviral therapy for hepatitis D patients. However, for clinical treatment, this analysis has shortcomings. There are eight genotypes of HDV RNA, and the genotypes prevalent in different regions vary, but most of the studies included in this meta-analysis did not cover all genotypes or were unclear about specific genotypes, and the efficiency of RNA detection for different genotypes could not be compared. In addition, as there is no uniform clinical standard for detection of. HDV RNA target genes and detection criteria, this analysis should have compared the detection efficiency of different target genes, but because of the lack of complete data, that as not possible.

Despite the emergence of many kits for the detection of hepatitis D, the study of HDV epidemiology on a global scale remains challenging. In the first place there is a lack of data from studies with large samples. Most existing studies have limited sample size and scope, and there is shortage of well-designed, global data studies with large samples.[38] Second, there are still deficiencies in the detection of HDV. Clinical practitioners are not sufficiently aware of HDV, do not include HDV testing as a routine screening indicator, and applicable guidelines lack appropriate recommendations. Furthermore, there is a lack of standardized methods for HDV detection. Most assays lack internationally standardized controls needed for uniform sensitivity and specificity. Therefore, it is particularly important to standardize HDV testing criteria.

This meta-analysis has some limitations of. First, although a systematic literature search was conducted, there may be some valuable studies that were missed and not included. Because only English-language databases were searched, there is a possibility of publication bias. Furthermore, the heterogeneity of this study was high, but the sources of heterogeneity could not be further explored owing to the limited data of the study.

Conclusions

In conclusion, the results suggest that HDV RNA detection using HDV RNA assays had high diagnostic performance. However, the analysis was limited by the number and the quality of the selected studies. There is a need for standardized protocols for the development of their assays by manufacturers, as well as larger studies on the use of the assays. The development of new diagnostic tools with higher accuracy and reliability in HDV diagnosis is important to guide

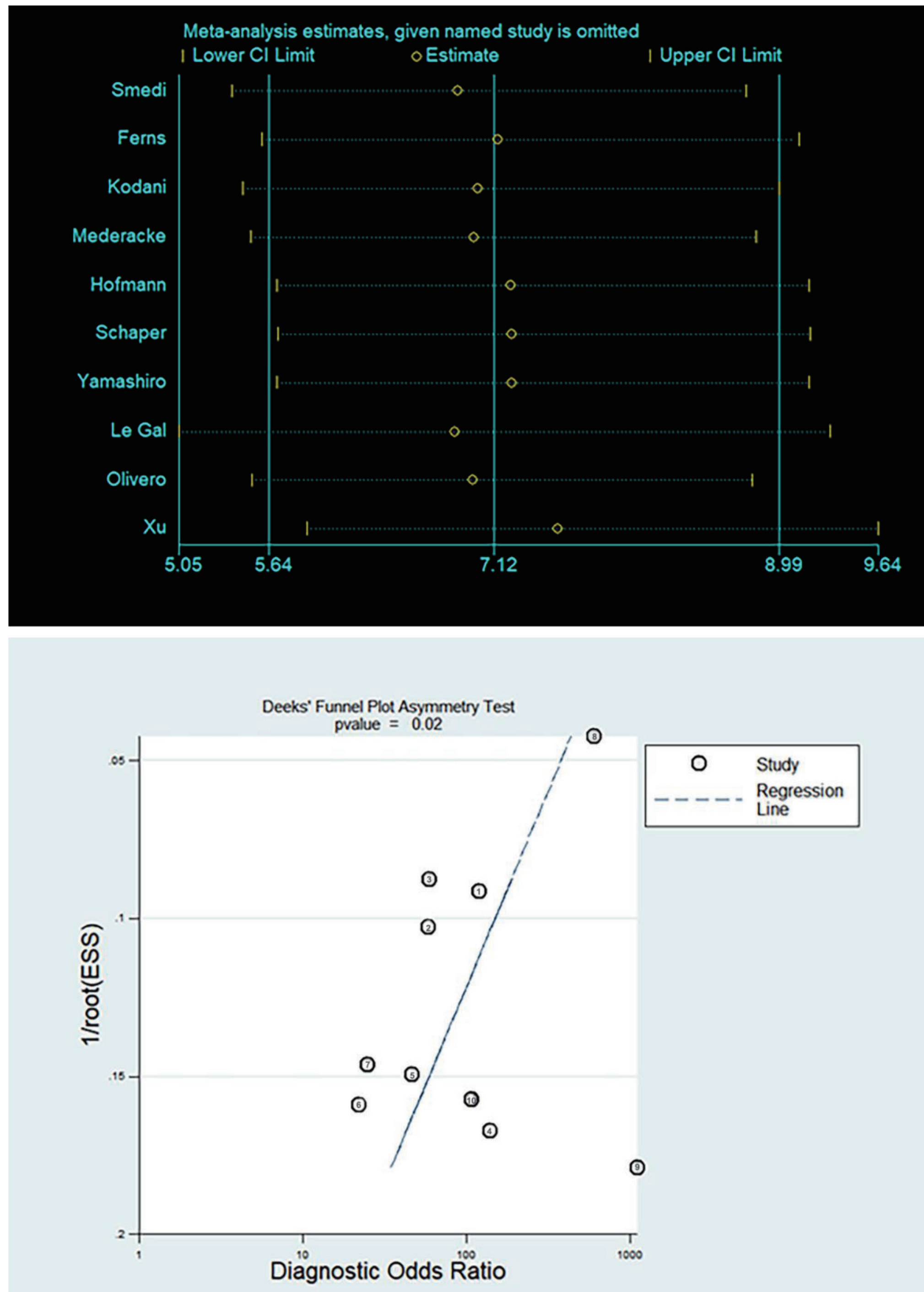


Fig. 4. Sensitivity analysis and Deek's funnel plot asymmetry test to assess publication bias. (A) Sensitivity analysis. (B) Deek's funnel plot asymmetry test.

the development and improvement of prevention and control strategies.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (FR, ML), acquisition of data (SC, XZ), analysis and interpretation of data (SC, XZ) drafting of the manuscript (SC, XZ), critical revision of the manuscript for important intellectual content (SZ, ZD), administrative, technical, or material support (LX, YT, ZF, YC, ZP), and study supervision (SZ, ZD). All authors have made a significant contribution to this study and have approved the final manuscript.

Data sharing statement

No additional data are available.

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