Contents

Appendix 1: Viral Hepatitis

The Hepatitis A virus (HAV) is most commonly transmitted through exposure to contaminated food or water, or exposure to infected people via the faecal-oral route.[6, 96] The course of infection is usually mild, with most people making a full recovery and remaining immune from further HAV infections. However, the virus can occasionally lead to life-threatening infection, the risk for which and is strongly dependent on age.[96] Approximately 11,000 deaths resulted from HAV in 2015 (0.8% of total mortality burden from viral hepatitis).[6] Safe and effective vaccines are available to prevent HAV.[6, 96]

The hepatitis B virus (HBV) is most prevalent in the World Health Organization (WHO) Western Pacific and African Regions. HBV is primarily transmitted through exposure to infective blood, semen, and other body fluids.[97] Most people clear the virus within a few months. However, in some cases it may cause life-long, chronic infection. Viral clearance is age-dependent; when babies and young children are infected they will often develop chronic infection. Combined, HBV and HCV are the leading cause of liver cirrhosis and cancer worldwide, and responsible for 96% of global mortality from viral hepatitis as well as significant morbidity and socio-economic losses.[6] Safe and effective vaccines are available to prevent HBV since 1982.[97] The widespread use of infant vaccination has significantly reduced the incidence of new chronic HBV infections.[6]

The Hepatitis C virus (HCV) is a blood-borne virus which infects the liver and commonly causes progressive liver disease. HCV infection can be mild, lasting only a few weeks (that is, acute HCV infection) or become a serious, lifelong illness.[14] HCV infection is often asymptomatic – only a minority of people experience mild symptoms such as fatigue, muscle and joint pain, jaundice, abdominal discomfort or itching.[15, 16] People with acute HCV infection may develop a vigorous antibody and cell-mediated immune response that spontaneously eradicates the virus. However, 55-85% of acutely infected individuals fail to clear the virus and develop chronic HCV infection.[2]

The hepatitis D virus (HDV) is an incomplete virus most commonly transmitted through contact with blood or other body fluids of an infected person. HDV infection only occurs in people who have already been infected with HBV.[6] As HDV worsens HBV outcomes, it is regarded as a co-factor of chronic liver disease with an estimated 5% of HBV-infected individuals co-infected with HDV.[98] Vaccines against HBV provide protection against HDV infection given the relationship between the viruses.[81] HDV is not currently a notifiable infection in Ireland.

The hepatitis E virus (HEV) is most commonly transmitted through the faecal-oral route, principally by contaminated water in developing countries. In Ireland, HEV is

rare and mostly a zoonotic food-borne disease linked to undercooked or processed pork. HEV is generally a self-limiting illness that does not cause any long term damage, but may progress to acute liver failure and can be fatal for pregnant women.[6] The WHO estimated that HEV infections, which most commonly occurs in South and East Asia, led to approximately 44,000 deaths in 2015 (3.3% of total viral hepatitis burden).[6] There are fewer than 100 cases of HAV and HEV reported per annum in Ireland.[81] A vaccine has been developed and is licensed in China, but not yet in most other countries.[99]

Appendix 2: Example of influence of prevalence on performance characteristics of HCV tests

The relationship between a screening test result and the occurrence of the condition is presented in Table 1.

Table 1. Relationship between a screening test result and the occurrence of the condition

Test result	Condition present*	Condition absent*
Positive	True positive (A)	False positive (B)
Negative	False negative (C)	True negative (D)

* As determined by the gold standard diagnostic test

The formulae used to calculate the diagnostic performance of a screening test (where the letters A, B, C and D indicate whether or not the condition is present according to Table 1) are presented in Table 2.

Table 2. Calculation of diagnostic performance characteristics

Characteristic	Formula*
Prevalence	Population with disease divided by total population. *Formula: (Total disease/Total population) x 100*
Sensitivity	Proportion of positive cases detected relative to the actual number of positive cases in the sample. *Formula: A/(A+C) x 100*
Specificity	Proportion of negative cases detected relative to the actual number of negative cases in the sample. *Formula: D/(B+D) x 100*
Positive predictive value	Proportion of cases with disease relative to those who have a positive test result in the sample. *Formula: A/(A+B) x 100*
Negative predictive value	Proportion of cases without disease relative to those who have a negative test result in the sample. *Formula: D/(C+D) x 100*

* The letters A, B, C and D indicate whether or not the condition is present according to Table 1.

An example of the influence of prevalence on these characteristics is described in Box 1.

Box 1. Influence of prevalence on predictive values

Example

- Assume that 1000 people are tested for a condition. Of these, 15 people have the condition. Therefore, 985 people do not have the disease. Applying the formula presented in Table 1, the prevalence is 1.5%. This is reflected in the table below.

Test result	Condition present*	Condition absent*	Total
Positive	A=10	B=463	473
Negative	C=5	D=522	527
Total	15	985	1000

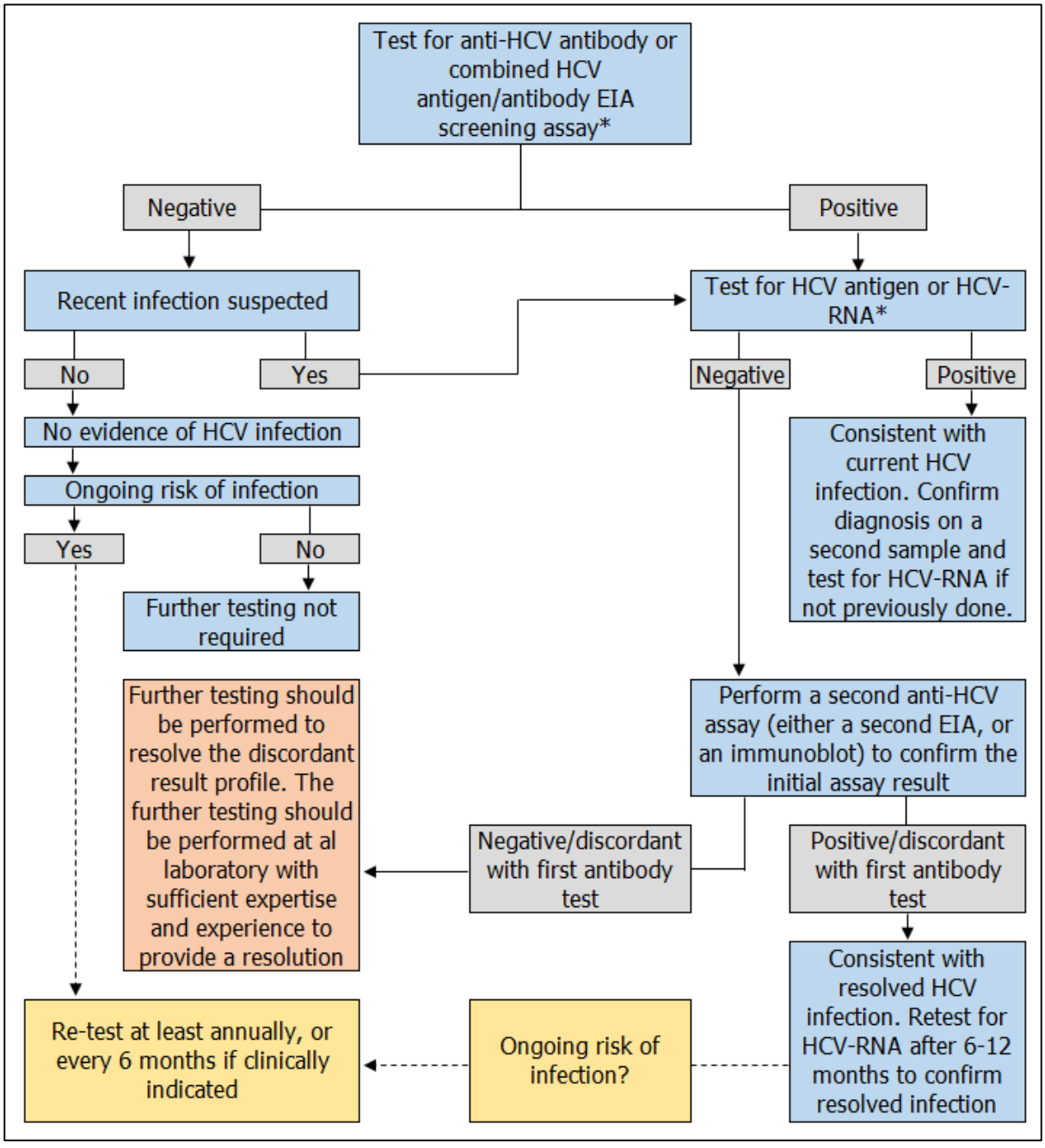
- Assume that the sensitivity of the test is 67%, as calculated by the formula presented in Table 2. In other words, 33% of people with the condition receive false negative test results.
- Assume that the specificity of the test is 53%, as calculated by the formula presented in Table 2. In other words, 53% of people with negative test results are truly negative and 47% of people tested receive false positive test results.
- The sensitivity and specificity are characteristics of the screening test. However, it is important for the clinician to know, among the people who test positive, the proportion that actually have the condition (i.e. the PPV) and, among those who test negative, the proportion that truly do not have the condition (i.e. the NPV).
- Applying the formula in Table 2, the PPV is approximately 2% (10/473 x 100). In other words, only 2% of people that tested positive truly have the condition (i.e. high rate of false positives).
- Applying the formula in Table 2, the NPV is 99% (522/527 x 100). In other words, 99% of people that tested negative do not truly have the condition (i.e. low rate of false negatives).

- If the prevalence was 3% (doubled) and the same rates of sensitivity and specificity were estimated, then the table would be updated as follows:

Test result	Condition present*	Condition absent*	Total
Positive	A=20	B=453	473
Negative	C=10	D=517	527
Total	30	970	1000

- However, the PPV and NPV will change. Using these updated figures, the PPV is estimated at approximately 4% (20/473 x 100) and the NPV is estimated at 98% (517/527 x 100).
- Therefore, the PPV improved and the NPV deteriorated when the prevalence increased despite using the same test in the population.

Appendix 3: Testing algorithm



Key: HCV – hepatitis C virus; EIA – enzyme immunoassay; RNA – ribonucleic acid.
* In certain patient groups, initial testing should routinely incorporate HCV antigen or RNA testing. Those are: immunocompromised individuals; individuals previously treated for HCV infection; and those at risk of infection in whom an antibody response might not yet have developed (RNA testing should be performed six weeks post-exposure).
Source: Hepatitis C Screening national clinical guideline No.15[11]

Appendix 4: Classification and prognosis of cirrhosis

The Child-Pugh classification system is used to grade the severity of cirrhosis and predict the risk of mortality in patients with cirrhosis (Table 3.x).[16] Child-Pugh incorporates five parameters:

- serum bilirubin (laboratory value)
- serum albumin (laboratory value)
- prothrombin time (laboratory value)
- severity of ascites (clinical parameter)
- grade of encephalopathy (clinical parameter).

Based on the sum of these parameters, patients are categorised as Child-Pugh class A, B or C. The system has been shown to accurately predict outcomes in patients with cirrhosis and portal hypertension.[13, 101, 102]

- class A (5-6 points) – compensated
- class B (7-9 points) – decompensated
- class C (10 to 15 points) – decompensated.

Child Pugh classification for severity of cirrhosis[103]

Criteria	1 point*	2 points*	3 points*
Encephalopathy	None	Grade 1 or 2	Grade 3 or 4
Ascites	None	Mild to moderate (diuretic responsive)	Severe (diuretic refractory)
Bilirubin (mg/dL)	<2	2-3	>3
Albumin (g/dL)	>3.5	2.8-3.5	<2.8
Prothrombin time: Seconds prolonged (international normalised ratio)	<4 (<1.7)	4-6 (1.7-2.3)	>6 (>2.3)

* Patients are classified as A, B or C based on their total points

The Model for End-Stage Liver Disease (MELD) is used to estimate survival for all patients with decompensated cirrhosis.[104, 105] The higher the MELD score, the lower the estimated three-month survival.[102] The MELD score has also been used to prioritise allocation of donor organs for liver transplant. Patients with a MELD score of 15 or greater are recommended for liver transplant evaluation.[106]

The MELD score ranges from four to 60 and is based on three laboratory parameters:

- serum bilirubin

- serum creatinine
- international normalised ratio (INR).

Interpretation of MELD score

MELD score	Observed mortality rate at 3 months
≤9	1.9%
10-19	6%
20-29	19.6%
30-39	52.6%
≥40	71.3%

Key: MELD – Model for End-stage Liver Disease.

Appendix 5: Supplementary epidemiology figures and tables

All HCV notifications in Ireland compared with notifications from the 1965-1985 birth cohort by sex, from 2004 to 2018*

	All HCV notifications						HCV notifications from 1965-1985 birth cohort					
	Male		Female		Unknown		Male		Female		Unknown	
Year	N	%	N	%	N	%	N	%	N	%	N	%
2004	689	62	411	37	19	1.7	474	59	320	40	16	2.0
2005	894	64	490	35	14	1.0	635	63	372	37	9	0.9
2006	778	64	414	34	16	1.3	551	63	316	36	11	1.3
2007	972	63	542	35	23	1.5	724	64	393	35	15	1.3
2008	953	63	523	35	27	1.8	721	64	381	34	24	2.1
2009	825	67	403	33	3	0.2	607	68	290	32	2	0.2
2010	818	67	387	32	9	0.7	612	68	279	31	7	0.8
2011	807	65	422	34	5	0.4	614	66	306	33	4	0.4
2012	579	66	290	33	5	0.6	408	69	183	31	4	0.7
2013	521	69	227	30	3	0.4	380	71	153	29	1	0.2
2014	484	70	205	30	1	0.1	335	73	126	27	1	0.2
2015	454	68	216	32	2	0.3	303	70	127	29	2	0.5
2016	456	71	180	28	3	0.5	299	74	102	25	2	0.5
2017	437	72	169	28	1	0.2	290	74	101	26	1	0.3
2018	419	71	168	29	2	0.3	275	76	85	24	1	0.3
Total	10,086	66	5,047	33	133	0.9	7,228	67	3,534	33	100	0.9

Source: Health Protection Surveillance Centre

* Percentages have been rounded and may be subject to minor error.

ICD-10 codes applicable to hepatitis C morbidity data

Code	Explanation	Code	Explanation
B182	Chronic hepatitis C	K729	Hepatic failure unspecified
C22	Malignant neoplasm of liver and intrahepatic bile ducts	K740	Hepatic fibrosis
I850	Bleeding oesophageal varices	K766	Portal hypertension
K704	Alcoholic hepatic failure	K767	Hepatorenal syndrome
K720	Acute and sub-acute hepatic failure	R18	Ascites
K721	Chronic hepatic failure		

Key: ICD-10 – International Statistical Classification of Diseases and Related Health Problems Version 10

Stage of HCC at diagnosis, 2009 to 2013*

Year	Stage 1 (n)	Stage 2 (n)	Stage 3 (n)	Stage 4 (n)	Unknown (n)
2009	6	12	16	23	23

2010	<5	9	12	20	20
2011	7	16	16	24	18
2012	9	15	14	36	18
2013	20	21	14	27	22

Key: HCC – hepatocellular carcinoma.
Source: National Cancer Registry Ireland
* Many liver cancers are clinically diagnosed without subtype-specification. Only liver cancers with a HCC subtype-specification are presented.

Treatments received for HCC, 2009 to 2016*

Year	Total cases (n)	Surgery (n)	Medical oncology (n)	Radiotherapy (n)
2009	80	19	34	5
2010	64	16	23	<5
2011	81	28	24	<5
2012	92	20	35	7
2013	104	35	35	6
2014	91	28	23	5
2015	82	25	30	<5
2016***	103	32	10	9

Key: HCC – hepatocellular carcinoma.
Source: National Cancer Registry Ireland
* Many liver cancers are clinically diagnosed without subtype-specification. Only liver cancers with a HCC subtype-specification are presented.
** Numbers refer to treatments administered within 1 year of diagnosis.
*** At the time of data extraction (mid 2018) treatment data for 2016 was not complete and figures are likely to change somewhat later, particularly for medical oncology.

Five-year net survival (age-standardised) for liver cancer in Ireland

Time period	Net survival (%)	95% CI
1994-1999	5.7	3.7-8.8
2000-2005	10.8	8.4-13.9
2006-2010	13.0	10.8-15.8
2011-2015	17.6	14.7-21.0

Key: CI – confidence interval.
Source: National Cancer Registry Ireland

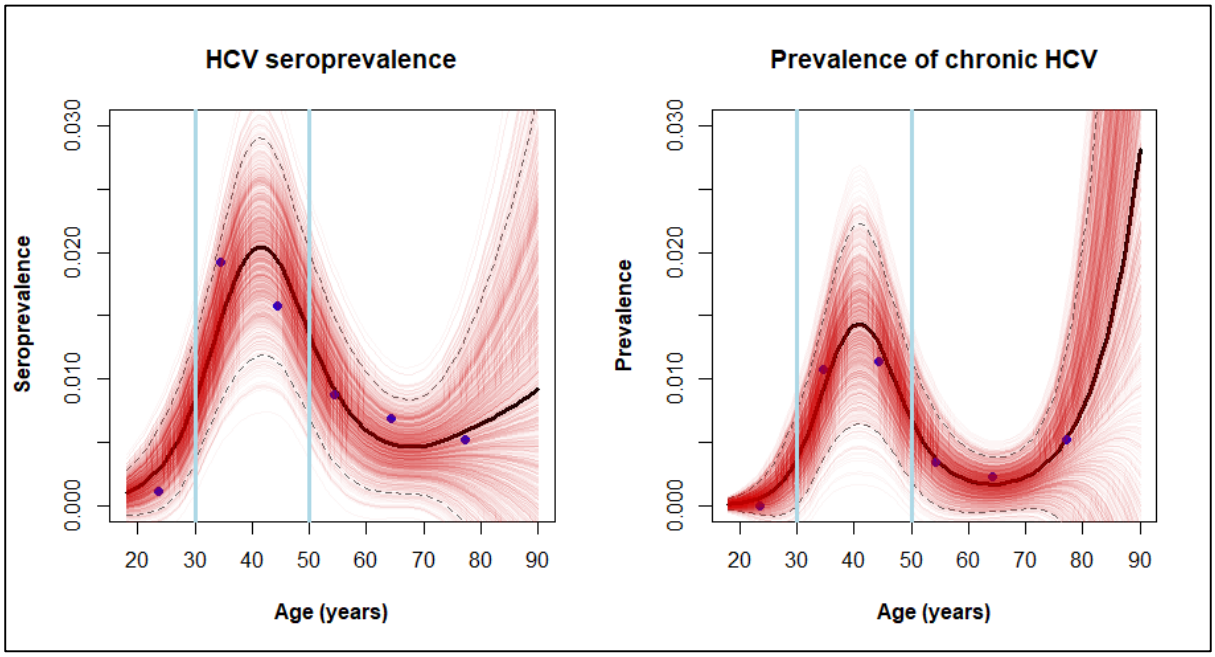
Appendix 6: Methods for estimating prevalence

The study by Garvey et al.[74] was identified as the best source of prevalence estimates for the 1965-1985 birth cohort. However, the anonymisation procedure used by Garvey et al.[74] on the patient-level data was irreversible, meaning that these data could not be disaggregated into age bands applicable to the 1965-1985 birth cohort. Therefore, an approximation method was used to generate prevalence estimates according to the appropriate age bands.

The approximation method involved the use of a generalised additive model (GAM). A GAM is a generalised linear model with a linear predictor involving a sum of smooth functions (that is, approximation to capture important patterns in the data) of covariates.[107] GAMs can be used to model non-linear relationships by fitting non-parametric smoothers to data without specification of a functional form to describe the observed non-linearity.

Four knots were used as the basis for fitting a trend line to the data observed in the Garvey study. Uncertainty in the prevalence estimates was then derived by repeated sampling of the observed mean estimates (n=6 observed point estimates). The model assumed that observations were independent and that variability in the point estimates was random rather than due to systematic bias in the sampling method. A graphical depiction of the models for approximating the seroprevalence of HCV infection and the prevalence of chronic HCV infection are presented below.

Capturing the line of best fit to approximate estimates of HCV seroprevalence and prevalence of chronic HCV infection*



Source: Results of generalised additive model fitted according to results presented by Garvey et al.[74]
*The population of interest was aged between 30 and 50 years in 2015

Two layers of uncertainty were assessed in the generalised additive model: age (weighted by CSO 2015 population estimates) and the study's sample size. The results from the generalised additive model were aggregated according to the age bands used by Garvey et al.[74] and compared against the original study to validate the model outputs. The sampled prevalence rates observed by Garvey et al.[74] were adjusted to account for weighting of the prevalence data applied in the original study. The aggregated results are presented below. No statistically meaningful differences (determined by overlap of the mean and 95% confidence intervals) were found between the estimates generated by the GAM and those by Garvey.

Comparison of HCV seroprevalence (chronic and resolved infections) results

Group	Generalised additive model		Garvey et al.[74]	
	Seroprevalence (%)	95% CI	Seroprevalence (%)	95% CI
18-29 years	0.12	0.00-0.35	0.07	0.01-0.47
30-39 years	1.96	1.10-2.93	1.94	1.21-3.10
40-49 years	1.60	0.73-2.62	1.53	0.96-2.43
50-59 years	0.88	0.18-1.76	0.83	0.33-2.09
60-69 years	0.70	0.00-1.62	0.69	0.29-1.66
70+ years	0.52	0.00-1.31	0.50	0.16-1.57
Overall	1.01	0.72-1.34	0.98	0.73-1.31

Key: CI – confidence interval.

In general, the confidence intervals estimated by the generalised additive model are wider than those estimated by Garvey et al.[74] due to uncertainty relating to the weighting applied to HCV cases within each five-year age band.

Comparison of prevalence of chronic HCV infection results

Group	Generalised additive model		Garvey et al.[74]	
	Prevalence of chronic HCV (%)	95% CI	Prevalence of chronic HCV (%)	95% CI
18-29 years	0	0-0	0	0-0
30-39 years	1.09	0.48-1.82	1.07	0.59-1.95
40-49 years	1.16	0.43-2.02	1.11	0.64-1.91
50-59 years	0.35	0.00-0.88	0.30	0.10-0.94
60-69 years	0.23	0.00-0.69	0.27	0.07-1.09
70+ years	0.52	0.00-1.31	0.50	0.16-1.57
Overall	0.59	0.35-0.85	0.57	0.40-0.81

Key: CI – confidence interval.

Appendix 7: Clinical effectiveness supplementary tables

Laboratory tests CE-marked for serological detection of anti-HCV in serum, plasma or whole-blood samples

Company	Name	Test type	Sensitivity (%)	Specificity (%)
Abbott Diagnostics	PRISM HCV assay (Abbott PRISMnEXT)	CIA	100	99.73
Abbott Diagnostics	AxSYM HCV3.0 (AxSYM Plus 5.0)	EIA	100	99.60
Abbott Diagnostics	ARCHITECT Anti-HCV (Abbott ARCHITECT i System)	CMIA	99.10	99.60
BHAT Biotech India	Hepa-Scan® HCV ELISA	EIA	>99	>98
Biokit S.A.*	Bioelisa HCV 4.0	EIA	100	99.6–99.8
Bio-Mérieux	VIDAS® Anti-HCV	EIA	99.70	>99.0
Bio-Rad Laboratories*	Monolisa® Anti-HCV PLUS Assay Version 3	EIA	100	99.90
DiaSorin	Murex anti-HCV	EIA	100	≥99.5
Fujirebio	INNOTEST® HCV Ab IV	EIA	100	99.80
HUMAN Diagnostics Worldwide	Anti-HCV ELISA (Elisys Quattro, Elisys Duo, Elisys Uno)	EIA	100	99.75
InTec® Products Inc	HCV Elisa Test Kit	EIA	99	99.80
MP Diagnostics	HCV BLOT 3.0	EIA	99.90	96.50
Roche Molecular Diagnostics*	Elecsys® Anti-HCV II Immunoassay and Elecsys® PreciControl Anti-HCV (COBAS® e 411/601/602, MODULAR ANALYTICS E170, Elecsys® 2010)	ECLIA	100	99.84
Siemens Healthcare Diagnostics	Enzygnost® Anti-HCV 4.0 (BEP® III System, BEP® 2000 Advance® System, Quadriga BeFree® System)	EIA	100	99.92

Key: CE – European Conformity; CIA – chemiluminescence assay; CMIA – chemiluminescent microparticle immunoassay; ECLIA – electrochemiluminescense immunoassay; EIA – enzyme immunoassay; HCV – hepatitis C virus.

Source: World Health Organization (additional CE-marked tests may since have become available).[14]

* Tests for detection of antibody only are presented. Assays which combine antibody and antigen detection are not presented. Estimates not verified by manufacturer.

Only tests that were CE-marked and had an estimate of sensitivity and specificity are presented.

Laboratory tests for confirmation of viraemic HCV infection in serum, plasma or whole-blood samples

Company	Name	Test target	Sensitivity	Specificity (%)
Abbott Diagnostics	i2000SR Platform/ARCHITECT Anti-HCV (Abbott ARCHITECT I System)	HCV core antigen	99.10%	99.60
Abbott Molecular	m2000 System (m2000sp (extraction) and m2000rt (amplification), RealTime Genotype II, RealTime HCV viral load assay	HCV-RNA	12 IU/mL for 0.5 mL sample; 30 IU/mL for 0.2 mL sample	100.00
Beckman Coulter	VERIS HCV ASSAY	HCV-RNA	12 IU/mL	100
Hologic®	Aptima® HCV Quant Dx Assay (Panther® System)	HCV-RNA	4.3 IU/mL for plasma; 3.9 IU/mL for serum	100
HUMAN Diagnostics Worldwide	HCV real-time PCR (HumaCycler)	HCV-RNA	9.5 IU/mL	100
QIAGEN N.V.	artus™ HCV RG RT-PCR (Rotor-Gene™ Q) artus™ HCV QS-RGQ (QIAsymphony® RGQ)	HCV-RNA	21 IU/mL	99.40
Roche Molecular Diagnostics*	COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test v2.0	HCV-RNA	15 IU/mL	100
Roche Molecular Diagnostics*	COBAS® HCV for use on the COBAS® 6800/8800 Systems	HCV-RNA	15 IU/mL	100
Sacace Biotechnologies	HCV Real-TM Quant Dx Assay (SaCycler-96™)	HCV-RNA	13 IU/mL	100
Siemens Healthcare Diagnostics	VERSANT® HCV 1.0 Assay (VERSANT® kPCR Molecular System)	HCV-RNA	15 IU/mL	100

Key: CE – European Conformity; HCV – hepatitis C virus; kPCR – kinetic polymerase chain reaction; RNA – ribonucleic acid.
Source: World Health Organization (additional CE-marked tests may since have become available).[14]
* Only tests that were CE-marked and had an estimate of sensitivity and specificity are presented. Only quantitative assays presented.

Quality appraisal of systematic reviews

AMSTAR 2 checklist item	USPSTF (2020)	WHO (2018)
Did the research questions and inclusion criteria for the review include the components of PICO?	Yes	Yes
Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Partial yes	No
Did the review authors explain their selection of the study designs for inclusion in the review?	Yes	Yes
Did the review authors use a comprehensive literature search strategy?	Yes	Yes
Did the review authors perform study selection in duplicate?	Yes	Yes
Did the review authors perform data extraction in duplicate?	Yes	Partial yes
Did the review authors provide a list of excluded studies and justify the exclusions?	Yes	Partial yes
Did the review authors describe the included studies in adequate detail?	Yes	Yes
Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	No	Yes
Did the review authors report on the sources of funding for the studies included in the review?	No	No
If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Yes	Yes
If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Yes	No
Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	No	Yes
Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	No	No
If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No	No
Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes	Yes
Quality outcome	**Critically low**	**Critically low**

Key: USPSTF – United States Preventive Services Task Force; WHO – World Health Organization.

Fixed-dose combination DAA therapies approved and recommended in Europe for treatment of chronic HCV infection

HCV genotype	Fixed-dose combination therapies (dose)	Administration
1a	Sofosbuvir (400 mg) + velpatasvir (100 mg)	Single tablet, once daily
	Glecaprevir (300 mg) + pibrentasvir (120 mg)	Three tablets (100 mg glecaprevir/40 mg pibrentasvir), once daily
	Sofosbuvir (400 mg) + ledipasvir (90 mg)	Single tablet, once daily
	Grazoprevir (100 mg) + elbasvir (50 mg)	Single tablet, once daily
1b	Sofosbuvir (400 mg) + velpatasvir (100 mg)	Single tablet, once daily
	Glecaprevir (300 mg) + pibrentasvir (120 mg)	Three tablets (100 mg glecaprevir/40 mg pibrentasvir), once daily
	Sofosbuvir (400 mg) + ledipasvir (90 mg)	Single tablet, once daily
	Grazoprevir (100 mg) + elbasvir (50mg)	Single tablet, once daily
	Ombitasvir (12.5mg) + paritaprevir (75 mg) + ritonavir (50 mg) + dasabuvir (250 mg)	Ombitasvir + paritaprevir + ritonavir in a single tablet (two tablets, once daily), and dasabuvir in a single tablet twice daily
2	Sofosbuvir (400 mg) + velpatasvir (100 mg)	Single tablet, once daily
	Glecaprevir (300 mg) + pibrentasvir (120 mg)	Three tablets (100 mg glecaprevir/40 mg pibrentasvir), once daily
3	Sofosbuvir (400 mg) + velpatasvir (100 mg)	single tablet administered once daily
	Glecaprevir (300 mg) + pibrentasvir (120 mg)	Three tablets (100 mg glecaprevir/40 mg pibrentasvir), once daily
	Sofosbuvir (400 mg) + velpatasvir (100 mg) + voxilaprevir (100 mg)	Single tablet, once daily
4	Sofosbuvir (400 mg) + velpatasvir (100 mg)	Single tablet, once daily
	Glecaprevir (300 mg) + pibrentasvir (120 mg)	Three tablets (100 mg glecaprevir/40 mg pibrentasvir), once daily
	Sofosbuvir (400 mg) + ledipasvir (90 mg)	Single tablet, once daily
	Grazoprevir (100 mg) + elbasvir (50 mg)	Single tablet, once daily
5/6	Sofosbuvir (400 mg) + velpatasvir (100 mg)	Single tablet, once daily
	Glecaprevir (300 mg) + pibrentasvir (120 mg)	Three tablets (100 mg glecaprevir/40 mg pibrentasvir), once daily
	Sofosbuvir (400 mg) + ledipasvir (90 mg)	Single tablet, once daily

Source: European Association for the Study of the Liver[13]

Key: DAA – direct-acting antiviral; HCV – hepatitis C virus; mg - microgram.

SVR rates of DAA therapies for treatment of chronic HCV infection

HCV genotype	Sofosbuvir/velpatasvir % (range)	Glecaprevir/pibrentasvir % (range)	Sofosbuvir/ledipasvir % (range)
1	99.0 (95.4- 99.8), n=3, I^2=27%	98.6 (94.1-99.7%), n=3, I^2=78%	99.4 (95.2-99.9%), n=6, I^2=89%
2	99.6 (97.6-99.95), n=3, I^2=0%	97.9 (95.0-99.1%), n=2, I^2=0%	NA
3	95.6 (87.1-98.6), n=4, I^2=82%	94.9 (90.2-97.8), n=1	NA
4	100 (95.9-100), n=1	93.5 (82.1-98.6), n=1	98.4 (93.7-99.6%), n=2, I^2=25%
5	96.6 (82.2-99.9), n=1	96.0 (76.4-99.4), n=2, I^2=0%	95.2 (76.2-99.9%) n=1
6	99.2 (94.9-99.9%), n=2, I^2=0%	97.2% (89.4% to 99.3%), n=2, I^2=42%	96.0 (79.6-99.9) n=1

Source: Agency for Healthcare Research and Quality[52]
Key: DAA – direct-acting antiviral; HCV – hepatitis C virus; I^2 – measure of heterogeneity; n – number of studies.
Combinations recommended in Ireland are highlighted.

Appendix 8: Search terms

The search terms for the economic search were modified from the clinical search undertaken in the 2016 systematic review of cost-effectiveness by Coward et al..[7] The search string was appraised using the Canadian Agency for Drugs and Technologies in Health's peer review checklist for search strings.[8]

The clinical search terms were combined with the relevant economic search filter for each database from the Scottish Intercollegiate Guidelines Network.[9] The search terms for the Medline and Embase databases are presented below. The Cochrane Library (which includes the Database of Systematic Reviews, the Database of Abstracts of Reviews of Effects (DARE), the Health Technology Assessment Database (HTA) and the National Health Service Economic Evaluation Database (NHS EED) are incorporated into Medline. Therefore, these databases were not searched separately.

Medline (via Ebsco Host)*		N=
Limiters: English Language; Human		
1.	MH Hepatitis C	43,387
2.	Hepacivirus	26,187
3.	TI ((hepatitis c OR hcv OR hepacivirus)) OR AB ((hepatitis c OR hcv OR hepacivirus)	82,156
4.	MH Hepatitis C Antigens OR MH Hepatitis C Antibodies OR MH Hepatitis C	44,890
5.	S2 OR S3 OR S4	89,495
6.	mass screening	114,937
7.	TI ((screen* OR test*)) AND AB ((screen* OR test*))	497,009
8.	S6 OR S7	566,802
9.	S5 AND S8	3,498
10.	S1 OR S9	44,794
11.	S10 AND SIGN economic search filter	2,286

Key: SIGN – Scottish Intercollegiate Guidelines Network.

*** Databases:** MEDLINE; CINAHL Plus with Full Text; Health Business Elite; PsychINFO; Library, Information Science & Technology Abstracts

Embase		N=
1.	'hepatitis c'/exp	106,192
2.	'hepatitis c virus'/exp	59,904
3.	#1 OR #2	130,330
4.	'hepatitis c antibody'/exp	8,340
5.	'hepatitis c antigen'/exp	836
6.	'hepatitis c':ab,ti OR 'hcv':ab,ti OR 'hepacivirus*':ab,ti	127,916
7.	#3 OR #4 OR #5 OR #6	154,997
8.	'screening'/exp	644,138
9.	screen*:ab,ti OR test*:ab,ti	4,548,526
10.	#8 OR #9	4,754,788
11.	#7 AND #10	40,835
12.	#3 OR #11	138,715
13.	(#3 OR #11) AND [humans]/lim AND [english]/lim AND ([article]/lim OR [article in press]/lim)	57,894
14.	#13 AND SIGN economic filter	1,975

Key: SIGN – Scottish Intercollegiate Guidelines Network.

Economic filters

Medline (via Ebsco Host)	
1.	Economics/
2.	"costs and cost analysis"/
3.	Cost allocation/
4.	Cost-benefit analysis/
5.	Cost control/
6.	Cost savings/
7.	Cost of illness/
8.	Cost sharing/
9.	"deductibles and coinsurance"/
10.	Medical savings accounts/

Medline (via Ebsco Host)	
11.	Health care costs/
12.	Direct service costs/
13.	Drug costs/
14.	Employer health costs/
15.	Hospital costs/
16.	Health expenditures/
17.	Capital expenditures/
18.	Value of life/
19.	Exp economics, hospital/
20.	Exp economics, medical/
21.	Economics, nursing/
22.	Economics, pharmaceutical/
23.	Exp "fees and charges"/
24.	Exp budgets/
25.	(low adj cost).mp.
26.	(high adj cost).mp.
27.	(health?care adj cost$).mp.
28.	(fiscal or funding or financial or finance).tw.
29.	(cost adj estimate$).mp.
30.	(cost adj variable).mp.
31.	(unit adj cost$).mp.
32.	(economic$ or pharmacoeconomic$ or price$ or pricing).tw.
33.	Or/1-32

Embase	
1.	Health economics/exp
2.	Socioeconomics/
3.	Cost benefit analysis/
4.	Cost effectiveness analysis/
5.	Cost minimi?ation analysis/
6.	Cost of illness/
7.	Cost control/
8.	Economic aspect/
9.	Financial management/
10.	Health care cost/
11.	Health care financing/
12.	hospital cost/
13.	(fiscal or financial or finance or funding):ti,ab.
14.	((cost NEXT/1 variable*) OR (cost NEXT/1 estimate*) OR (unit NEXT/1 cost*)):ab,ti
15.	or/1-14

Appendix 9: Grey literature search

The following electronic sources were searched for economic evaluations relevant to the research questions of this systematic review:

- Centre for Health Economics and Policy Analysis (CHEPA); Available from http://www.chepa.org/
- Cost Effectiveness Analysis Registry; Available from http://healtheconomics.tuftsmedicalcenter.org/cear4/SearchingtheCEARegistry/SearchtheCEARegistry.aspx
- HTAi vortal; Available from https://www.htai.org/index.php?id=579
- Google Scholar and Google; Available from https://scholar.google.com/, https://www.google.ie
- Health Service Executive (HSE); Available from https://www.hse.ie/eng/
- Health Information and Quality Authority (HIQA); Available from https://www.hiqa.ie/
- Health Research Board (HRB) Ireland; Available from http://www.hrb.ie/home/
- Institute of Health Economics (Alberta Canada); Available from https://www.ihe.ca/
- Lenus; Available from http://www.lenus.ie/hse/
- National Coordinating Centre for Health Technology Assessment (NCCHTA) ; Available from https://www.nihr.ac.uk/funding-and-support/funding-for-research-studies/funding-programmes/health-technology-assessment/
- National Centre for Pharmacoeconomics (NCPE); Available from http://www.ncpe.ie/
- National Institute for Health and Clinical Excellence (NICE); Available from https://www.nice.org.uk/
- NHS Evidence database (UK); Available from https://www.evidence.nhs.uk/
- Open Grey; Available from http://www.opengrey.eu/
- World Health Organization (WHO); Available from http://www.who.int/en/

Appendix 10: Modelled treatment regimens

Study	Genotype	Fibrosis	Treatment	Duration	SVR (%)	Cost (Irish 2019 €)
Barocas (2018)	1-6	F0-F3	SOF+VEL	12 weeks	99	€58,177
Barocas (2018)	1-6	F4	SOF+VEL	12 weeks	93	€58,177
Buti (2018)	1-6	F0-F4	SOF+VEL	NA	98	€31,493
Coffin (2012)	1	F0-F3	Antiviral therapy (unspecified)	NA	46	€32,836
Coffin (2012)	2/3	F0-F3	Antiviral therapy (unspecified)	NA	80	€20,341
Coffin (2012)	1	F4	Antiviral therapy (unspecified)	NA	20	€32,836
Coffin (2012)	2/3	F4	Antiviral therapy (unspecified)	NA	43	€20,341
Crespo(2019)	NA	NA	DAAs (unspecified)	NA	96	€9,122
Deuffic-Burban (2018)	NA	F0-F4	DAA (unspecified)	12 weeks	95	€31,259
Dimitrova (2019)	NA	F0-F2	Antiviral therapy (unspecified)	8 weeks	95	€19,930
Dimitrova (2019)	NA	F3-F4	Antiviral therapy (unspecified)	12 weeks	95	€30,481
Eckman (2013)	2-6	F0-F3	INF+RBV	24 weeks	87	€21,674
Eckman (2013)	2-6	F4	INF+RBV	24 weeks	75	€21,674
Eckman (2013)	1	NA	INF+RBV+BOC	28 weeks	88	€57,122
Eckman (2013)	1	NA	INF+RBV+BOC	48 weeks	NA	€101,487
Eckman (2013)	1	NA	INF+RBV+TVR	28 weeks	92	€78,537
Eckman (2013)	1	NA	INF+RBV+TVR	48 weeks	64	€101,841
Eckman (2018)	1a	F0-F4	SOF+VEL	12 weeks	98	€19,939
Eckman (2018)	1b	F0-F4	SOF+VEL	12 weeks	99	€19,939
Eckman (2018)	2	F0-F4	SOF+VEL	12 weeks	99	€19,939

Study	Genotype	Fibrosis	Treatment	Duration	SVR (%)	Cost (Irish 2019 €)
Eckman (2018)	3	F0-F4	SOF+VEL	12 weeks	93	€19,939
Eckman (2018)	4	F0-F4	SOF+VEL	12 weeks	100	€19,939
Eckman (2018)	1a	F0-F3	GLE+PIB	8 weeks	99	€16,152
Eckman (2018)	1b	F0-F3	GLE+PIB	8 weeks	99	€16,152
Eckman (2018)	2	F0-F3	GLE+PIB	8 weeks	98	€16,152
Eckman (2018)	3	F0-F3	GLE+PIB	8 weeks	95	€16,152
Eckman (2018)	4	F0-F3	GLE+PIB	8 weeks	99	€16,152
Eckman (2018)	1a	F4	GLE+PIB	12 weeks	98	€24,227
Eckman (2018)	1b	F4	GLE+PIB	12 weeks	99	€24,227
Eckman (2018)	2	F4	GLE+PIB	12 weeks	99	€24,227
Eckman (2018)	3	F4	GLE+PIB	12 weeks	100	€24,227
Eckman (2018)	4	F4	GLE+PIB	12 weeks	100	€24,227
Eckman (2018)	1-6	F0-F3	SOF+VEL+VOX	12 weeks	93	€47,531
Eckman (2018)	1-6	F4	SOF+VEL+VOX	12 weeks	99	€47,531
Ethgen (2017)	NA	F2-F4	IFN+RBV+PI	NA	NA	€17,905
Ethgen (2017)	NA	F2-F4	IFN+RBV+SOF	12 weeks	NA	€48,039
Ethgen (2017)	NA	F2-F4	OBV+PTV+RTV+DAS	12 or 24 weeks	NA	€46,792
Ethgen (2017)	NA	F0-F4	OBV+PTV+RTV+DAS	12 or 24 weeks	NA	€46,792
Kim (2017)	1 (non-1b)	F0-F3	DCV+SOF	12 weeks	100	€19,012
Kim (2017)	1 (non-1b)	F0-F3	LDV+SOF	12 weeks	100	€18,999
Kim (2017)	1 (non-1b)	F4	DCV+SOF	12 weeks	91	€19,012
Kim (2017)	1 (non-1b)	F4	LDV+SOF+RBV	12 weeks	100	€19,021

Study	Genotype	Fibrosis	Treatment	Duration	SVR (%)	Cost (Irish 2019 €)
Kim (2017)	1b	F0-F4	DCV+ASV	24 weeks	96	€6,513
Kim (2017)	1b	F0-F3	DCV+SOF	12 weeks	100	€19,012
Kim (2017)	1b	F0-F3	LDV+SOF	12 weeks	100	€18,999
Kim (2017)	1b	F4	DCV+SOF	12 weeks	91	€19,012
Kim (2017)	1b	F4	LDV+SOF+RBV	12 weeks	100	€19,021
Kim (2017)	2	F0-F3	SOF+RBV	12 weeks	97	€16,435
Kim (2017)	2	F4	SOF+RBV	16 weeks	100	€21,915
Kim (2018)	1b	F0-F4	DCV+ASV	24 weeks	95	€6,389
Kim (2018)	1	F0-F4	LDV+SOF	12 weeks	99	€16,145
Kim (2018)	2	F0-F3	SOF+RBV	12 weeks	95	€16,209
Kim (2018)	2	F4	SOF+RBV	16 weeks	95	€23,037
Kim (2019)	NA	NA	DAAs (unspecified)	NA	95	€8,422
Kim (2020)	1/2	NA	LDV+SOF	12 weeks*	NA	€10,149
Kim (2020)	2	NA	SOF+RBV	12 weeks*	NA	€15,224
Kim (2020)	1/2	NA	GLE+PIB	12 weeks*	NA	€9,889
Kondili (2020)	NA	F0-F4	DAA (unspecified)	NA	NA	€3,331
Liu (2013)	1	F0-F2	INF+RBV	Unclear	NA	NA
Liu (2013)	1	F0-F2	INF+RBV+PI (unspecified)	Unclear	NA	NA
Liu (2013)	2/3	F0-F2	INF+RBV+PI (unspecified)	Unclear	80	NA
Liu (2013)	2/3	F3-F4	INF+RBV+PI (unspecified)	Unclear	64	NA
McEwan (2013)	1	F0-F2	NA	12 weeks	78	NA
McEwan (2013)	1	F3-F4	NA	12 weeks	62	NA

Study	Genotype	Fibrosis	Treatment	Duration	SVR (%)	Cost (Irish 2019 €)
McEwan (2013)	2/3	F0-F2	NA	12 weeks	76	NA
McEwan (2013)	2/3	F3-F4	NA	12 weeks	61	NA
McEwan (2013)	2/3	F4	NA	12 weeks	57	NA
McGarry (2012)	1	F0-F2	INF+RBV+PI (unspecified)	12-48 weeks	78	NA
McGarry (2012)	1	F3	INF+RBV+PI (unspecified)	12-48 weeks	62	NA
McGarry (2012)	1	F4	INF+RBV+PI (unspecified)	12-48 weeks	62	NA
McGarry (2012)	2/3	F0-F2	INF+RBV+PI (unspecified)	12-48 weeks	76	NA
McGarry (2012)	2/3	F3	INF+RBV+PI (unspecified)	12-48 weeks	61	NA
McGarry (2012)	2/3	F4	INF+RBV+PI (unspecified)	12-48 weeks	57	NA
Mendlowitz (2020)	3	F0-F3	SOF+VEL	12 weeks	97	€40,078
Mendlowitz (2020)	3	F4	SOF+VEL	12 weeks	91	€40,078
Mendlowitz (2020)	1-2,4-6	F0-F3	SOF+VEL	12 weeks	99	€40,078
Mendlowitz (2020)	1-2,4-6	F4	SOF+VEL	12 weeks	99	€40,078
Nagai (2020)	1	F0-F3	IFN+RBV+SMV	24 weeks	89	€17,420
Nagai (2020)	1	F4	IFN+RBV	48 weeks	19	€17,593
Nagai (2020)	2	F0-F3	IFN+RBV	24 weeks	79	€8,797
Nagai (2020)	2	F4	IFN+RBV	48 weeks	83	€17,593
Nagai (2020)	1	F0-F3	LDV+SOF	12 weeks	100	€36,011
Nagai (2020)	1	F0-F3	OBV+PTV+RTV	12 weeks	94	€30,305
Nagai (2020)	1	F0-F3	DCV+ASV	24 weeks	87	€17,872
Nagai (2020)	1	F4	LDV+SOF	12 weeks	100	€36,011
Nagai (2020)	1	F4	OBV+PTV+RTV	12 weeks	91	€30,305

Study	Genotype	Fibrosis	Treatment	Duration	SVR (%)	Cost (Irish 2019 €)
Nagai (2020)	1	F4	DCV+ASV	24 weeks	87	€17,872
Nagai (2020)	2	F0-F3	RBV+SOF	12 weeks	98	€29,283
Nagai (2020)	2	F4	RBV+SOF	12 weeks	98	€29,283
Nakamura (2008)	1	F0-F3	IFN+RBV	48 weeks	80	€32,155
Nakamura (2008)	1	F0-F3	IFN+RBV	72 weeks	29	€34,145
Nakamura (2008)	2/3	F0-F3	IFN+RBV	24 weeks	71	€20,084
Opstaele (2019)	1	F0-F4	DAAs (unspecified)	NA	NA	€43,974
Rein (2012)	1	NA	IFN+RBV	NA	33	€12,144
Rein (2012)	1	NA	IFN+RBV+DAA (unspecified)	12 weeks	54	€48,468
Rein (2012)	2/3	NA	IFN+RBV	NA	69	€6,480
Ruggeri (2013)	1/4	NA	IFN+RBV	NA	46	€17,181
Ruggeri (2013)	2/3	NA	IFN+RBV	NA	76	€17,181
Ruggeri (2013)	1/4	NA	IFN+RBV	NA	36	€17,181
Ruggeri (2013)	2/3	NA	IFN+RBV	NA	61	€17,181
Williams (2019)	1-6	F0-F3	1st line DAAs (unspecified)	NA	93	€12,393
Williams (2019)	1-6	F4	1st line DAAs (unspecified)	NA	91	€12,393
Williams (2019)	1-6	F0-F3	2nd line DAAs (unspecified)	NA	94	€18,589
Williams (2019)	1-6	F4	2nd line DAAs (unspecified)	NA	86	€18,589
Wong (2015)	1	F0-F3	IFN+RBV+SMV	24 week	96	€32,721
Wong (2015)	1	F4	IFN+RBV+SMV	48 weeks	94	€39,565
Wong (2015)	1	F0-F3	IFN+RBV+PTV	NA	96	€35,446
Wong (2015)	1	F4	IFN+RBV+PTV	NA	94	€35,446

Study	Genotype	Fibrosis	Treatment	Duration	SVR (%)	Cost (Irish 2019 €)
Wong (2015)	1	F0-F2	IFN+RBV	48 weeks	49	€14,141
Wong (2015)	1	F3-F4	IFN+RBV	48 weeks	37	€14,141
Wong (2015)	2/3	NA	IFN+RBV	24 weeks	77	€7,071
Wong (2015)	4-6	NA	IFN+RBV	48 weeks	65	€14,141
Wong (2015)	2	F0-F3	IFN+RBV+SOF	12 weeks	97	€38,990
Wong (2015)	2	F4	IFN+RBV+SOF	12 weeks	100	€38,990
Wong (2015)	3	F0-F3	IFN+RBV+SOF	24 weeks	95	€38,990
Wong (2015)	3	F4	IFN+RBV+SOF	24 weeks	92	€38,990
Wong (2017)	1	F0-F3	OBV+PTV+RTV+DAS	12 weeks	95	€38,997
Wong (2017)	1	F0-F3	LDV+SOF	12 weeks	97	€46,774
Wong (2017)	1	F4	OBV+PTV+RTV+DAS	12 weeks	94	€38,997
Wong (2017)	1	F4	LDV+SOF	12 weeks	95	€46,774
Wong (2017)	2	F0-F3	RBV+SOF	12 weeks	95	€38,397
Wong (2017)	2	F4	RBV+SOF	12 weeks	85	€38,397
Wong (2017)	3	F0-F3	RBV+SOF	24 weeks	93	NA
Wong (2017)	3	F0-F3	DCV+SOF	12 weeks	97	NA
Wong (2017)	3	F4	RBV+SOF	24 weeks	91	NA
Wong (2017)	4-6	NA	IFN+RBV	48 weeks	65	€13,317
Wong (2017)	4-6	NA	SOF+VEL	12 weeks	NA	NA
Younossi (2017)	1	F0	Hypothetical DAAs	12 weeks	97	€62,962
Younossi (2017)	1	F1	Hypothetical DAAs	12 weeks	97	€62,962
Younossi (2017)	1	F2	Hypothetical DAAs	12 weeks	97	€62,962

Study	Genotype	Fibrosis	Treatment	Duration	SVR (%)	Cost (Irish 2019 €)
Younossi (2017)	1	F3	Hypothetical DAAs	12 weeks	97	€62,962
Younossi (2017)	1	F4	Hypothetical DAAs	12 weeks	100	€62,962
Younossi (2017)	2	F0	Hypothetical DAAs	12 weeks	99	€62,962
Younossi (2017)	2	F1	Hypothetical DAAs	12 weeks	99	€62,962
Younossi (2017)	2	F2	Hypothetical DAAs	12 weeks	99	€62,962
Younossi (2017)	2	F3	Hypothetical DAAs	12 weeks	99	€62,962
Younossi (2017)	2	F4	Hypothetical DAAs	12 weeks	99	€62,962
Younossi (2017)	3	F0	Hypothetical DAAs	12 weeks	99	€62,962
Younossi (2017)	3	F1	Hypothetical DAAs	12 weeks	99	€62,962
Younossi (2017)	3	F2	Hypothetical DAAs	12 weeks	99	€62,962
Younossi (2017)	3	F3	Hypothetical DAAs	12 weeks	99	€62,962
Younossi (2017)	3	F4	Hypothetical DAAs	12 weeks	92	€62,962
Younossi (2017)	4	F0	Hypothetical DAAs	12 weeks	100	€62,962
Younossi (2017)	4	F1	Hypothetical DAAs	12 weeks	100	€62,962
Younossi (2017)	4	F2	Hypothetical DAAs	12 weeks	100	€62,962
Younossi (2017)	4	F3	Hypothetical DAAs	12 weeks	100	€62,962
Younossi (2017)	4	F4	Hypothetical DAAs	12 weeks	100	€62,962
Younossi (2017)	5/6	F0	Hypothetical DAAs	12 weeks	98	€62,962
Younossi (2017)	5/6	F1	Hypothetical DAAs	12 weeks	98	€62,962
Younossi (2017)	5/6	F2	Hypothetical DAAs	12 weeks	98	€62,962
Younossi (2017)	5/6	F3	Hypothetical DAAs	12 weeks	98	€62,962
Younossi (2017)	5/6	F4	Hypothetical DAAs	12 weeks	100	€62,962

Key: ASV – Asunaprevir; BOC – Boceprevir; DAA – direct-acting antiviral; DAS – Dasabuvir; DCV – Daclatasvir; F – fibrosis level; GLE – Glecaprevir; IFN – Interferon; LDV – Ledipasvir; NA – not available; OBV – Ombitasvir; PI – protease inhibitor; PIB – Pibrentasvir; PTV – Paritaprevir; RBV – Ribavirin; RTV – Ritonavir; SMV – Simeprevir; SOF – Sofosbuvir; TVR – Telaprevir; VEL – Velpatasvir; VOX – Voxilaprevir.
* Assumption.

Appendix 11: Decision tree equations

Formulae to predict outcomes of birth cohort testing strategy

Antibody test (one-step strategy)		Antibody test + test for confirmation of viraemic infection (two-step strategy)	
TP1	N x sero x snAb	TP2	TP1 x (chronic ÷ sero) x snCAg
TN1	(N x (1 – sero) x spAb	TN2	(TP1 x (1 – (chronic ÷ sero)) x spCAg) + (FP1 x spCAg))
FP1	N x (1 – sero) x (1 – spAb)	FP2	(TP1 x (1 – (chronic ÷ sero)) x (1 – spCAg)) + (FP1 x (1 – spCAg))
FN1	N x sero x (1 – snAb)	FN2	TP1 x (chronic ÷ sero) x (1 – snCAg)
PPV1	TP1 ÷ (TP1 + FP1)	PPV2	TP2 ÷ (TP2 + FP2)
NPV1	TN1 ÷ (TN1 + FN1)	NPV2	TN2 ÷ (TN2 + FN2)
R1	TP1 ÷ FP1	R2	TP2 ÷ FP2

Key: Ab – antibody; cAg – core antigen; chronic – prevalence of chronic infection; FN – false negative; FP – false positive; N – population; NPV – negative predictive value. ; PPV – positive predictive value; R – ratio of true to false positive tests; sero – seroprevalence; sn – sensitivity; sp – specificity; TN – true negative; TP – true positive.

Appendix A: Systematic review and meta-analysis of diagnostic accuracy of laboratory-based HCV tests using dried blood spot samples

Key points

- The conventional approach to the diagnosis of chronic HCV infection requires sequential healthcare attendances to provide samples and receive a diagnosis, thus increasing the risk of patient drop-off along the clinical pathway, which may culminate in a missed opportunity for diagnosis and treatment. Reflex testing, whereby the second test (to detect active infection) is performed on the same sample used for the serological test, offers a potential solution to this challenge. However, in order to prevent degradation of venous serum or plasma samples, reflex testing necessitates centrifugation, freezing and cold-chain storage of samples within six to 24 hours following phlebotomy.
- Testing of dried blood spot (DBS) samples, which involves depositing a finger prick of whole blood on filter paper (as opposed to a conventional blood sample collected by venepuncture), is a potential mechanism for enabling reflex testing that circumvents the need for venepuncture, centrifugation and freezing of samples. A systematic review and meta-analysis was undertaken to assess the diagnostic accuracy of DBS samples compared with conventional blood samples for detection of HCV using laboratory-based tests (that is, anti-HCV, RNA and core antigen).
- The sensitivity and specificity of anti-HCV in DBS was estimated at 0.95 (95% CI: 0.92 to 0.97) and 0.98 (95% CI: 0.98 to 0.99), respectively, compared with anti-HCV in serum, plasma or whole-blood. The sensitivity and specificity of HCV-RNA in DBS was estimated at 0.95 (95% CI: 0.93 to 0.97) and 0.97 (95% CI: 0.94 to 0.98), respectively, compared with HCV-RNA in serum, plasma or whole-blood. The sensitivity and specificity of core antigen in DBS was estimated at 0.87 (95% CI: 0.80 to 0.91) and 0.99 (95% CI: 0.96 to 1.00), respectively, compared with HCV-RNA in serum, plasma or whole-blood.
- Although the results of the meta-analysis support the use of DBS for HCV testing, the international evidence was of variable quality and is not directly applicable given that many of the studies were conducted in at-risk populations in developing countries. The use of DBS would require independent validation in the healthcare setting of intended use prior to incorporation in a birth cohort testing programme.

A1 Introduction

A.1.1 Background

Diagnosis of chronic HCV infection typically involves:

1. an initial serological test to indicate an antibody response following exposure to HCV (that is, anti-HCV positive)
2. if anti-HCV positive, a second test to verify active HCV infection using either a nucleic acid amplification technology test ((NAAT) to detect viral ribonucleic acid (RNA)) or a core antigen test (to detect the HCV viral protein).

Both tests are based on venous blood samples (that is, serum or plasma) obtained by venepuncture.[1] The conventional approach requires separate samples which means that two healthcare visits are required to provide samples, increasing the risk of patient drop-off along the clinical pathway, which may culminate in a missed opportunity for diagnosis and treatment.[2] Reflex testing, whereby the second test is performed on the same sample used for the initial serological test, offers a potential solution to this challenge. However, in order to prevent degradation of venous serum or plasma samples prior to their being processed, reflex testing necessitates centrifugation, freezing and cold-chain storage of samples within six to 24 hours following phlebotomy.[3, 4]

Dried blood spot (DBS) testing, which involves depositing finger pricks of whole blood on filter paper, may facilitate reflex testing.[2] As DBS can be prepared using capillary blood, it circumvents the need for venepuncture, centrifugation and freezing of samples.[3] Despite its clear logistical advantages, the diagnostic accuracy of laboratory assays using DBS compared with that of conventional blood samples for diagnosis of chronic HCV infection is subject to uncertainty. Determination of diagnostic accuracy is a key step in informing the clinical effectiveness of incorporating DBS testing into the diagnostic pathway with the aim of correctly and efficiently identifying people with currently undiagnosed chronic HCV infection.

A1.2 Description of the intervention

For collection of DBS samples, a skin puncture on the person's fingertip is made with a retractable lancet or a finger puncture device. Drops of blood are then applied to filter paper and dried at room temperature for up to four hours.[5] After drying, the blood remains stable on the DBS card and can be inserted into moisture-protected packaging for transportation to a centralised laboratory. In the laboratory, the DBS is

manually extracted from the card using a sterile hole-puncher and the whole-blood is eluted, the sample is then run using the standard automated platforms.[2, 4, 5] DBS facilitates the sampling process by avoiding venepuncture (that is, it is less invasive), removing the need to separate serum and plasma, requiring smaller volumes of blood and blood components, and obviating the need for cold-chain storage.[2, 6-8] As multiple spots can be collected at once, reflex RNA or core antigen testing can be undertaken using the second or third spot (where the initial spot is anti-HCV positive).[9]

A1.3 Purpose of the systematic review

The aim of this systematic review was to assess the diagnostic accuracy of DBS samples compared with venous blood samples for detection of HCV using laboratory-based tests (that is, anti-HCV, RNA and core antigen).

A2 Methods

A protocol, developed in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) criteria,[10] was prospectively registered with PROSPERO and the Health Research Board Open Research. The reporting of this systematic review adheres to the PRISMA of Diagnostic Test Accuracy studies (PRISMA-DTA) criteria,[11] and conforms to national Health Technology Assessment (HTA) guidelines for evaluating the clinical effectiveness of health technologies.[12]

A2.1 Review question

The systematic review question was formulated using the Population, Index test, Reference test, Diagnosis (PIRD) framework and presented in Table A1.[13] The systematic review sought to answer:

- What is the diagnostic accuracy of laboratory-based HCV testing using DBS compared with venous blood (whole blood, serum or plasma) samples among patients with chronic or resolved HCV infection?

Table A1. Systematic review question defined using PIRD framework

Population	Adults exposed to, having or suspected of having chronic HCV
Index test	DBS tested for the presence of anti-HCV antibodies, HCV-RNA or HCV core antigen in a laboratory setting*
Reference test	Venous blood (serum, plasma or whole blood) samples tested for the presence of anti-HCV antibodies, HCV-RNA or HCV core antigen in a laboratory setting
Diagnosis of interest	Diagnosis of chronic or resolved HCV infection

Key: DBS – dried blood spot; HCV – hepatitis C virus; RNA – ribonucleic acid.
* In a deviation from the original protocol, the scope was extended to include DBS using a venous

sample which can be done as part of a routine blood sample collection.

A2.2 Inclusion and exclusion criteria

Inclusion criteria

Cross-sectional and case-control studies which compared the index test (based on a DBS sample) with the reference test (based on a serum, plasma or whole blood sample) in the population of interest were included in the systematic review. Studies were eligible for inclusion if they assessed the detection of at least one anti-HCV antibody, HCV-RNA or HCV core antigen using a DBS sample, and reported sufficient data to estimate sensitivity and specificity (that is, sufficient data must be presented to construct 2x2 contingency tables to calculate the number of true positives (TPs), true negatives (TNs), false positives (FPs) and false negatives (FNs)).

Exclusion criteria

The following exclusion criteria were applied:

- studies in children only
- studies that presented insufficient data to construct 2x2 contingency tables to calculate the number of TPs, TNs, FPs and FNs
- point-of-care tests conducted outside of laboratory settings that used DBS samples
- studies where DBS results had not been compared against a reference standard method in serum, plasma or whole blood samples
- letters to the editor, case reports, commentaries, expert opinion, conference abstracts and literature reviews
- animal studies
- studies where an English translation could not be retrieved.

A2.3 Search methods

Electronic searches were conducted in PubMed, Embase, Scopus, Web of Science, Lilacs and the Cochrane library (which includes the Database of Systematic Reviews, the Database of Abstracts of Reviews of Effects (DARE), the HTA and the National Health Service Economic Evaluation Database (NHS EED)) up to July 17 2020, supplemented by a grey literature search of national and international electronic sources. The search terms were based on those used in a previously published systematic review and are in line with Cochrane guidance for identifying diagnostic accuracy studies.[6, 14] Forward citation searching and hand-searching of the reference lists of included studies were also undertaken. The full search strategy is presented in Appendix A1.1.

A2.4 Data collection and analysis

Selection of studies

All citations (titles and abstracts) as well as full texts of potentially eligible studies were screened independently by two reviewers as per the inclusion criteria, with disagreements resolved by discussion. Screening was undertaken using EndNote X8 software.

Data extraction and management

Data extraction was performed independently using Microsoft Excel software by two people with disagreements resolved by discussion. A standardised data extraction template, based on the standards for the reporting of diagnostic accuracy studies (STARD) checklist,[15] was developed prior to undertaking the systematic review. Key data extracted include:

- population characteristics (country, sample size, age, gender, HCV genotype, fibrosis levels, and HCV risk factors such as HCV/HIV co-infection, sexual orientation, intravenous drug misuse)
- index test (assay type, manufacturer assay, cut-off and limit of detection, viral load threshold, filter paper)
- reference test (assay type, manufacturer assay, cut-off and limit of detection)
- outcomes (TPs, FPs, TNs, FNs)
- setting (healthcare setting of sample collection, storage conditions and transportation, spoilage)
- author conflicts of interest.

Where cut-off or threshold values were not specified in the journal article, authors were contacted and manufacturer websites were reviewed for further information.

Risk of bias assessment

The methodological quality of included studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool.[16] Study risk of bias in each domain of QUADAS-2 was graded as low, high or some concerns (moderate). The risk of bias assessment was conducted independently by two reviewers, with disagreements resolved by discussion.

GRADE certainty of the evidence assessment

GRADE summary of findings tables were developed using GRADEpro software. The body of evidence was independently assessed by two reviewers for each primary outcome (that is, sensitivity and specificity) according to risk of bias, inconsistency, indirectness, imprecision and publication bias in accordance with previously published GRADE guidance.[17-19] The overall certainty of the evidence was graded accordingly (high, moderate, low, very low).

Data synthesis and statistical analysis

All statistical analyses were conducted in R Studio version 4.0.2 using the *meta* and *mada* packages.[20, 21]

Study-level means and 95% confidence intervals of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (PLR), and diagnostic odds ratios (DORs) were estimated for each pair of diagnostic outcomes in each included study. Definitions of these outcomes are presented in Table A2.

To facilitate meta-analysis (that is, to ensure independence of the included studies), one pair of diagnostic outcomes were selected from each study. Where it was unclear which of the reported data pairs should be included as the primary study analysis, a conservative approach was adopted by selecting the data pair which produced the lowest estimate of sensitivity and specificity. Diagnostic outcomes reported at the threshold specified by the manufacturer were included when available.

Table A2 Definitions of diagnostic outcomes presented

Outcome	Calculation	Definition (interpretation)
Sensitivity	TP/(TP+FN)	Proportion of all people who have the disease that receive a positive index test result (sn=0: no diagnostic value; sn=1: perfect test)
Specificity	TN/(TN+FP)	Proportion of all people who do not have the disease that receive a negative index test result (sp=0: no diagnostic value; sp=1: perfect test)
PLR	Sn/(1-Sp)	The odds of a positive test result in an individual who has the disease compared with that of a non-diseased individual (PLR=1 no diagnostic value; PLR >10: reliably rules in disease)
NLR	(1-Sn)/Sp	The odds of a negative test result in an individual who has the disease compared with that of a non-diseased individual (NLR=1 no diagnostic value; NLR < 0.1 reliably rules out disease)
PPV*	(TP/(TP+FP)	Proportion of all people that receive a positive index test result that actually have the disease (PPV=0 has no clinical value; PPV=1 clinical value is equivalent to the reference standard)
NPV*	(TN/(TN+FN)	Proportion of all people that receive a negative index test result that do not have the disease (NPV=0 has no clinical value; NPV=1 clinical value is equivalent to the reference standard)
DOR	(TP x TN) / (FP x FN)	The odds of a positive test result in an individual with the disease compared with that of a non-diseased individual (DOR ≥0 and ≤1: ; DOR=1 no diagnostic value)

Key: DOR – diagnostic odds ratio; FN – false negative; FP – false positive; NLR – negative likelihood ratio; NPV – negative predictive value; PLR – positive likelihood ratio; PPV – positive predictive value; sn – sensitivity; sp – specificity; TP – true positive; TN – true negative.

* PPV and NPV are prevalence-dependent measures of diagnostic accuracy (that is, PPV will increase and NPV will decrease if prevalence increases, holding all else equal). A worked example of this interaction is presented in Appendix 3 of Chapter 2.

Pooled estimates were derived by meta-analysis, using both univariate and bivariate approaches. As the observed heterogeneity was generally larger than would be expected by chance, a random-effects model (which estimates the average rather than common effect) was employed in the meta-analysis. In the univariate analysis, a random-effects model was used to estimate sensitivity, specificity, log-transformed PLRs, NLRs and log-transformed DORs. Between-study variance was estimated in the univariate analysis using the Mantel-Haenszel method for calculation of Cochran's Q test and the inconsistency index (I^2), with the DerSimonian-Laird estimator used for calculation of tau^2. A continuity correction of 0.5 was applied to included studies with zero cell frequencies.

In the bivariate analysis a linear mixed-effects model, with known variances of the random effects, was used to account for correlation between pairs of sensitivity and specificity.[22] Variance components were estimated by restricted maximum likelihood. Pooled log-transformed DORs, log-transformed PLRs and NLRs were estimated from Markov Chain Monte Carlo (MCMC) sampling based on the parameters fitted to the bivariate model.[23]

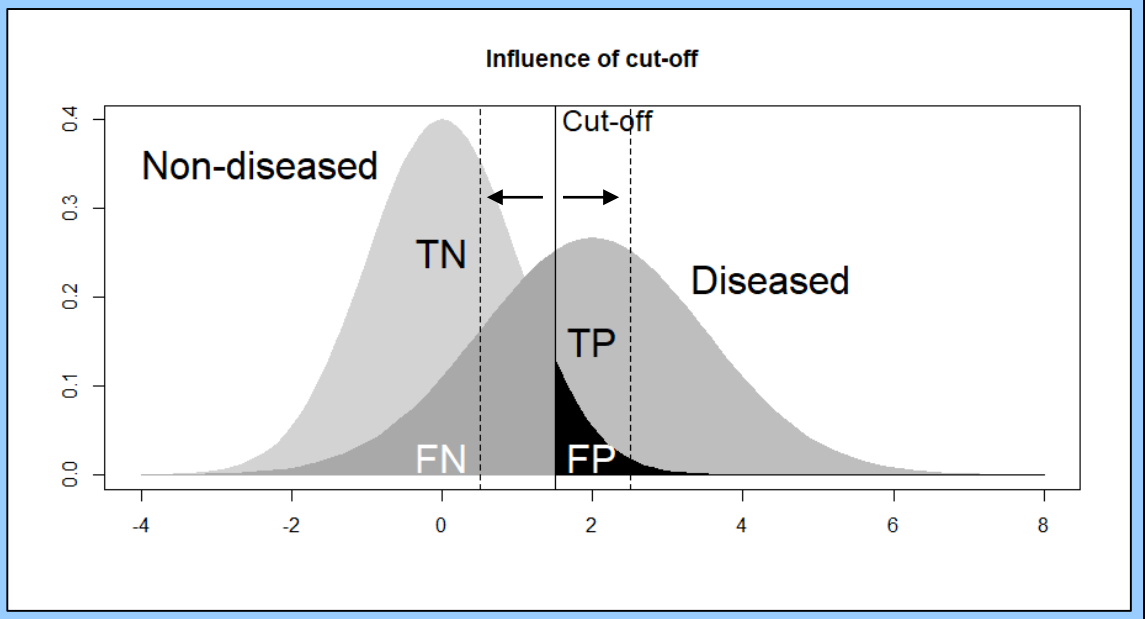
Within each of the HCV detection categories (that is, anti-HCV, HCV-RNA and HCV core antigen), subgroup analyses were performed according to the type of laboratory assay (that is, enzyme-linked immunoassay (ELISA), chemiluminescence assay (CLIA), reverse-transcription polymerase chain reaction (RT-PCR), transcription-mediated amplification (TMA), etc.) and according to commonly reported threshold values. The application and influence of a threshold on diagnostic accuracy is outlined in Box A.1.

Sensitivity analysis was also undertaken to investigate the impact of study design, risk of bias and small-study effects (publication bias). Small-study effects were assessed using Deek's regression test of asymmetry, visually inspected with Deek's funnel plot and further investigated using the trim-and-fill method.[24-27] Deek's funnel plot presents the log-transformed DOR against the inverse of the square root of the effective sample size. The trim-and-fill method involves first trimming the studies that cause asymmetry (to minimise the impact of publication bias) and then imputing missing studies based on the bias-corrected overall estimate.

Box A.1. Application of a threshold to determine diagnostic test accuracy

- A **threshold or cut-off** (for example, a continuous biomarker) is generally applied to distinguish diseased from non-diseased individuals. Typically, a person's test result is interpreted as positive when their result is above the threshold or negative when their result is below the threshold.
- As illustrated in the hypothetical example in Figure A1, sensitivity increases and specificity decreases if the threshold is moved to the left. This is because the total number of positive results increases leading to a higher true positive rate, but also a higher false positive rate.
- In contrast, if the threshold moves to the right sensitivity decreases and specificity increases. This is because the total number of negative results increases leading to a higher true negative rate, but also a higher false negative rate.
- It follows that, for each possible cut-off, there is a different 2x2 table, and therefore a distinct pair of sensitivity and specificity. It also implies a trade-off between sensitivity and specificity when these outcomes are negatively correlated within a study.

Figure A1. Influence of a threshold on sensitivity and specificity



Source: Adapted from Schwarzer et al.[28]

A2.5 Overview of laboratory-based assay principles

The included studies are described in section A3.1 to A3.3 according to the detection target of the test under assessment (that is, anti-HCV, HCV-RNA or core antigen). Alternative laboratory-based tests exist within each of these categories which are distinguished by their test principle and method for achieving endpoint detection. The alternatives included in this systematic review are outlined below according to the following categories:

- serology platforms for detection of anti-HCV and or HCV core antigen
- molecular detection of HCV-RNA.

Serology platforms for detection of anti-HCV and or HCV core antigen

Serology-based assays can detect the presence of anti-HCV antibodies, HCV core antigen, or both simultaneously.[4] There are a variety of serology-based immunoassays that can be used to detect anti-HCV antibodies, including:

- chemiluminescent immunoassay (CLIA)
- chemiluminescent micro-particle immunoassay (CMIA)
- electro-chemiluminescence immunoassay (ECLIA)
- enzyme-linked immunosorbent assay (ELISA).

The test principle used in these assays is the same, but the end-point detection differs. In enzyme immunoassays (that is, ELISA), endpoint detection is measured as colour change or fluorescence. Chemiluminescence assays represent a variation of the enzyme immunoassay principle in which chemiluminescence (that is, photons of light produced by a chemical reaction) is measured as the endpoint. CMIA and ECLIA comprise enhanced chemiluminescence immunoassays which use different types of luminescence to achieve the chemical reaction. Chemiluminescence immunoassays generally have a greater sensitivity than enzyme immunoassays.

Molecular detection of HCV-RNA

Detection of genetic material, otherwise known as molecular testing, commonly requires amplification when viral genetic material (such as RNA) is suspected to be present. A nucleic acid amplification test (NAAT) for detection of HCV-RNA represents the gold standard for the detection of active HCV infection.

In NAATs, amplification techniques are used to increase the number of target molecules (viral nucleic acids) or increase the signal generated to a level that permits detection of nucleic acids that occur naturally in the blood, commonly referred to as target amplification and signal amplification, respectively.[4] Target amplification techniques include reverse transcription polymerase chain reaction (RT-PCR) and transcription-mediated amplification (TMA), the key distinction being that RT-PCR

requires thermal cycling (repeated heating and cooling) while TMA is isothermal (the temperature of the nucleic acid does not change).

A3 Results

A3.1.1 Search results

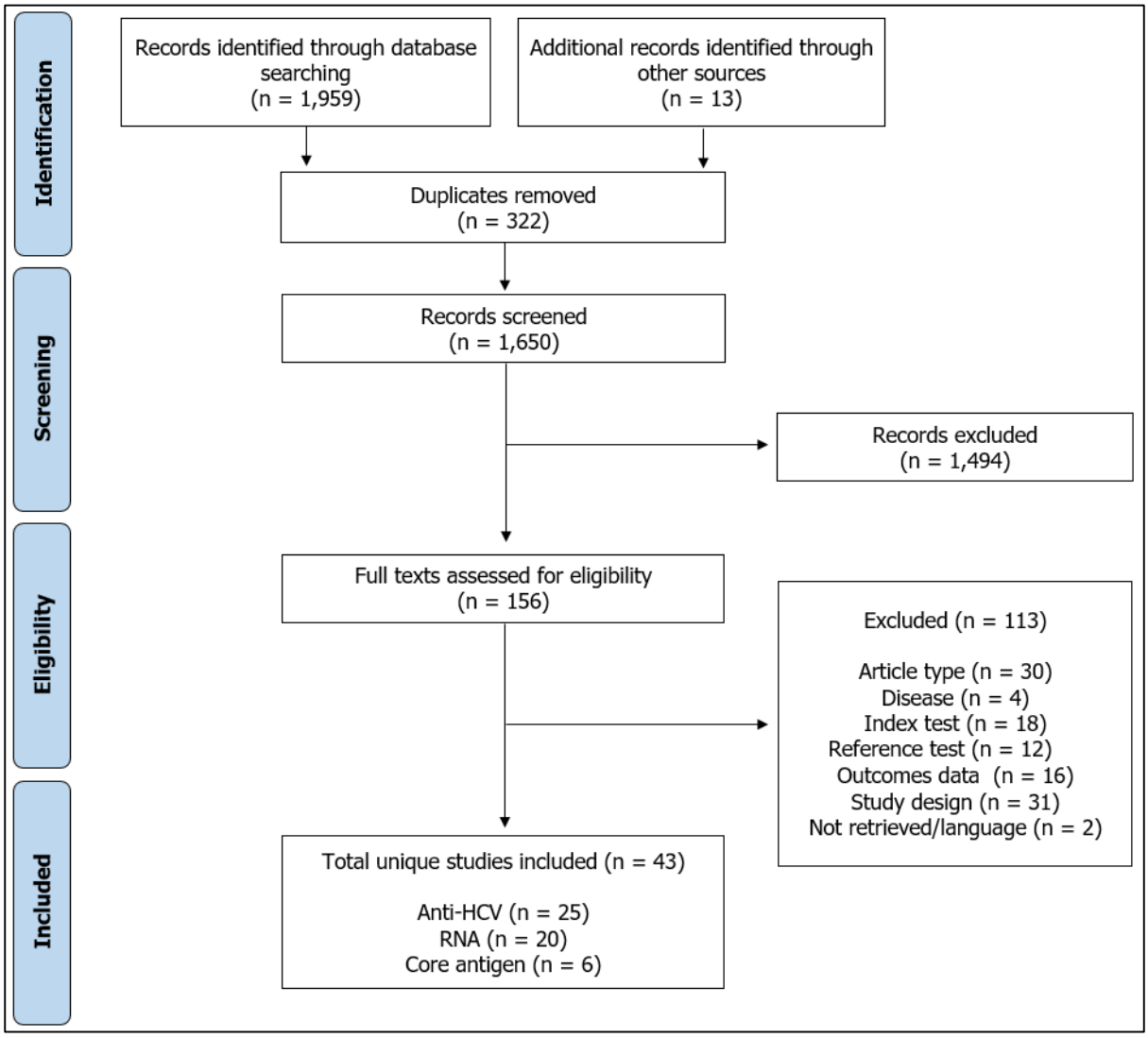
Overall, a total of 1,972 citations were returned from database and grey literature searching. Of these, 322 were removed as duplicate citations. A further 1,494 citations were excluded following title and abstract screening. Of the 156 citations that underwent full-text review, 43 individual studies were included in the synthesis.[29-71]

Of these 43 studies, 25 assessed the diagnostic accuracy of anti-HCV tests in DBS,[29-53] 20 assessed the diagnostic accuracy of HCV-RNA tests in DBS,[29, 33, 44, 47, 48, 51, 54-67] and six assessed the diagnostic accuracy of core antigen tests in DBS compared with conventional blood samples.[48, 61, 68-71]

The included studies are described in section A3.1 to A3.3 according to the detection target of the laboratory test under assessment (that is, anti-HCV, HCV-RNA or core antigen).

The PRISMA flow diagram,[72] depicting the flow of information through the various phases of the systematic review process, is presented in Figure A2.

Figure A2. PRISMA flow diagram



Key: HCV – hepatitis C virus; PRISMA – Preferred Reporting Items for Systematic Review and Meta-Analysis; RNA – ribonucleic acid.

A3.1.2 Overview of included studies assessing anti-HCV in DBS

Overall, 25 individual studies, published between 1999 and 2020, were included in the synthesis of anti-HCV tests in DBS.[29-53] Six of the studies were conducted in Brazil,[30, 32, 34, 41, 42, 52] three in France,[38, 48, 50] three in the US,[33, 45, 49] two in the UK,[43, 46] two in China,[39, 40] and one each from Denmark,[44] Germany,[47] Spain,[51] Australia,[31] South Africa,[35] Argentina,[53] Cameroon,[37] Burkina Faso,[36] and Pakistan.[29] Eight studies were cross-sectional,[35, 36, 39, 40, 45, 47, 52, 53] and 17 were case-control studies.[29-34, 37, 38, 41-44, 46, 48-51]

Overall, there was a total study population of 7,894 participants with study sample size ranging from 40 to 1,090 participants. Eleven studies reported the gender of study participants,[30, 34-36, 38, 41, 42, 45, 48, 51, 53] in which the proportion of male participants ranged from 37% to 77% with a weighted mean of 47%. Eleven studies reported the age of study participants, with the average ranging from 30 to 52 years.[30, 34-36, 38, 41, 42, 45, 48, 51, 53] One study reported that the study population comprised young adults, aged under 30.[33]

Anti-HCV prevalence (based on the reference standard) in the included studies ranged from 2% to 78%, with a weighted mean of 37%. Positive predictive value and negative predictive value ranged from 0.74 to 1.00 and 0.76 to 1.00, respectively. Disease severity of HCV-infected study participants was not reported in any of the included studies.

The HCV genotype of HCV-infected participants was reported in seven studies, with HCV genotype 1 and HCV genotype 3 reported in 75% and 17% of study participants, respectively.[35, 38, 42, 49-51, 53] However, the reported genotype data was generally from only a subset of study participants or based on a larger cohort study (for example, where oral samples were also analysed as part of the study), therefore its applicability is limited.

Study population risk factors for HCV acquisition were reported in 10 studies.[31, 33-36, 38, 39, 44, 51, 53] From these, the most commonly reported risk factors were co-infection with HBV and or HIV, and a history of injecting drug use. However, a detailed breakdown of risk factor data was often not provided.

Eleven studies reported diagnostic outcomes for capillary DBS samples.[29, 33, 36-38, 44-46, 49, 51, 52] Twelve studies reported diagnostic outcomes for venous DBS samples (for example, pipetting venous blood onto filter paper).[31, 32, 34, 35, 39, 40, 43, 47, 48, 50, 53] Two studies reported results using a combination of both sample collection types.[30, 41]

The 25 included studies contributed 39 unique pairs of diagnostic outcomes (that is, sensitivity and specificity) of anti-HCV tests in DBS to the synthesis.[29-53] Nineteen

studies contributed only one pair of diagnostic outcomes.[29, 31, 33-36, 38-40, 42, 44-48, 50-53] Six studies contributed multiple pairs of diagnostic outcomes.[30, 32, 37, 41, 43, 49] The reasons for individual studies contributing multiple pairs of diagnostic outcomes included reporting by:

- assay principle (for example, CLIA versus ELISA)[49]
- manufacturer assay[30, 32, 41, 49]
- threshold applied.[30, 37, 41, 43]

The diagnostic accuracy may vary according to the type of assay used. Nineteen studies reported results using ELISA,[29-34, 36, 38-43, 45, 46, 49-51, 53] four studies reported results using CMIA,[35, 37, 44, 47] two studies reported results using CLIA,[48, 49] and one study reported results using ECLIA.[52]

Nineteen studies reported the use of a threshold at which samples were considered reactive.[29-32, 34-38, 40, 41, 43, 45, 47-50, 52, 53] These were generally reported in terms of a signal-to-cut-off ratio. The signal-to-cut-off ratios reported ranged from ≥0.11 to ≥2.00. The most commonly reported threshold (n=8) was a signal-to-cut-off ratio of ≥1.00.[31, 32, 35, 37, 43, 45, 49, 52] Six studies reported that they interpreted the results according to the manufacturer's instructions,[29, 30, 34, 40, 47, 53] but the explicit threshold value used could not be identified. Seven studies did not report the threshold used.[33, 39, 42, 44, 46, 51, 73]

Additional study characteristics are presented in Appendix A1.2.

A3.1.3 Study-level estimates of DBS for detection of anti-HCV

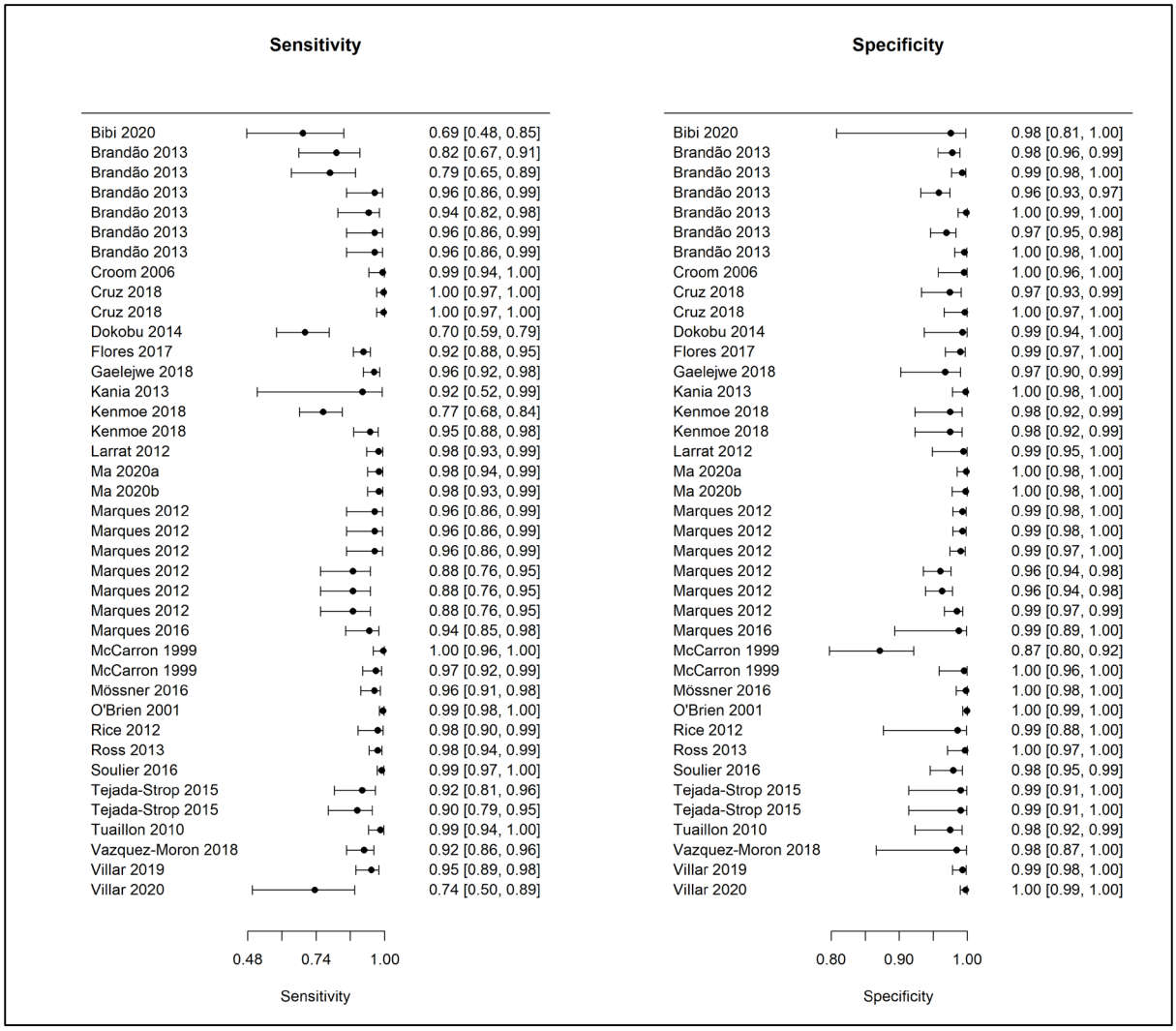
Six studies reported multiple pairs of diagnostic outcomes.[30, 32, 37, 41, 43, 49] The range (that is, the minimum and maximum) of the study-level point estimates is presented in Table A3. Forest plots of the study-level estimates of sensitivity and specificity are presented in Figure A3.

Table A3. Summary of study-level estimates of anti-HCV in DBS

Outcome	Lowest reported estimate (95% CI)	Highest reported estimate (95% CI)	N*
Sensitivity	0.69 (95% CI: 0.48 to 0.85)	1.00 (95% CI: 0.97 to 1.00)	39
Specificity	0.87 (95% CI: 0.80 to 0.92)	1.00 (95% CI: 0.99 to 1.00)	39
NLR	0.00 (95% CI: 0.00 to 0.05)	0.32 (95% CI: 0.17 to 0.60)	39
PLR**	2.05 (95% CI: 1.57 to 2.53)	7.22 (95% CI: 4.45 to 9.99)	39
DOR**	4.52 (95% CI: 1.56 to 7.47)	12.31 (95% CI: 9.27 to 15.35)	39

Key: CI – confidence interval; DBS – dried blood spot; DOR – diagnostic odds ratio; HCV – hepatitis C virus; NLR – negative likelihood ratio; PLR – positive likelihood ratio.
* Total number of data pairs across 25 individual studies.
** Estimates are log-transformed.

Figure A3. Forest plot of study-level estimates of sensitivity and specificity of anti-HCV in DBS



Key: DBS – dried blood spot; HCV – hepatitis C virus.

Note: Some studies reported multiple pairs of diagnostic outcomes. Pooling of these data would break the assumption of independence in meta-analysis.

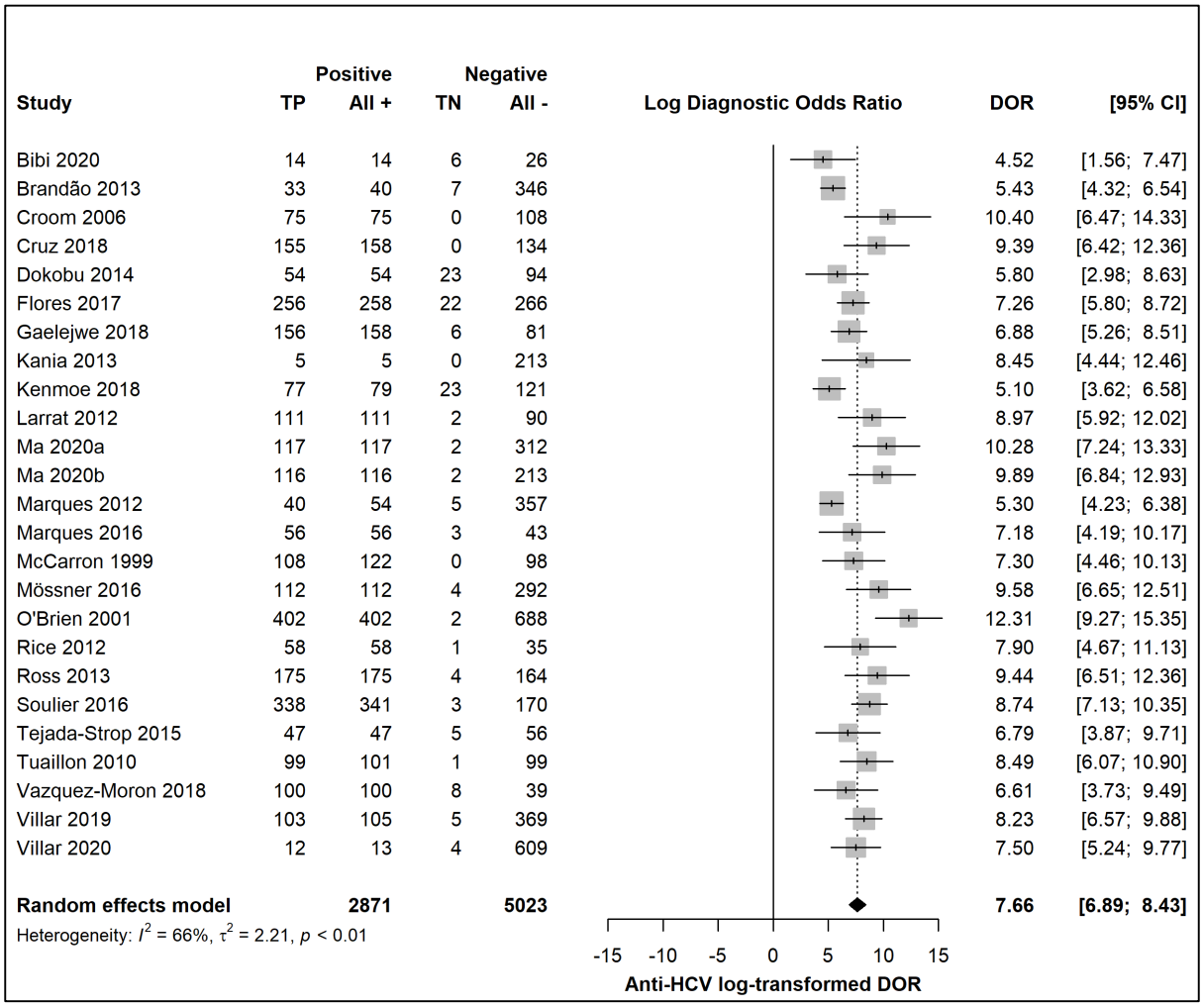
A3.1.4 Univariate meta-analysis of DBS for detection of anti-HCV

As described in section A2.4, to facilitate pooling of study estimates one data pair was selected from studies that reported multiple pairs of diagnostic outcomes.

From the 25 included studies comparing anti-HCV in DBS with anti-HCV in serum or plasma, the pooled sensitivity and specificity were estimated at 0.96 (95% CI: 0.94 to 0.98) and 1.00 (95% CI: 0.99 to 1.00), respectively. The pooled log-transformed positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were estimated at 4.26 (95% CI: 3.72 to 4.80) and 0.05 (95% CI: 0.03 to 0.08), respectively. The log-transformed DOR was estimated at 7.66 (95% CI: 6.89 to 8.43). The forest plots of DORs, sensitivity and specificity are presented in Figure A4-A5.

The I^2 index of sensitivity and specificity (estimated at 90% and 86%, respectively) indicated a high degree of statistical heterogeneity in the univariate meta-analysis. From visual inspection of Figure A5, variability in the estimates of sensitivity was driven by five studies.[29, 30, 33, 37, 53] The study population in one of these studies was relatively small (n=40),[29] while anti-HCV prevalence was relatively low (3%) in one of these,[53] but otherwise these studies were not systematically different to the other included studies. Variability in the estimates of specificity was mainly driven by three studies.[30, 41, 43] One of these studies (McCarron 1999),[43] which was published in 1999, can be considered an outlier in the analysis.

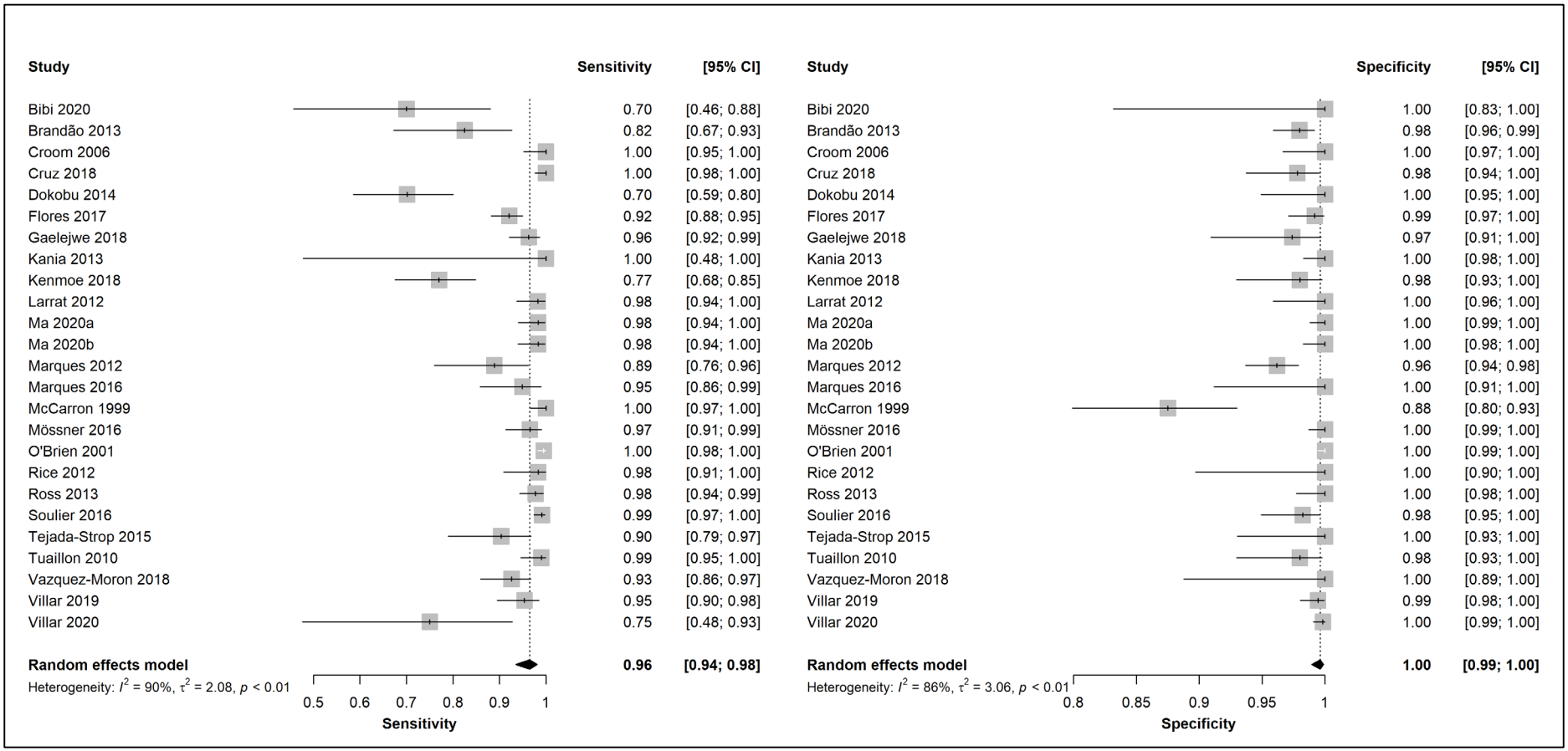
Figure A4. Forest plot of DORs of anti-HCV in DBS



Key: DBS – dried blood spot; DOR – diagnostic odds ratio; HCV – hepatitis C virus.

Note: Although the DOR provides a single indicator of diagnostic performance, it does not adequately reflect the potential correlation between sensitivity and specificity, and univariate tests for heterogeneity such as the inconsistency index (I^2) and tau^2, can be misleading.[74] Heterogeneity is considered the rule rather than expectation in meta-analyses of diagnostic accuracy.[75, 76]

Figure A5. Forest plot of sensitivity and specificity of anti-HCV tests in DBS



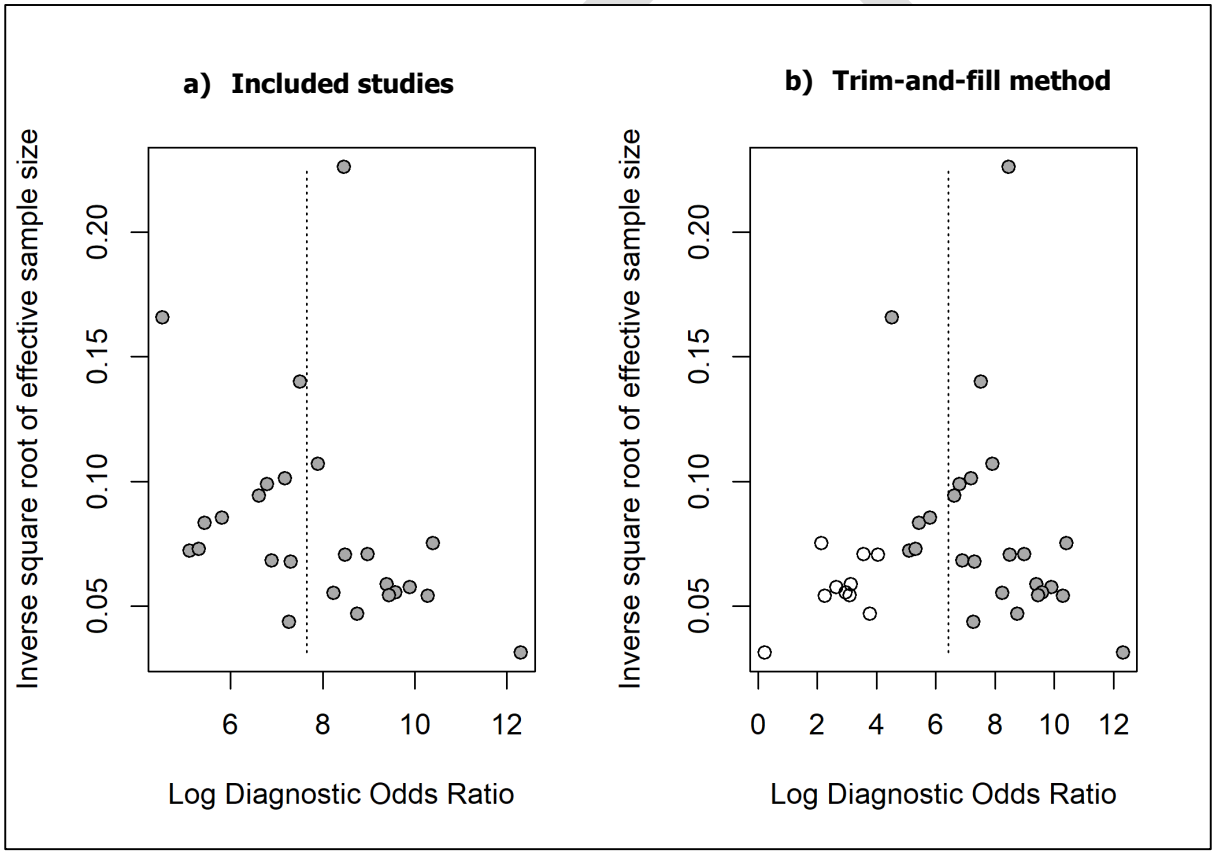
Key: DBS – dried blood spot; HCV – hepatitis C virus.

Note: Univariate meta-analysis does not account for correlation between pairs of sensitivity and specificity.[75-77] Heterogeneity is considered the rule rather than expectation in meta-analyses of diagnostic accuracy.[75, 76] Univariate tests for heterogeneity, such as the inconsistency index (I^2) and tau^2, can be misleading.

Publication bias

Publication bias was assessed using Deek's regression test of asymmetry and funnel plot.[24, 25] The regression test indicated the presence of publication bias (p-value of less than 0.01), as did the asymmetry in the funnel plot. A sensitivity analysis using the trim-and-fill method was undertaken to investigate the impact of publication bias. This involves first "trimming" the studies that cause asymmetry (to minimise the impact of publication bias) and then imputing missing studies based on the bias-corrected overall estimate.[26, 27] Imputed values were added for 10 studies, resulting in an adjusted log-transformed DOR of 6.42 (95% CI: 5.61 to 7.23) compared with 7.66 (95% CI: 6.89 to 8.43) in the main analysis. Although the trim-and-fill estimate is lower than the DOR estimated in the main analysis, it is still significantly greater than 1 demonstrating clinical utility. The funnel plots are presented in Figure A6.

Figure A6. Funnel plots of anti-HCV tests in DBS



Key: DBS – dried blood spot; HCV – hepatitis C virus.
Note: The trim-and-fill method is a sensitivity analysis that requires imputation to correct for publication bias. The imputed study estimates are coloured white in b). The vertical line represents the estimate from the random-effects model.

Subgroup and sensitivity analysis

The robustness of the meta-analysis estimates was investigated by subgroup and sensitivity analysis. A statistically meaningful difference between subgroups was determined by a p-value of less than 0.05. The subgroup and sensitivity analysis included analyses:

- by assay principle (that is, ELISA versus CMIA)
- by DBS sample type (that is, capillary versus venous)
- of DBS in ELISA, stratified by DBS sample type
- by study design (cross-sectional versus case-control studies)
- stratified by risk of bias
- according to signal-to-cut-off ratio of 1.00.

The results of these analyses are summarised in Table A4. Notably, a statistically significant difference was found in the DOR when comparing subgroups by study design (p-value=0.02) and in sensitivity when comparing subgroups by risk of bias (p-value = 0.03).

Table A4. Summary of subgroup and sensitivity analyses of anti-HCV in DBS

Subgroup	N	Sensitivity (95% CI)	Specificity (95% CI)	DOR* (95% CI)
Assay principle				
ELISA	19	0.97 (0.93-0.99)	1.00 (0.99-1.00)	7.64 (6.69-8.58)
CMIA	4	0.95 (0.86-0.98)	1.00 (0.96-1.00)	7.43 (5.37-9.49)
DBS sample type				
Capillary	11	0.94 (0.87-0.98)	1.00 (0.99-1.00)	7.54 (6.19-8.89)
Venous	12	0.98 (0.96-0.99)	0.99 (0.98-1.00)	8.12 (7.45-8.78)
DBS in ELISA, stratified by DBS sample type				
Capillary	8	0.94 (0.83-0.98)	0.99 (0.98-1.00)	7.61 (5.93-9.29)
Venous	9	0.97 (0.93-0.99)	0.99 (0.96-1.00)	8.15 (7.32-8.98)
Study design				
Case-control	17	0.96 (0.91-0.98)	0.99 (0.98-1.00)	7.07 (6.23-7.92)**
Cross-sectional	8	0.97 (0.94-0.99)	1.00 (0.99-1.00)	8.83 (7.58-10.08)**
Risk of bias (see section A3.1.6)				
Low	3	0.96 (0.93-0.98)**	0.99 (0.98-1.00)	7.6 (6.50-8.73)
Moderate	16	0.98 (0.94-0.99)**	1.00 (0.99-1.00)	8.16 (7.13-9.19)
High	6	0.91 (0.85-0.95)**	1.00 (0.94-1.00)	6.32 (5.08-7.56)
Common threshold				
S/Co=1.00	8	0.99 (0.93-1.00)	0.99 (0.97-1.00)	8.00 (6.47-9.54)

Key: CI – confidence interval; CMIA – chemiluminescent micro-particle immunoassay; DBS – dried blood spot; DOR – diagnostic odds ratio; ELISA – enzyme-linked immunosorbent assay; HCV – hepatitis C virus; NLR – negative likelihood ratio; PLR – positive likelihood ratio; S/Co – signal to cut off ratio.

* Estimates are log-transformed.

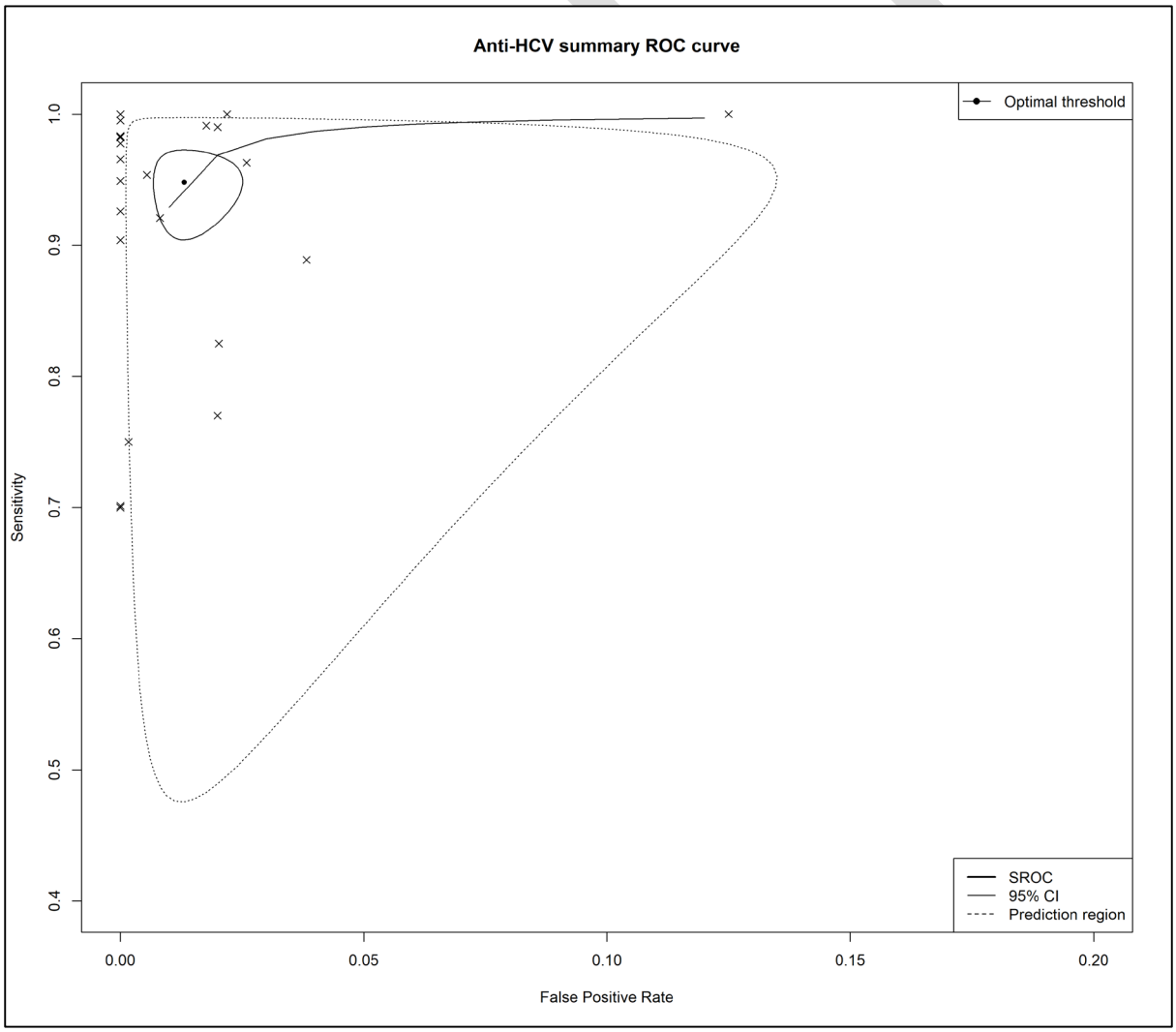
** Subgroup differences were found to be statistical significant at the 5% level.

A3.1.5 Bivariate meta-analysis of DBS for detection of anti-HCV

Fitting of the bivariate model is considered standard practice in meta-analysis of diagnostic accuracy to account for correlation between pairs of sensitivity and specificity.[22, 75, 78]

In the bivariate meta-analysis, the pooled sensitivity and specificity were estimated at 0.95 (95% CI: 0.92 to 0.97) and 0.98 (95% CI: 0.98 to 0.99), respectively. The correlation co-efficient was estimated at 0.019 (indicating that the univariate meta-analysis represents an acceptable estimate), the area under the curve (AUC) was 0.988 and the Akaike information criterion (AIC) was -218.95. The pooled log-transformed PLR, NLR and log-transformed DOR were estimated at 4.3 (95% CI: 3.8 to 4.8), 0.05 (95% CI: 0.03 to 0.08) and 7.3 (95% CI: 6.5-8.0). The summary receiver operating characteristic (SROC) curve, based on the parameters of the bivariate model, is presented in Figure A7.

Figure A7. SROC curve of anti-HCV in DBS



Key: DBS – dried blood spot; HCV – hepatitis C virus; SROC – summary receiver operating characteristic.
Note: The SROC is plotted according to the generalisation of the Rutter and Gatsonis curve.[79]

A3.1.6 Methodological quality of studies assessing anti-HCV in DBS

Study risk of bias was assessed using the QUADAS-2 tool. Of the 25 included studies, three were rated as low risk of bias,[35, 36, 52] 16 as moderate risk of bias,[29-34, 41-43, 45-48, 50, 53] and six as high risk of bias.[37, 41, 42, 44, 49, 51]

Participant selection was rated as introducing a high risk of bias in 15 of the 25 included studies.[29-34, 37, 41-44, 48-51] This was due to non-consecutive enrolment of study participants and or inappropriate exclusions (which may have results in a biased study sample). Although three studies reported a cross-sectional design,[33, 37, 51] study design appeared to be consistent with that of a case-control study as the diagnosis of patients (that is, the presence or absence of anti-HCV) was known in advance, indicating a potential source of bias. Eight studies were consistent with a cross-sectional design.[35, 36, 39, 40, 45, 47, 52, 53] Study recruitment methods were unclear in five studies.[32, 39, 40, 45, 52]

The index test was rated as introducing a moderate risk of bias in:

- four studies due to the use of a threshold to interpret DBS results that was not pre-specified[36, 38, 45, 48]
- six studies which did not report the threshold used[33, 39, 42, 44, 46, 51]
- six studies which reported that they interpreted the results in accordance with the manufacturer's instruction, but without disclosing the explicit threshold used.[29, 30, 34, 40, 47, 53]

Nine studies which pre-specified the threshold used to interpret results were rated at a low risk of bias.[31, 32, 35, 37, 41, 43, 49, 50, 52] Blinding of those interpreting DBS results (that is, specifying they were without knowledge of the results in the reference standard) was reported in only three studies.[45, 46, 50] Blinding was unclear in all other studies.[29-44, 47-49, 51-53] However, it should be noted that interpretation of laboratory-based anti-HCV results is automated (rather than subjective) and therefore potential bias is likely minimal.

All of the studies compared the results of anti-HCV immunoassays in DBS with that in serum, plasma or whole blood (considered acceptable reference standards). However, one study which compared anti-HCV in DBS against two alternative reference standard assays (that is, Radim HCV antibody assay and DiaSorin ETI HCV-antibody assay) was considered at a moderate risk of bias.[41] This is because the total number of positive samples detected by each reference standard assay differed (n=40 versus n=45) when comparing results from the same study sample.[41] Blinding during interpretation of results was reported in only four studies[45, 46, 48, 50] As interpretation of laboratory-based anti-HCV immunoassay results is automated (rather than subjective) the potential bias is likely minimal and therefore study quality was not downgraded on the

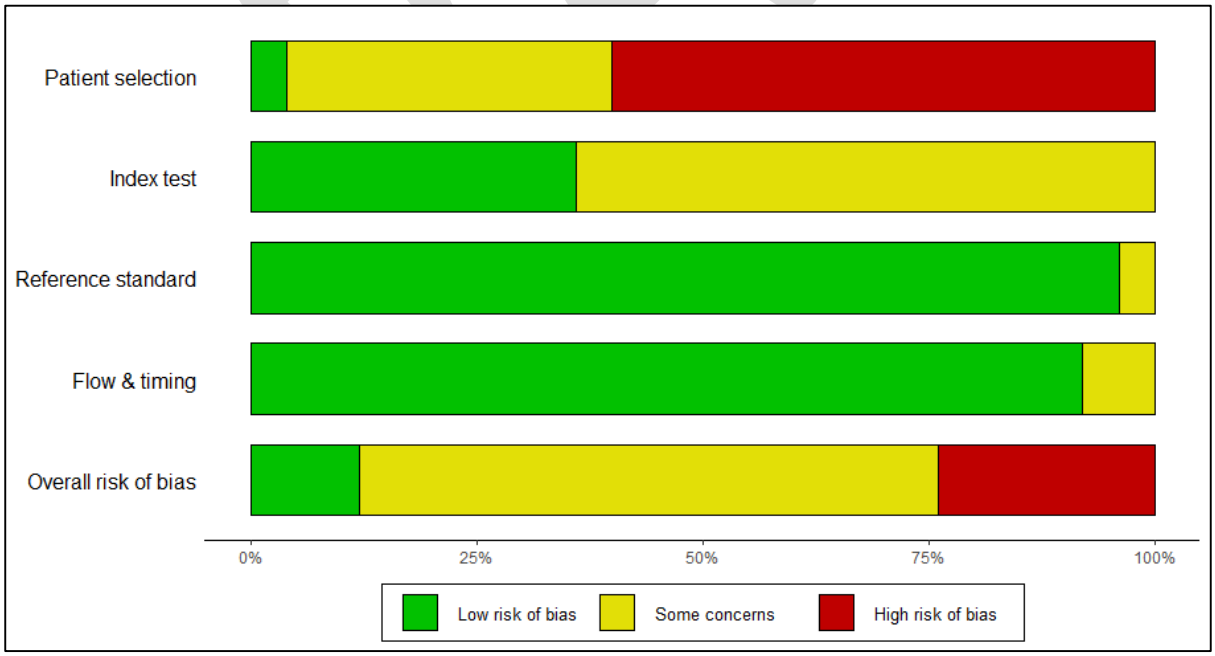
basis of blinding.

In the patient flow and timing domain, 23 of the 25 studies were rated at a low risk of bias.[29-32, 34-43, 45-53] One study was rated at moderate risk of bias as some study participants received different reference standard enzyme-immunoassays (that is, some patient diagnoses were confirmed in serum, plasma or whole blood with ELISA, others were confirmed with CLIA).[33] One study was rated at moderate risk of bias due to the exclusion of patient results without explanation.[44]

Two studies declared potential conflicts of interest due to the receipt of financial support from industry,[44, 48] eighteen studies declared no competing interests,[29, 30, 32-34, 36-40, 42, 46, 47, 49-53] while five studies did not report any conflict of interest statement.[31, 35, 41, 43, 45] Study risk of bias outcomes are summarised according to the QUADAS-2 domains in Figure A8 and Table A5.

The summary of findings table, based on the GRADE framework, is presented in Table A6. Overall, the certainty of the evidence was assessed for the primary outcomes (that is, sensitivity and specificity) and judged to be low. The certainty of the evidence was downgraded for risk of bias (based on QUADAS-2 assessment) and indirectness due to a lack of representativeness of the study populations.

Figure A8. Overall risk of bias of studies assessing anti-HCV in DBS, by QUADAS-2 domain



Key: DBS – dried blood spot; HCV – hepatitis C virus; QUADAS – Quality Assessment of Diagnostic Accuracy Studies.
Note: "Some concerns" indicates that the study was at moderate risk of bias.

Table A5. Risk of bias of included studies assessing anti-HCV in DBS, by QUADAS-2 domain

Study	Patient Selection	Index Test	Reference standard	Flow and timing
Bibi 2020	High	Some concerns	Low	Low
Brandão 2013	High	Some concerns	Low	Low
Croom 2006	High	Low	Low	Low
Cruz 2018	High	Low	Low	Low
Dokobu 2014	High	Some concerns	Low	Some concerns
Flores 2017	High	Some concerns	Low	Low
Gaelejwe 2018	Some concerns	Low	Low	Low
Kania 2013	Low	Some concerns	Low	Low
Kenmoe 2018	High	Low	Low	Low
Larrat 2012	Some concerns	Some concerns	Low	Low
Ma 2020a	Some concerns	Some concerns	Low	Low
Ma 2020b	Some concerns	Some concerns	Low	Low
Marques 2012	High	Low	Some concerns	Low
Marques 2016	High	Some concerns	Low	Low
McCarron 1999	High	Low	Low	Low
Mössner 2016	High	Some concerns	Low	Some concerns
O'Brien 2001	Some concerns	Some concerns	Low	Low
Rice 2012	Some concerns	Some concerns	Low	Low
Ross 2013	Some concerns	Some concerns	Low	Low
Soulier 2016	High	Some concerns	Low	Low
Tejada-Strop 2015	High	Low	Low	Low
Tuaillon 2010	High	Low	Low	Low
Vazquez-Moron 2018	High	Some concerns	Low	Low
Villar 2019	Some concerns	Low	Low	Low
Villar 2020	Some concerns	Some concerns	Low	Low

Key: DBS – dried blood spot; HCV – hepatitis C virus; QUADAS – Quality Assessment of Diagnostic Accuracy Studies.

Note: "Some concerns" indicates that the study was at moderate risk of bias.

Table A6. GRADE summary of findings table for diagnostic accuracy anti-HCV in DBS

Outcome	Study design	Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	Certainty of evidence
Sensitivity: 0.95 (95% CI: 0.92 to 0.97)							
Studies: 25 Sample size: 2,957	Cross-sectional, case-control	Serious*	Serious**	Not serious	Not serious	Not serious***	⊕⊕◯◯ LOW
Specificity: 0.98 (95% CI: 0.98 to 0.99)							
Studies: 25 Sample size: 4,937	Cross-sectional, case-control	Serious*	Serious**	Not serious	Not serious	Not serious***	⊕⊕◯◯ LOW
Prevalence of anti-HCV: 0.37 (95% CI: 0.32 to 0.43)							

Key: CI – confidence interval; DBS – dried blood spot; HCV – hepatitis C virus.
* Overall certainty of the evidence downgraded one level for risk of bias due to limitations in study design and execution (based on risk of bias assessment in QUADAS-2).
** Overall certainty of the evidence downgraded one level for indirectness due to lack of representativeness of the study population.
*** Publication bias was suspected, but not considered serious enough for downgrading certainty of evidence.

A3.2.1 Overview of included studies HCV-RNA in DBS

Overall, 20 individual studies assessing the diagnostic accuracy of HCV-RNA tests in DBS were included in the synthesis.[29, 33, 44, 47, 48, 51, 54-67] The studies were published between 2002 and 2020. Four of the studies were from Spain,[51, 59, 62, 63] two each were from Australia,[55, 56] France,[48, 67] Italy,[57, 65] UK,[54, 64] Vietnam,[61, 66] and there was one each from Denmark,[44] Germany,[47] India,[60] Pakistan,[29] Saudi Arabia,[58] and the USA.[33] Nine studies were cross-sectional,[47, 55, 56, 59-63, 66] and eleven were case-control studies.[29, 33, 44, 48, 51, 54, 57, 58, 64, 65, 67]

Overall, there was a total study population of 2,940 participants with study sample size ranging from 25 to 511 participants. Ten studies reported the gender of study participants.[48, 51, 55, 58-63, 66] The proportion of male participants ranged from 55% to 96%, with a mean of 74%. Nine studies reported the age of study participants, with the average ranging from 39 to 52 years.[48, 51, 55, 58-61, 63, 66]

Study-level HCV-RNA prevalence ranged from 26% to 85%, with a weighted mean of 62%. Positive predictive value and negative predictive value ranged from 0.84 to 1.00 and 0.68 to 1.00, respectively. Only two studies reported the disease severity (that is, fibrosis distribution) of HCV-infected participants.[55, 61] In both studies, the majority (>65%) of patients that received fibrosis staging were between METAVIR fibrosis stages F0 to F2.

The HCV genotype of HCV-infected participants was reported in 10 studies,[48, 56, 59-65, 67] with HCV genotype 1 and HCV genotype 3 reported in 58% and 23% of study participants, respectively. However, the reported genotype data was generally from only a subset of participants or based on a larger cohort study (for example, where oral samples were also analysed as part of the study) therefore its applicability is limited.

Study population risk factors for HCV acquisition were reported in 10 studies.[33, 44, 51, 55, 57, 59, 61-63, 66] From these, the most commonly reported risk factors were co-infection with HBV and or HIV, and a history of injecting drug use. However, a detailed breakdown of risk factor data was often not provided. Four studies reported the treatment status of HCV-infected patients.[48, 61-63] In two of these,[48, 61] all HCV-infected patients were treatment-naïve.

Nine studies reported diagnostic outcomes for capillary DBS samples.[29, 33, 44, 51, 55, 58, 59, 62, 63] Nine studies reported diagnostic outcomes for venous DBS samples (for example, pipetting venous blood onto filter paper).[47, 48, 56, 57, 60, 61, 64, 65, 67] One study reported diagnostic outcomes for both capillary and venous DBS samples.[66] The DBS sample type was unclear in one study.[54]

The 20 included studies contributed 34 unique pairs of diagnostic outcomes (that is, sensitivity and specificity) of HCV-RNA tests in DBS to the synthesis. Twelve studies contributed only one pair of diagnostic outcomes.[29, 33, 44, 47, 51, 54, 57-61, 67] Eight studies contributed multiple 2x2 data pairs.[48, 55, 56, 62-66] The reasons for individual studies contributing multiple pairs of diagnostic outcomes included reporting:

- paired outcomes by assay principle[65]
- paired outcomes by manufacturer assay[48, 55]
- different DBS sample types[66]
- paired outcomes by threshold applied.[56, 62-64]

The diagnostic accuracy may vary according to the type and technique principle of assay used. Target amplification techniques were reported by the vast majority of studies, with fourteen studies reporting the use of reverse transcription polymerase chain reaction (RT-PCR),[29, 48, 51, 54, 57-64, 66, 67] and studies reporting the use of transcription-mediated amplification (TMA),[33, 44, 47, 56] while one reported the use of both.[65] The assay principle of the assay used in one study was unclear.[55]

Twelve studies reported the use of a viral load threshold, reported in terms of international units per millilitre (IU/ml).[29, 44, 47, 55-57, 60, 62-64, 66, 67] The viral load thresholds reported ranged from 10 IU/ml to 50,000 IU/ml. The most commonly reported threshold (n=5) was a viral load threshold of ≥1,000 IU/ml.[56, 60, 62-64] Four studies reported that they interpreted the results according to the manufacturer's instructions, but the explicit threshold value used could not be identified.[29, 44, 47, 67]

Additional study characteristics are presented in Appendix A2.

A3.2.2 Study-level estimates of DBS for detection of HCV-RNA

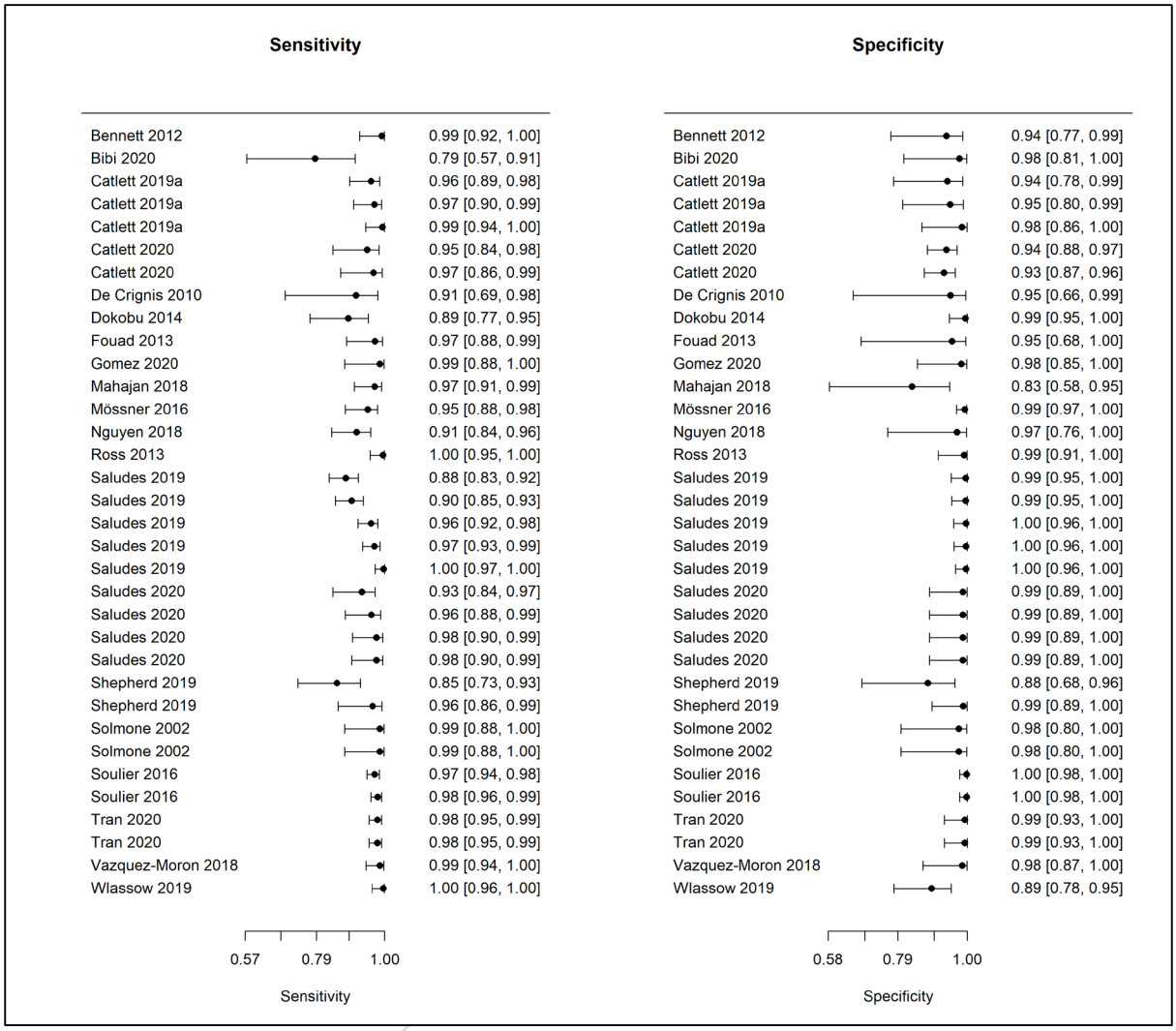
Eight studies reported multiple pairs of diagnostic outcomes.[55, 56, 62-66, 73] The range (that is, the minimum and maximum) of the study-level point estimates is presented in Table A7. Forest plots of the study-level estimates of sensitivity and specificity of HCV-RNA in DBS are presented in Figure A9.

Table A7. Summary of study-level estimates of HCV-RNA in DBS

Outcome	Lowest reported estimate (95% CI)	Highest reported estimate (95% CI)	N*
Sensitivity	0.79 (95% CI: 0.57 to 0.91)	1.00 (95% CI: 0.96 to 1.00)	34
Specificity	0.83 (95% CI: 0.58 to 0.95)	1.00 (95% CI: 0.98 to 1.00)	34
NLR	0.00 (95% CI: 0.00 to 0.07)	0.22 (95% CI: 0.10 to 0.50)	34
PLR**	1.76 (95% CI: 0.63 to 2.89)	5.96 (95% CI: 3.19 to 8.72)	34
DOR**	3.76 (95% CI: 2.23 to 5.29)	11.33 (95% CI: 7.40 to 15.26)	34

Key: CI – confidence interval; DBS – dried blood spot; DOR – diagnostic odds ratio; HCV – hepatitis C virus; NLR – negative likelihood ratio; PLR – positive likelihood ratio; RNA – ribonucleic acid.
* Total number of data pairs across 20 individual studies.
** Estimates are log-transformed.

Figure A9. Forest plot of study-level estimates of the sensitivity and specificity of HCV-RNA tests in DBS



Key: DBS – dried blood spot; HCV – hepatitis C virus; RNA – ribonucleic acid.

Note: Some studies reported multiple pairs of diagnostic outcomes. Pooling of these data would break the assumption of independence in meta-analysis.

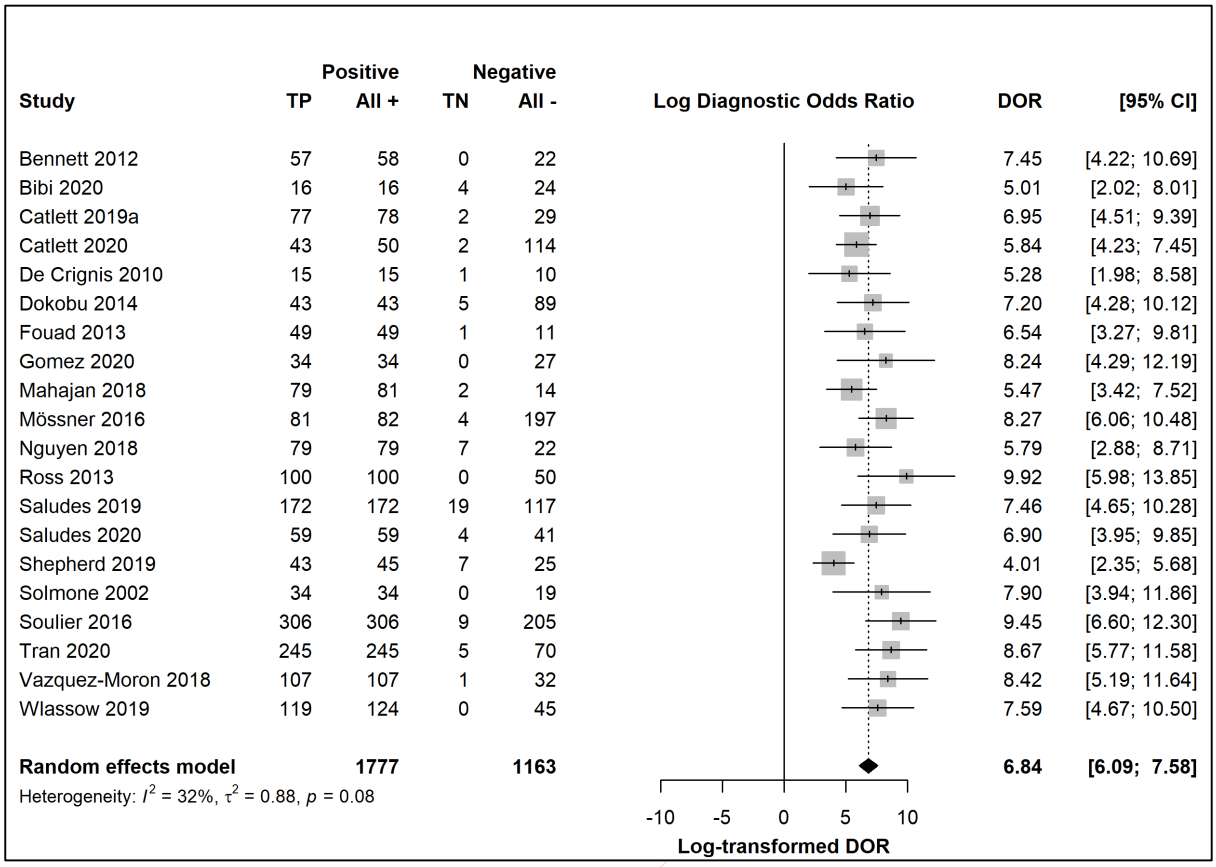
A3.2.3 Univariate meta-analysis of DBS for detection of HCV-RNA

As described in section A2.4, to facilitate pooling of study estimates one data pair was selected from studies that reported multiple pairs of diagnostic outcomes.

Across all included studies comparing HCV-RNA in DBS with HCV-RNA in serum, plasma or whole-blood the pooled sensitivity and specificity were estimated at 0.97 (95% CI: 0.95 to 0.98) and 0.99 (95% CI: 0.97 to 1.00), respectively. The pooled log-transformed PLR and NLR were estimated at 3.22 (95% CI: 2.70 to 3.74) and 0.05 (95% CI: 0.04 to 0.08), respectively. The log-transformed DOR was estimated at 6.84 (95% CI: 6.09 to 7.58). The forest plots of DORs, sensitivity and specificity are presented in Figure A10-A11.

The I^2 index of sensitivity and specificity (estimated at 77% and 79%, respectively) indicated a high degree of statistical heterogeneity in the univariate meta-analysis. From visual inspection of Figure A11, variability in the estimates of sensitivity was driven by five studies.[29, 33, 61, 62, 64] The study population was relatively small (n=40) in the study appeared to be the main outlier in the analysis.[29] Variability in the estimates of specificity was driven by four studies.[55, 60, 64, 67]

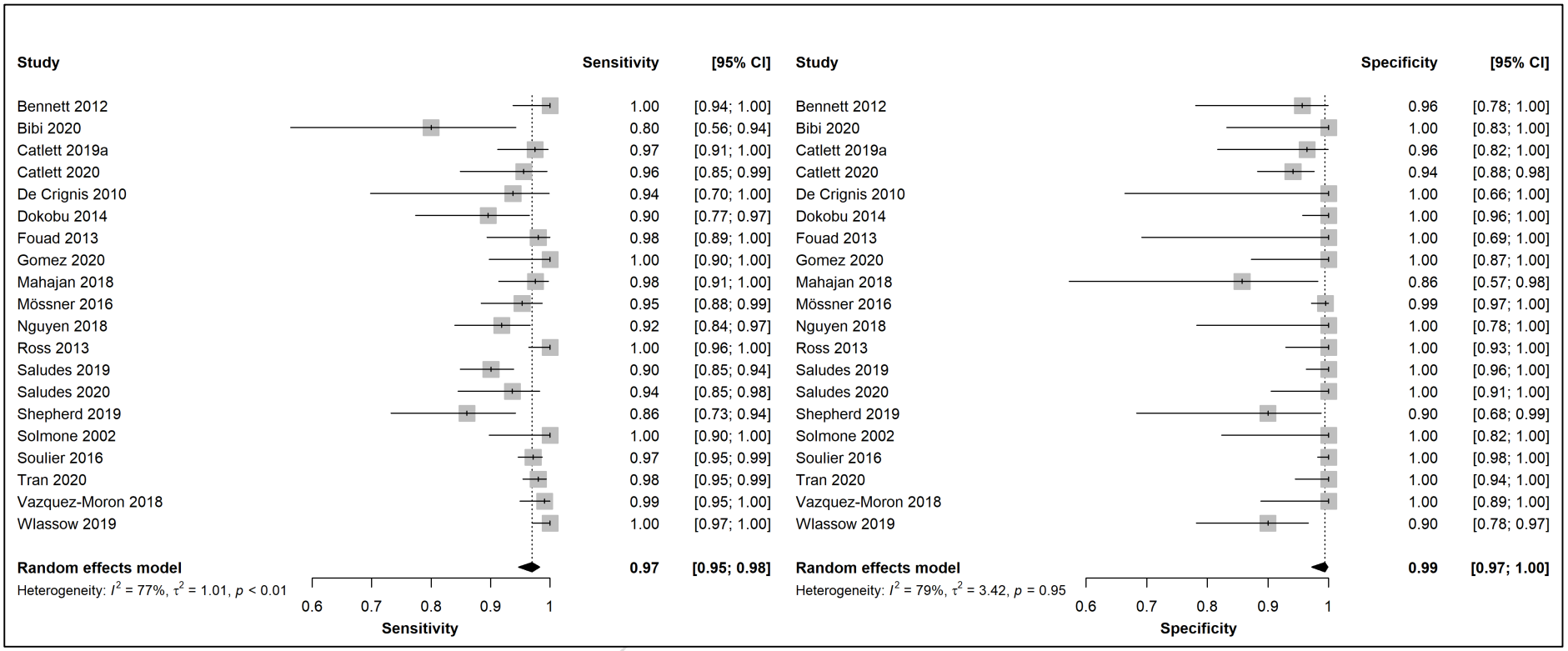
Figure A10. Forest plot of DORs of HCV-RNA in DBS



Key: DBS – dried blood spot; DOR – diagnostic odds ratio; HCV – hepatitis C virus; RNA – ribonucleic acid.

Note: Although the DOR provides a single indicator of diagnostic performance, it does not adequately reflect variability between studies and univariate tests for heterogeneity, such as the inconsistency index (I^2) and tau^2, can be misleading.[74] Heterogeneity is considered the rule rather than expectation in meta-analyses of diagnostic accuracy.[75, 76]

Figure A11. Forest plot of sensitivity and specificity of HCV-RNA tests in DBS



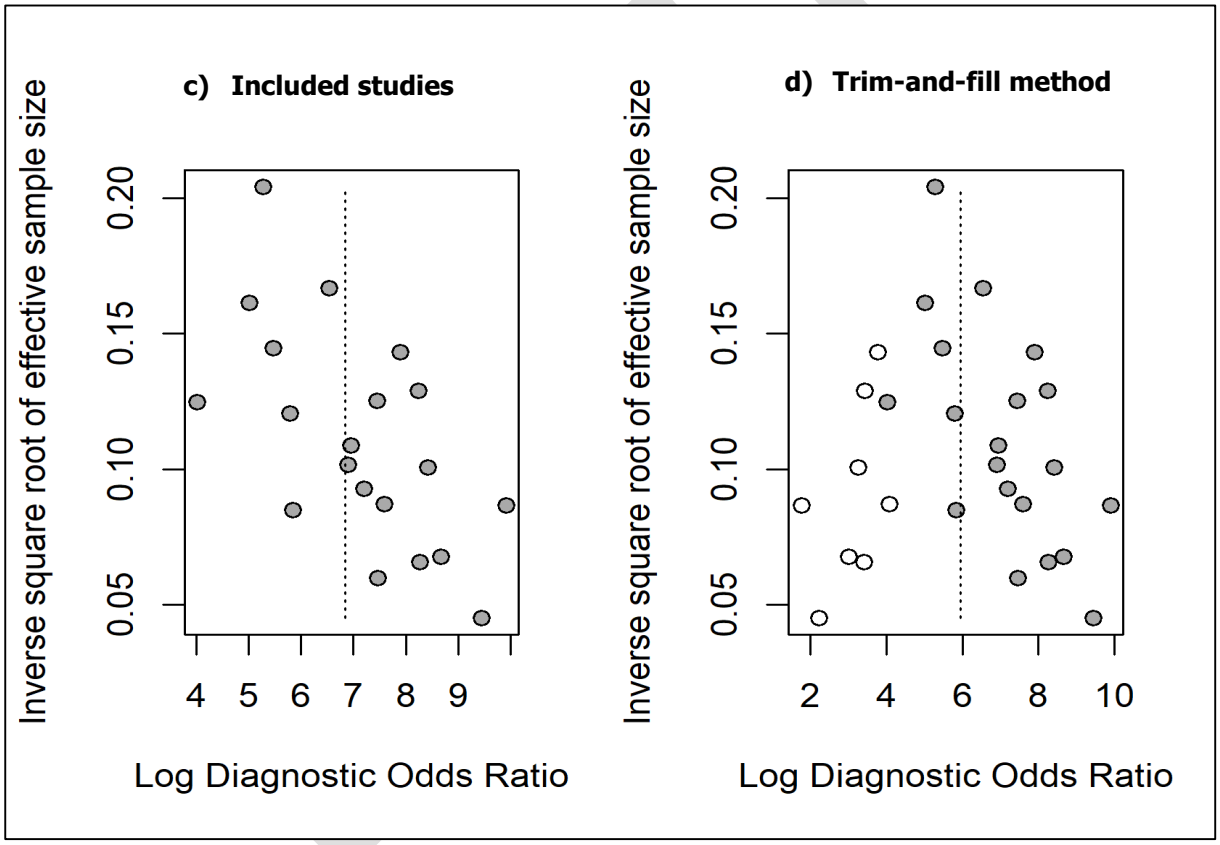
Key: DBS – dried blood spot; HCV – hepatitis C virus; RNA – ribonucleic acid.

Note: Univariate meta-analysis does not account for correlation between pairs of sensitivity and specificity.[75-77] Heterogeneity is considered the rule rather than expectation in meta-analyses of diagnostic accuracy.[75, 76] Univariate tests for heterogeneity, such as the inconsistency index (I^2) and tau^2, can be misleading.

Publication bias

Publication bias was assessed using Deek's regression test of asymmetry and funnel plot.[24, 25] The regression test indicated the presence of publication bias ($p<0.01$), as did the asymmetry presented in the funnel plot. A sensitivity analysis using the trim-and-fill method was undertaken to investigate the impact of publication bias. The trim-and-fill method involves first trimming the studies that cause asymmetry (to minimise the impact of publication bias) and then imputing missing studies based on the bias-corrected overall estimate.[26, 27] Imputed values were added for eight studies, resulting in an adjusted log-transformed DOR of 5.94 (95% CI: 5.15 to 6.74) compared with 6.84 (95% CI: 6.09 to 7.58) in the main analysis. The funnel plots are presented in Figure A12.

Figure A12. Funnel plots of HCV-RNA tests in DBS



Key: DBS – dried blood spot; HCV – hepatitis C virus; RNA – ribonucleic acid.

Note: The trim-and-fill method is a sensitivity analysis that requires imputation to correct for publication bias. The imputed study estimates are coloured white in b). The vertical line represents the estimate from the random-effects model.

Subgroup and sensitivity analysis

The robustness of the univariate meta-analysis estimates was investigated by subgroup and sensitivity analysis. A statistically meaningful difference between subgroups was determined by a p-value of less than 0.05. The subgroup and sensitivity analysis included analyses:

- by target amplification method (that is, RT-PCR versus TMA)
- by DBS sample type (that is, capillary versus venous)
- by study design (cross-sectional versus case-control)
- stratified by risk of bias (low, moderate or high)
- according to common viral load threshold of 1,000 IU/ml.

The results of these analyses are summarised in Table A8. None of these analyses produced a statistically significant change in the meta-analysis estimates.

Table A8. Summary of subgroup and sensitivity analyses of HCV-RNA in DBS

Subgroup	N	Sensitivity (95% CI)	Specificity (95% CI)	DOR* (95% CI)
Target amplification method				
RT-PCR	15	0.97 (0.94-0.99)	1.00 (0.96-1.00)	6.71 (5.78-7.64)
TMA	5	0.98 (0.92-0.99)	0.99 (0.98-1.00)	7.84 (6.57-9.11)
DBS sample type				
Capillary	10	0.96 (0.92-0.98)	1.00 (0.96-1.00)	7.02 (6.19-7.86)
Venous	10	0.98 (0.95-0.99)	0.99 (0.93-1.00)	6.84 (5.51-8.17)
Study design				
Case-control	11	0.97 (0.93-0.99)	0.99 (0.96-1.00)	6.89 (5.69-8.09)
Cross-sectional	9	0.97 (0.94-0.98)	0.99 (0.93-1.00)	6.71 (5.83-7.58)
Risk of bias				
Low	6	0.96 (0.93-0.98)	0.99 (0.93-0.98)	6.5 (2.53-3.79)
Moderate	10	0.96 (0.91-0.99)	1.00 (0.91-0.99)	6.64 (2.32-4.27)
High	4	0.99 (0.94-1.00)	0.99 (0.94-1.00)	8.08 (2.76-6.74)
Viral load threshold				
1,000 IU/ml	5	0.97 (0.92-0.99)	0.99 (0.83-1.00)	6.66 (4.59-8.74)

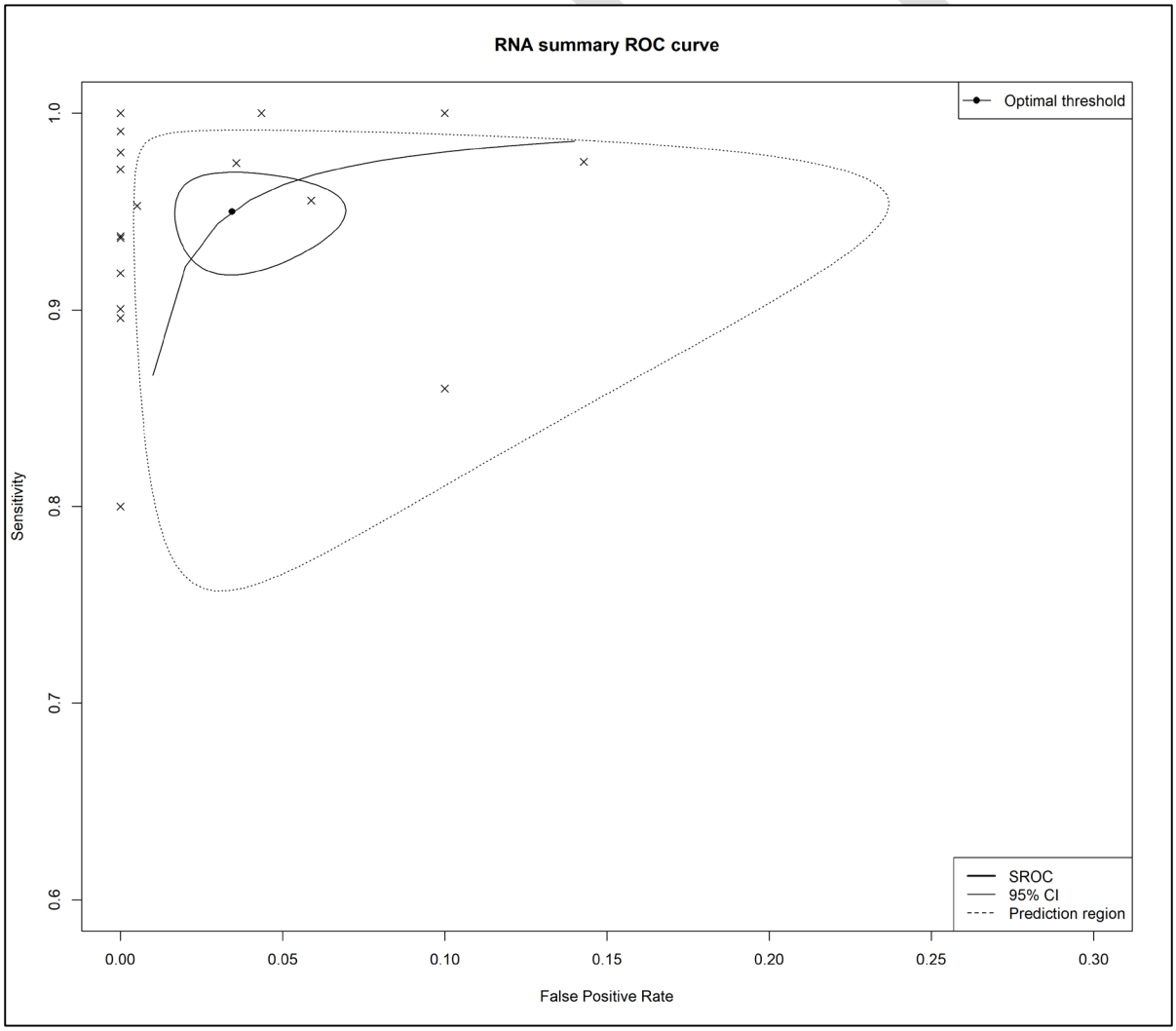
Key: CI – confidence interval; DBS – dried blood spot; DOR – diagnostic odds ratio; HCV – hepatitis C virus; IU/ml; NLR – negative likelihood ratio; PLR – positive likelihood ratio; RT-PCR – reverse transcription polymerase chain reaction; TMA – transcription-mediated amplification.
* Estimates are log-transformed.

A3.2.4 Bivariate meta-analysis of DBS for detection of HCV-RNA

Fitting of the bivariate model, to account for correlation between pairs of sensitivity and specificity, is considered standard practice in meta-analysis of diagnostic accuracy.[22, 75]

In the bivariate meta-analysis, the pooled sensitivity and specificity were estimated at 0.95 (95% CI: 0.93 to 0.97) and 0.97 (95% CI: 0.94 to 0.98), respectively. The correlation co-efficient was estimated at 0.052 (indicating that the univariate meta-analysis estimates are acceptable), the area under the curve (AUC) was 0.982 and the Akaike information criterion (AIC) was -152.29. The pooled log-transformed PLR, NLR and log-transformed DOR were estimated at 3.4 (95% CI: 2.8 to 3.9), 0.05 (95% CI: 0.03-0.07) and 6.3 (95% CI: 5.6 to 7.0), respectively. The summary receiver operating characteristic (SROC) curve, based on the parameters of the bivariate model, is presented in Figure A13.

Figure A13. SROC curve of HCV-RNA in DBS



Key: SROC – summary receiver operating characteristic.
Note: The SROC is plotted according to the generalisation of the Rutter and Gatsonis curve.[79]

A3.2.5 Methodological quality of studies assessing HCV-RNA in DBS

Risk of bias was assessed using the QUADAS-2 tool. Overall, six studies were rated as having a low risk of bias,[55, 56, 60, 62, 63, 66] 10 were considered to have a moderate risk of bias,[29, 33, 47, 48, 54, 57-59, 61, 64] and four were deemed to have a high risk of bias.[44, 51, 65, 67]

Participant selection was rated as introducing a high risk of bias in 12 of the 20 included studies.[29, 33, 44, 48, 51, 54, 57-59, 64, 65, 67] This was due to non-consecutive enrolment of study participants (such as convenience sampling) and or inappropriate exclusions (which may have results in a biased study sample). Study recruitment methods were unclear in five studies.[54, 55, 59, 61, 63] Although three studies reported a cross-sectional design,[33, 51, 58] study design appeared to be consistent with that of a case-control study as the diagnosis of patients (that is, the presence or absence of HCV-RNA) was known in advance, indicating a potential source of bias. Nine studies were consistent with a cross-sectional design.[47, 55, 56, 59-63, 66]

The index test (that is, the molecular assay used in the DBS sample) was rated as introducing a moderate risk of bias in:

- eight studies which did not report the use of a threshold[33, 48, 51, 54, 58, 59, 61, 65]
- five studies due to the use of a threshold to interpret DBS results that was not pre-specified[56, 57, 60, 64, 66]
- four studies which reported that they interpreted the results in accordance with the manufacturer's instruction, but without disclosing the explicit threshold used.[29, 44, 47, 67]

Three studies which pre-specified the threshold used to interpret results were rated at a low risk of bias.[55, 62, 63] Blinding of those interpreting DBS results (that is, without knowledge of the results in the reference standard) was reported in only three studies.[62, 63, 66] Blinding was unclear in all other studies. However, it should be noted that interpretation of HCV-RNA results is automated by molecular assays (rather than subjective) and therefore potential bias is likely minimal.

All of the studies used molecular assays in serum, plasma or whole blood as the reference standard which were considered at low risk of bias. Blinding during interpretation of results was reported in only two studies.[48, 66] As interpretation of HCV-RNA results is automated (rather than subjective) the potential bias is likely minimal and study quality was not downgraded on the basis of blinding.

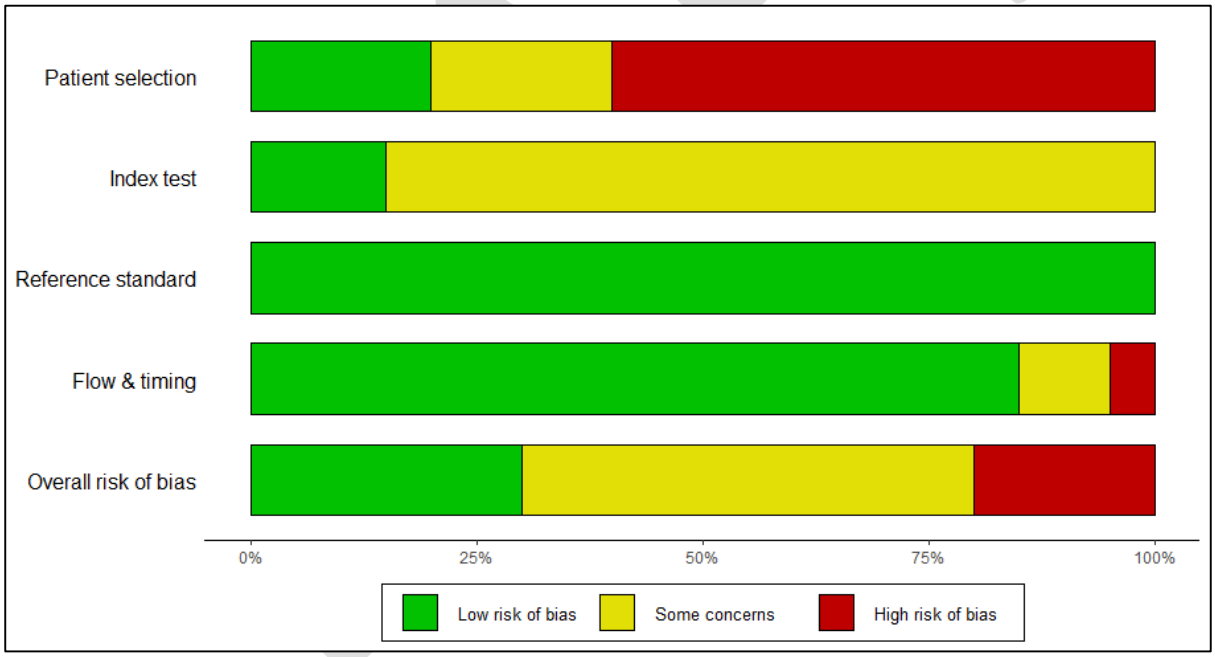
In the patient flow and timing domain, 17 of the 20 studies were rated at a low risk of bias.[29, 33, 47, 48, 51, 54-64, 66] One study was rated at moderate risk of bias as it was unclear if study participants all received the same reference standard assay.[67] One

study was rated at moderate risk of bias as it was unclear if all patients were included in the analysis of HCV-RNA.[44] One study was rate at a high risk of bias due to the inclusion of multiple DBS samples per patient in the analysis.[65]

Ten studies declared potential conflicts of interest due to the receipt of financial support from industry,[44, 48, 55, 56, 60, 62-64, 67] eight studies declared no competing interests,[29, 33, 47, 51, 54, 57, 59, 61] while two studies did not report any conflict of interest statement.[58, 65] The QUADAS-2 risk of bias domains are summarised for all studies in Figure A14 and Table A9.

The GRADE summary of findings table is presented in Table A10. Overall, the certainty of the evidence was assessed for the primary outcomes (that is, sensitivity and specificity) and judged to be low. The certainty of the evidence was downgraded for risk of bias (based on QUADAS-2 assessment) and indirectness due to a lack of representativeness of the study populations.

Figure A14. Overall risk of bias of studies assessing HCV-RNA in DBS, by QUADAS-2 domain



Key: DBS – dried blood spot; HCV – hepatitis C virus; QUADAS – Quality Assessment of Diagnostic Accuracy Studies; RNA – ribonucleic acid.
Note: "Some concerns" indicates moderate risk of bias.

Table A9. Risk of bias of included studies assessing HCV-RNA in DBS, by QUADAS-2 domain

Study	Patient Selection	Index Test	Reference standard	Flow and timing
Bennett 2012	High	Some concerns	Low	Low
Bibi 2020	High	Some concerns	Low	Low
Catlett 2019a	Some concerns	Low	Low	Low
Catlett 2020	Low	Some concerns	Low	Low
De Crignis 2010	High	Some concerns	Low	Low
Dokobu 2014	High	Some concerns	Low	Low
Fouad 2013	High	Some concerns	Low	Low
Gomez 2020	High	Some concerns	Low	Low
Mahajan 2018	Low	Some concerns	Low	Low
Mössner 2016	High	Some concerns	Low	Some concerns
Nguyen 2018	Some concerns	Some concerns	Low	Low
Ross 2013	Some concerns	Some concerns	Low	Low
Saludes 2019	Low	Low	Low	Low
Saludes 2020	Some concerns	Low	Low	Low
Shepherd 2019	High	Some concerns	Low	Low
Solmone 2002	High	Some concerns	Low	High
Soulier 2016	High	Some concerns	Low	Low
Tran 2020	Low	Some concerns	Low	Low
Vazquez-Moron 2018	High	Some concerns	Low	Low
Wlassow 2019	High	Some concerns	Low	Some concerns

Key: DBS – dried blood spot; HCV – hepatitis C virus; QUADAS – Quality Assessment of Diagnostic Accuracy Studies.

Note: "Some concerns" indicates moderate risk of bias.

Table A10. GRADE summary of findings table for diagnostic accuracy of HCV-RNA tests in DBS

Outcome	Study design	Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	Certainty of evidence
Sensitivity: 0.95 (95% CI: 0.93 to 0.97)							
Studies: 20 Sample size: 1,831	Cross-sectional, case-control	Serious*	Serious**	Not serious	Not serious	Not serious***	⊕⊕◯◯ LOW
Specificity: 0.97 (95% CI: 0.94 to 0.98)							
Studies: 20 Sample size: 1,109	Cross-sectional, case-control	Serious*	Serious**	Not serious	Not serious	Not serious***	⊕⊕◯◯ LOW
Prevalence of HCV-RNA: 0.62 (95% CI: 0.54 to 0.70)							

Key: CI – confidence interval; DBS – dried blood spot; HCV – hepatitis C virus.
* Overall certainty of evidence downgraded one level for risk of bias due to limitations in study design and execution (based on risk of bias assessment in QUADAS-2).
** Overall certainty of evidence downgraded one level for indirectness due to lack of representativeness of the study population.
*** Publication bias was suspected, but not considered serious enough for downgrading certainty of evidence.

A3.3.1 Overview of included studies HCV-core antigen in DBS

Overall, six individual studies assessing the diagnostic accuracy of HCV core antigen tests in DBS were included in the synthesis.[48, 61, 68-71] The included studies were published between 2016 and 2019. Two of the studies were from Australia,[69, 70] and one each was from Canada,[68] France,[48] Tanzania,[71] and Vietnam.[61]

There was a total study population of 1,154 participants across the included studies, with sample size ranging from 83 to 511 participants. Five studies reported the gender of study participants.[48, 61, 68, 69, 71] The proportion of male participants ranged from 55% to 96%, with a mean of 70%. Five studies reported the age of study participants, with the average ranging from 34 to 56 years.[48, 61, 68, 69, 71]

Prevalence of HCV-RNA (based on the reference standard) ranged from 28% to 85%, with a weighted mean of 65%. Positive predictive value and negative predictive value ranged from 0.99 to 1.00 and 0.54 to 1.00, respectively. Three studies reported the disease severity (that is, fibrosis distribution) of HCV-infected participants.[61, 68, 69] The majority (>66%) of patients that received fibrosis staging were between METAVIR fibrosis stages F0 to F2.

The HCV genotype of HCV-infected participants was reported in three studies,[68, 70, 71] with HCV genotype 1, HCV genotype 3 and HCV genotype 4 reported in 65%, 16% and 12% of study participants, respectively. However, the reported genotype data was generally from only a subset of participants or based on a larger cohort study (for example, where oral samples were also analysed as part of the study) therefore its applicability is limited.

Study population risk factors for HCV acquisition were reported in four studies.[61, 68, 69, 71] From these, the most commonly reported risk factors were co-infection with HBV and or HIV, and a history of injecting drug use. However, a detailed breakdown of risk factor data was often not provided. Three studies reported the treatment status of HCV-infected patients.[48, 61, 68] In two of these,[48, 61] all HCV-infected patients were treatment-naïve. In the other, 76% of HCV-infected patients were treatment-naïve.[68]

Five studies compared the diagnostic outcomes of HCV core antigen tests in DBS with RNA tests in serum or plasma.[61, 68-71] One study compared the diagnostic outcomes of HCV core antigen tests in DBS with HCV core antigen tests in serum or plasma.[48]

Five studies reported diagnostic outcomes for venous DBS samples.[48, 61, 68, 70, 71] One study reported use of capillary DBS samples.[69] All of the studies reported the use of CMIA.

The included studies contributed 18 unique pairs of diagnostic outcomes (that is,

sensitivity and specificity) of core antigen tests in DBS to the synthesis. Three studies contributed only one pair of diagnostic outcomes.[48, 61, 71] Three studies contributed multiple 2x2 data pairs.[68-70] The reasons for individual studies contributing multiple pairs of diagnostic outcomes included:

- reporting paired outcomes by threshold applied or analytical sensitivity (e.g. qualitative versus quantitative)[68-70]
- storage conditions (storage temperature ranged from -80 degrees Celsius to 37 degrees Celsius)[68]

Three studies reported the use of a viral load threshold,[68-70] reported in terms of international units per millilitre (IU/ml). The viral load thresholds reported ranged from 12 IU/ml to 3,000 IU/ml. The most commonly reported threshold (n=3) was a viral load threshold of 3,000 IU/ml. The other studies did not reported the use of an explicit threshold during the interpretation of results.[48, 61, 71]

Additional study characteristics are presented in Appendix A2.

A3.3.2 Study-level estimates of DBS for detection of HCV-core antigen in DBS

Five studies reported multiple pairs of diagnostic outcomes comparing the HCV core antigen in DBS with HCV-RNA in serum, plasma or whole blood. The range (that is, the minimum and maximum) of the study-level estimates is presented in Table A9. Forest plots of the study-level estimates of sensitivity and specificity of HCV-core antigen in DBS compared with HCV-RNA in serum or plasma are presented in Figure A15.

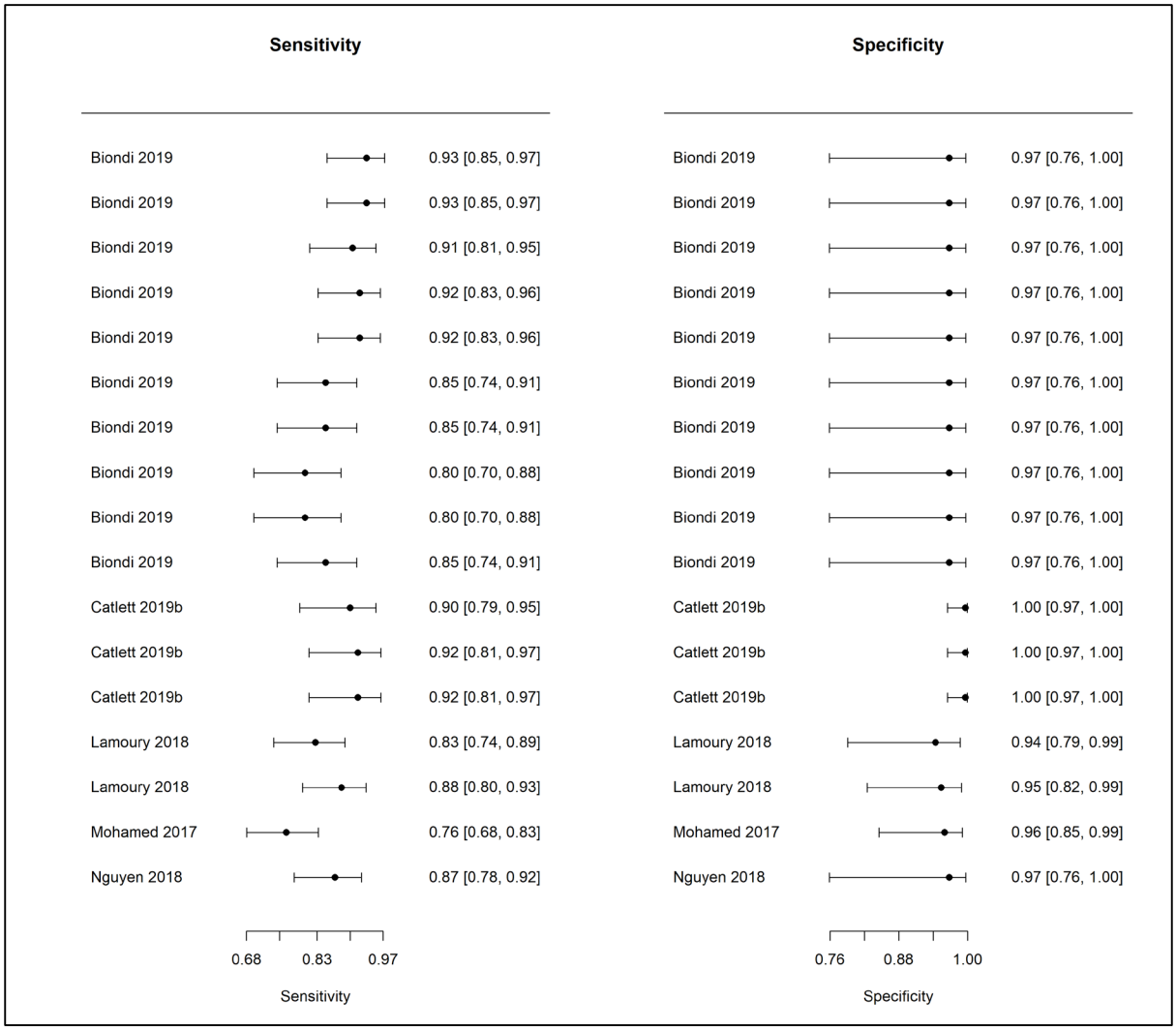
One study,[48] which compared HCV core antigen in DBS with HCV core antigen in serum and whole blood, estimated a sensitivity of 0.64 (95% CI: 0.58 to 0.69) and a specificity of 1.00 (95% CI: 0.98 to 1.00). This study is excluded from Table A11 and Figure A9 due to the use of a different reference standard to that of the other studies.

Table A11. Summary of study-level estimates of HCV core antigen in DBS compared with HCV-RNA in serum or plasma

Outcome	Lowest reported estimate (95% CI)	Highest reported estimate (95% CI)	N*
Sensitivity	0.76 (95% CI: 0.68 to 0.83)	0.93 (95% CI: 0.85 to 0.97)	18
Specificity	0.94 (95% CI: 0.79 to 0.99)	1.00 (95% CI: 0.97 to 1.00)	18
NLR	0.07 (95% CI: 0.03 to 0.17)	0.24 (95% CI: 0.18 to 0.34)	18
PLR**	2.70 (95% CI: 1.14 to 4.26)	5.50 (95% CI: 2.74 to 8.27)	18
DOR**	4.37 (95% CI: 2.68 to 6.06)	7.99 (95% CI: 5.05 to 10.93)	18

Key: CI – confidence interval; DBS – dried blood spot; DOR – diagnostic odds ratio; HCV – hepatitis C virus; NLR – negative likelihood ratio; PLR – positive likelihood ratio.
* Total number of data pairs across six individual studies.
** Estimates are log-transformed.

Figure A15. Forest plot of study-level estimates of HCV core antigen sensitivity and specificity



Key: HCV – hepatitis C virus.

Note: Some studies reported multiple pairs of diagnostic outcomes. Pooling of these data would break the assumption of independence in meta-analysis.

A3.3.3 Univariate meta-analysis of DBS for detection of HCV-core antigen in DBS

As described in section A2.4, to facilitate pooling of study estimates one data pair was selected from studies that reported multiple pairs of diagnostic outcomes.

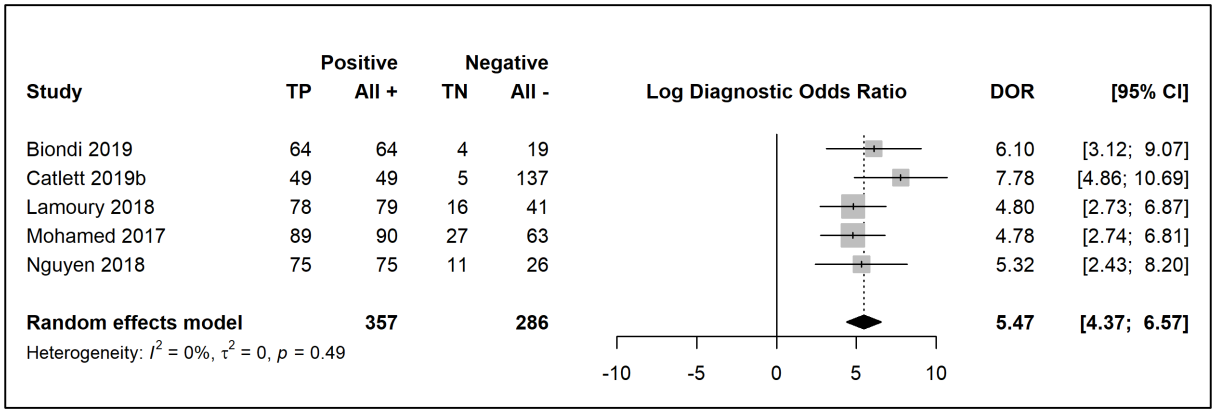
Five studies compared the diagnostic outcomes of HCV core antigen tests in DBS with RNA tests in serum or plasma.[61, 68-71] One study compared the diagnostic outcomes of HCV core antigen tests in DBS with HCV core antigen tests in serum or plasma.[48] This study was excluded from the meta-analysis.

The pooled sensitivity and specificity were estimated at 0.87 (95% CI: 0.80 to 0.91) and 0.99 (95% CI: 0.96 to 1.00), respectively, across all included studies comparing HCV-core antigen in DBS with HCV-RNA in serum, plasma or whole-blood. The pooled log-transformed PLR and NLR were estimated at 3.23 (95% CI: 2.33 to 4.14) and 0.15 (95% CI: 0.10 to 0.23), respectively. The log-transformed DOR was estimated at 5.47 (95% CI: 4.37 to 6.57). The forest plots of DORs, sensitivity and specificity are presented in Figure A16-A17.

The I^2 index of sensitivity and specificity was estimated at 61% and 22%, respectively, indicating a high degree of statistical heterogeneity in sensitivity (indicating variability in the point estimates of sensitivity), but low heterogeneity in specificity estimates (which were relatively consistent).

A bivariate meta-analysis of HCV core antigen tests in DBS was not undertaken due to the small number of included studies (n=5). Similarly, publication bias was not investigated as too few studies were included in the meta-analysis. The general rule of thumb is that there should be a minimum of 10 studies to assess publication bias.[80]

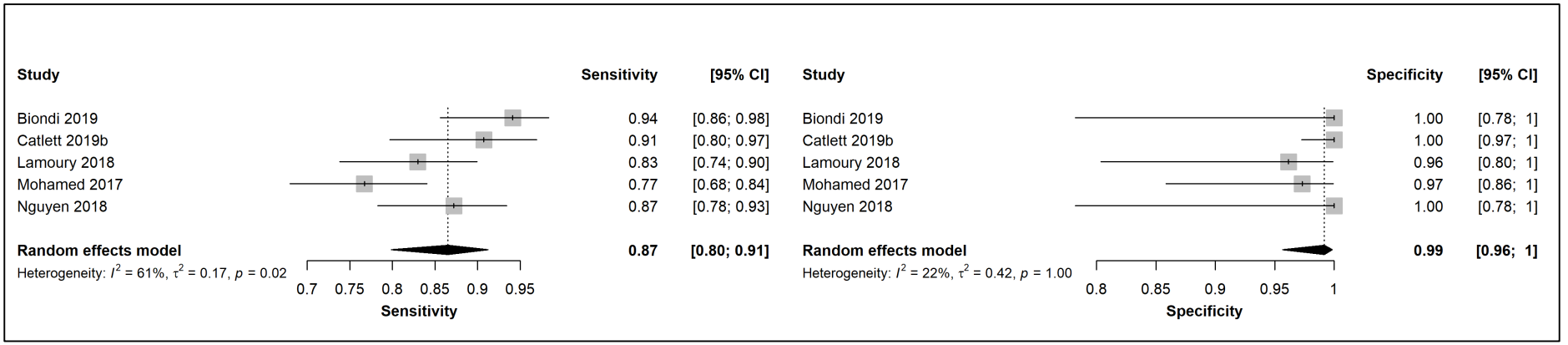
Figure A16. Forest plot of DORs of HCV-core antigen in DBS compared with HCV-RNA in serum, plasma or whole blood



Key: DBS – dried blood spot; DOR – diagnostic odds ratio; HCV – hepatitis C virus; RNA – ribonucleic acid.

Note: Although the DOR provides a single indicator of diagnostic performance, it cannot weight the true positive and false positive rates separately, and does not adequately reflect variability between studies.[74] The pooled estimate does not account for correlation between pairs of sensitivity and specificity.[75-77] Heterogeneity is considered the rule rather than expectation in meta-analyses of diagnostic accuracy.[75, 76] Univariate tests for heterogeneity, such as the inconsistency index (I^2) and tau^2, can be misleading.

Figure A17. Forest plot of sensitivity and specificity of HCV core antigen tests in DBS compared with HCV-RNA in serum, plasma or whole blood



Key: DBS – dried blood spot; HCV – hepatitis C virus; RNA – ribonucleic acid.

Note: Univariate meta-analysis does not account for correlation between pairs of sensitivity and specificity.[75-77] Heterogeneity is considered the rule rather than expectation in meta-analyses of diagnostic accuracy.[75, 76] Univariate tests for heterogeneity, such as the inconsistency index (I^2) and tau^2, can be misleading.

A3.3.4 Methodological quality of studies assessing HCV-core antigen in DBS

Risk of bias was assessed using the QUADAS-2 tool. Overall, two studies were rated as having a low risk of bias,[69, 70] three as having a moderate risk of bias,[48, 61, 71] and one as having a high risk of bias.[68]

Participant selection was judged as introducing a high risk of bias in one of the six included studies.[48] This was due to the non-consecutive enrolment of study participants and potentially inappropriate exclusions (which may have results in a biased study sample). Study recruitment methods were unclear in two studies, and rated as introducing a moderate risk of bias.[61, 70] Four of the studies were consistent with a cross-sectional design,[61, 69-71] and the other two were case-control studies.[48, 68]

The index test (that is, the immunoassay used in the DBS sample) was rated as introducing a moderate risk of bias in three studies which did not report the use of a threshold.[48, 61, 71] Three studies which pre-specified the threshold used to interpret results were rated at a low risk of bias.[68-70] Blinding of those interpreting DBS results (that is, without knowledge of the results in the reference standard) was not reported in any of the studies. However, it should be noted that interpretation of HCV core antigen results is automated by immunoassays (rather than subjective) and therefore potential bias is likely minimal.

Five studies used RT-PCR in serum or plasma as the reference standard.[61, 68-71] One study used a HCV core antigen immunoassay in serum and whole-blood as the reference standard.[48] All of these studies were considered to have a low risk of bias. Blinding during interpretation of results was reported in only two studies.[48, 68] As interpretation of HCV core antigen results is automated (rather than subjective) the potential bias is likely minimal and study quality was not downgraded on the basis of blinding.

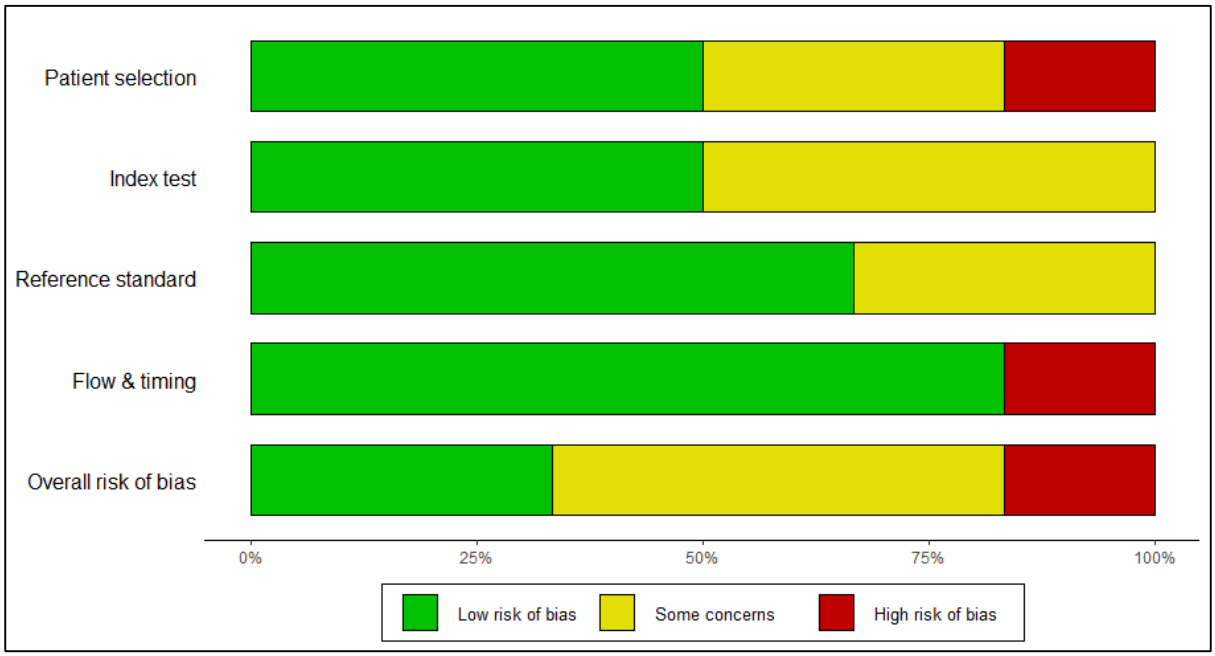
In the patient flow and timing domain, one study was rated as having a high risk of bias due to exclusion of study participants from the analysis, without appropriate explanation.[68] Five of the six studies were rated as having a low risk of bias.[48, 61, 69-71]

Five studies declared potential conflicts of interest due to the receipt of financial support from industry,[48, 68-71] while one study declared no competing interests.[61] The risk of bias for all studies is summarised according to each of the QUADAS-2 domains in Figure A18 and Table A12.

The GRADE summary of findings table is presented in Table A13. Overall, the certainty of the evidence was assessed for the primary outcomes (that is, sensitivity and

specificity) and judged to be very low. The certainty of the evidence was downgraded for risk of bias (based on QUADAS-2 assessment), indirectness due to a lack of representativeness of the study populations and imprecision due to the limited number of studies, the small sample sizes and variation in the effect estimates.

Figure A18. Overall risk of bias of studies assessing HCV-core antigen in DBS, by QUADAS-2 domain



Key: DBS – dried blood spot; HCV – hepatitis C virus; QUADAS – Quality Assessment of Diagnostic Accuracy Studies.
Note: "Some concerns" indicates moderate risk of bias.

Table A12. Risk of bias of included studies assessing HCV-core antigen in DBS, by QUADAS-2 domain

Study	Patient Selection	Index Test	Reference standard	Flow and timing
Biondi 2019	Some concerns	Low	Low	High
Catlett 2019b	Low	Low	Low	Low
Lamoury 2018	Low	Low	Low	Low
Mohamed 2017	Low	Some concerns	Some concerns	Low
Nguyen 2018	Some concerns	Some concerns	Some concerns	Low
Soulier 2016	High	Some concerns	Low	Low

Key: DBS – dried blood spot; HCV – hepatitis C virus; QUADAS – Quality Assessment of Diagnostic Accuracy Studies.
Note: "Some concerns" indicates moderate risk of bias.

Table A13. GRADE summary of findings table for diagnostic accuracy HCV-core antigen in DBS

Outcome	Study design	Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	Certainty of evidence
Sensitivity: 0.95 (95% CI: 0.93 to 0.97)							
Studies: 5 Sample size: 418	Cross-sectional, case-control	Serious*	Serious**	Not serious	Serious***	Not serious****	⊕◯◯◯ VERY LOW
Specificity: 0.97 (95% CI: 0.94 to 0.98)							
Studies: 5 Sample size: 225	Cross-sectional, case-control	Serious*	Serious**	Not serious	Serious***	Not serious****	⊕◯◯◯ VERY LOW
Prevalence of HCV-core antigen: 0.65 (95% CI: 0.57 to 0.73)							

Key: CI – confidence interval; DBS – dried blood spot; HCV – hepatitis C virus.

* Overall certainty of the evidence downgraded one level for risk of bias due to limitations in study design and execution (based on risk of bias assessment in QUADAS-2).

** Overall certainty of the evidence downgraded one level for indirectness due to lack of representativeness of the study population.

*** Overall certainty of the evidence downgraded one level for imprecision due to the limited number of studies included in the meta-analysis, the small sample sizes and variation in the effect estimates and 95% CIs.

**** Publication bias was suspected, but not considered serious enough for downgrading certainty of evidence.

A4 Discussion

Recent advances in HCV therapies have led to a shift towards identifying and curing those currently living with chronic HCV infection with the aim of achieving the World Health Organization's viral hepatitis elimination targets by 2030.[81, 82] A key logistical challenge for identifying the currently undiagnosed population is how best to integrate reflex testing into the care pathway with a view to minimising patient drop-off along the cascade of care.[2] While no DBS assays are currently commercially available for diagnosis of HCV infection,[83, 84] growing interest in its "off-label" use has led to the development of standardised laboratory protocols for using DBS samples in serological and molecular techniques.[85]

When considering the implementation of reflex testing, the use of DBS samples offers potential logistical advantages over the current standard of conventional blood samples collected by phlebotomy. This is because the enhanced stability of a DBS sample provides greater flexibility for reflex testing (that is, unlike conventional blood samples neither cold-chain storage nor time-dependent centrifugation are required to ensure that samples are suitable for reflex testing).[4, 5] In other words, a DBS sample does not need to be spun down and frozen upon arrival at a laboratory, instead it can be stored for when and if the need arises for a reflex test of a positive anti-HCV blood sample. The use of a reflex test in this manner could mitigate against potential patient drop-off that may occur when two separate healthcare attendances are required to receive a positive diagnosis of chronic HCV infection.

This systematic review and meta-analysis assessed the diagnostic accuracy of DBS samples compared with conventional blood samples for detection of HCV using laboratory-based tests (that is, anti-HCV, RNA and core antigen). The analyses found a high diagnostic accuracy for each of anti-HCV and HCV-RNA tests in DBS compared with these tests in serum, plasma or whole blood samples collected by venepuncture. This finding was generally robust during subgroup and sensitivity analysis. Therefore, these tests may provide a useful alternative in situations where reflex testing of conventional blood samples is not logistically feasible.

The diagnostic accuracy of HCV core antigen in DBS was lower than that of anti-HCV and HCV-RNA tests in DBS, but fewer studies were included in the meta-analysis. Therefore, further research is required to determine if HCV antigen testing in DBS provides a useful alternative.

A clear limitation in the general applicability of these estimates is that many of the included studies were conducted in at-risk populations in developing countries. Therefore, any decision to implement reflex HCV-RNA testing using DBS samples in a birth cohort testing programme would first require independent validation in the

healthcare setting of intended use. In addition, any deliberative decision-making process will need to consider the additional time required in the laboratory for manual processing of DBS samples.

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Appendix A1.1: Search strategy

A1.1.1 Database search (run July 17th 2020)

PubMed	
A	("hepatitis C") OR (HCV)
B	((((((DBS) OR (Whatman)) OR ("filter paper")) OR ("dried blood spot")) OR ("dried blood filter")) OR ("dried blood")) OR ("dried sample")
C	(((((((sensitivity) OR (specificity)) OR ("positive predictive value")) OR ("negative predictive value")) OR (AUROC)) OR (AUCROC)) OR (diagnostic)) OR (screening)
D	A AND B AND C

Embase	
A	'hepatitis c'/exp OR 'hepatitis c' OR 'hepatitis c virus'/exp OR 'hepatitis c virus'
B	'dbs' OR 'whatman' OR 'filter paper' OR 'dried blood spot testing' OR 'dried blood spots' OR 'dried blood spot sampling' OR 'dried sample' OR 'dried blood filter'
C	'sensitivity and specificity' OR 'sensitivity' OR 'specificity' OR 'predictive value' OR 'area under the curve' OR 'diagnosis' OR 'screening'
D	A AND B AND C

Cochrane Library	
A	"hepatitis C" OR HCV
B	"DBS" OR "Whatman" OR "filter paper" OR "dried blood spot" OR "dried blood filter" OR "dried blood" OR "dried sample"
C	sensitivity OR specificity OR "positive predictive value" OR "negative predictive value" OR auroc OR aucroc OR diagnostic OR screening
D	A AND B AND C

Lilacs	
A	"hepatitis C" OR HCV
B	"DBS" OR "Whatman" OR "filter paper" OR "dried blood spot" OR "dried blood filter" OR "dried blood" OR "dried sample"
C	sensitivity OR specificity OR "positive predictive value" OR "negative predictive value" OR auroc OR aucroc OR diagnostic OR screening
D	A AND B AND C

Web of Science	
A	TS=("hepatitis C" OR HCV)
B	TS=("DBS" OR "Whatman" OR "filter paper" OR "dried blood spot" OR "dried blood filter" OR "dried blood" OR "dried sample")
C	TS=(sensitivity OR specificity OR "positive predictive value" OR "negative predictive value" OR auroc OR aucroc OR diagnostic OR screening)
D	A AND B AND C

A1.1.2 Grey literature search

The following electronic sources will also be searched to identify grey literature relevant to this systematic review's research question:

- American Association for Clinical Chemistry (AACC); Available from: https://www.aacc.org/science-and-practice/practice-guidelines
- Centers for Disease Control and Prevention; Available from: https://www.cdc.gov/
- Cochrane Register of Diagnostic Test Accuracy Studies; Available from https://methods.cochrane.org/sdt/cochrane-diagnostic-test-accuracy-reviews
- Google Scholar and Google; Available from https://scholar.google.com/, https://www.google.ie
- Health Service Executive (HSE); Available from https://www.hse.ie/eng/
- Health Information and Quality Authority (HIQA); Available from https://www.hiqa.ie/
- Health Research Board (HRB) Ireland; Available from http://www.hrb.ie/home/
- Lenus; Available from http://www.lenus.ie/hse/
- MedNar; Available from https://mednar.com/mednar/desktop/en/search.html
- National Coordinating Centre for Health Technology Assessment (NCCHTA) ; Available from https://www.nihr.ac.uk/funding-and-support/funding-for-research-studies/funding-programmes/health-technology-assessment/
- National Health Service (NHS) Evidence; Available from https://www.evidence.nhs.uk/
- National Institute for Health and Clinical Excellence (NICE); Available from https://www.nice.org.uk/
- Ontario Association of Medical Laboratories; Available from: https://oaml.com/guidelines/
- Open Grey; Available from http://www.opengrey.eu/
- World Health Organization (WHO); Available from http://www.who.int/en/

Appendix A1.2: Study characteristics

Table A2.1. Summary of study characteristics of included studies assessing anti-HCV in DBS

Study, country	Study design	Filter paper brand	DBS volume	Storage	Index test (in DBS)	Reference standard (in serum or plasma)
Bibi 2020, Pakistan	CC	Whatman® 903	NA	Packed in zip-locked bag with 2 silica gel desiccants, stored in an airtight box at -20°C.	Abbott Murex HCV AgAb	
Brandão 2013, Brazil	CC	Whatman® 903	75 µL	Air-dried at rt for 4 hours, packed in sealable plastic bags with silica desiccant and stored at −20°C.	Bio-Rad MonolisaTM HCV AgAb ULTRA	
Croom 2006, Australia	CC	Schleicher and Schuell cards (Grade 903)	80 µl	Air-dried at rt, packed in envelope inside zip lock bag with silica desiccant, stored at −20°C.	Bio-Rad Monolisa anti-HCV PLUSVersion 2 EIA	
Cruz 2018, Brazil	CC	Whatman® 903	105 µL	NA	Multiple assays evaluated: MBiolog Diagnósticos Imunoscreen HCV SS EIAs; MBiolog Diagnósticos Optimised EIA	Multiple assays evaluated: Radim, DiaSorin HCV Ab (Radim) or ETI-AB-HCVK-4 (DiaSorin)
Dokobu 2014, USA	CC	Whatman® 903	50 µL	Air-dried for 2 hours, packaged and stored at −70◦C.	Ortho-Clinical Diagnostics ELISA HCV v3.0	Third-generation ELISA or CLIA (unspecified)
Flores 2017, Brazil	CC	Whatman® 903	75 µL	Air-dried at rt for 4 hours, placed in sealable plastic bag with silica desiccant sachets, stored at −20°C.	DiaSorin HCV Murex Ab	
Gaelejwe 2018, South Africa	CS	Whatman® 903	50 µL	Stored at -20˚C.	Abbott ARCHITECT HCV Ab assay	
Kania 2013, Burkina Faso	CS	Whatman® 903	50 µl	NA	Bio-Rad Monolisa HCV Ab-Ag ULTRA assay	

Study, country	Study design	Filter paper brand	DBS volume	Storage	Index test (in DBS)	Reference standard (in serum or plasma)
Kenmoe 2018, Cameroon	CC	Whatman® 903	50 µl	NA	Abbott ARCHITECT HCV Ab assay	
Larrat 2012, France	CC	Whatman® 903	NA	Air-dried at rt for 24 hours, placed in zip-locked plastic bag, stored at −20°C.	Bio-Rad Monolisa HCV-Ag-Ab-ULTRA	
Ma 2020a, China	CS	Whatman® 903	70/100 µL	Air-dried at rt for 4 hours, stored at −20°C.	WANTAI BioPharm Diagnostic Kit for Antibody to Hepatitis C Virus	
Ma 2020b, China	CS	Whatman® 903	70 µL	Air-dried for 3 hours at rt, stored at −20°C.	WANTAI BioPharm Diagnostic Kit for Antibody to Hepatitis C Virus	
Marques 2012, Brazil	CC	Whatman® 903	75 µL	Air-dried for 4 hours at rt, placed in zip-locked plastic bag with silica gel desiccant, stored at −20°C.	Multiple assays evaluated: Radim HCV Ab; DiaSorin ETI-AB-HCVK-4	
Marques 2016, Brazil	CC	Whatman® 903	75 µL	Air-dried for 4 hours at rt, placed in zip-locked plastic bag with silica gel desiccant, stored at −20°C.	Radim HCV Ab	
McCarron 1999, UK	CC	Guthrie filter paper	NA	Stored at 4°C.	Sanofi Monolisa anti-HCV	Abbott AxSYM HCV version 3.0
Mössner 2016, Denmark	CC	Whatman® 903	75 µL	Air-dried for 24 hours at rt.	Abbott ARCHITECT HCV Ab assay	
O'Brien 2001, USA	CS	NA	NA	Air-dried for 30 minutes, packed in zip-locked bag with desiccant, sent by mail.	Third-generation EIA (unspecified)	
Rice 2012, UK	CC	Guthrie filter paper	NA	NA	Ortho-Clinical Diagnostics Chiron V3.0 SaVE assay	Unspecified
Ross 2013, Germany	CS	Whatman® 903	100 µL	Dried and eluted overnight at rt then centrifuged.	Abbott ARCHITECT HCV Ab assay	
Soulier 2016, France	CC	Whatman® 903	50 µL	Air-dried at rt for 1 hour, placed in sealed plastic bag with desiccant, stored at −80°C.	Ortho-Clinical Diagnostics aHCV Vitros Eci	

Study, country	Study design	Filter paper brand	DBS volume	Storage	Index test (in DBS)	Reference standard (in serum or plasma)
Tejada-Strop 2015, USA	CC	Whatman® 903	75 µL	Packed in Bitran® bags with desiccant, stored at −20°C until use 5 years later.	Multiple assays evaluated: Ortho-Clinical Diagnostics VITROS anti-HCV assay; Ortho-Clinical Diagnostics HCV 3.0 enzyme immunoassay	
Tuaillon 2010, France	CC	Whatman® 903	50 µL	Dried for 18 hours at rt, placed in zipped plastic bag with desiccant, stored at 20°C.	Ortho-Clinical Diagnostics Ortho HCV 3.0 ELISA Test	
Vazquez-Moron 2018, Spain	CC	Whatman® 903	NA	Air-dried at rt for 4 hours, placed in zipped plastic bag with desiccant, stored at 4°C.	DiaSorin Murex anti-HCV kit, version 4.0	Siemens ADVIA Centaur HCV assay
Villar 2019, Brazil	CS	Whatman® 903	75 µL	NA	Roche Elecsys anti-HCV II	
Villar 2020, Argentina	CS	Whatman® 903	75 µL	Air-dried at rt for 4 hours, placed in sealable plastic bag with silica desiccant, stored at −20°C.	DiaSorin Murex HCV Ab	

Key: CC – case control; CLIA – chemiluminescent immunoassay; CS – cross sectional; DBS – dried blood spot; EIA – enzyme immunoassay; ELISA – enzyme-linked immunosorbent assay; HCV – hepatitis C virus; NA – not available; rt – room temperature.

Table A2.2. Summary of study characteristics of included studies assessing HCV-RNA in DBS

Study, country	Study design	Filter paper brand	DBS volume	Storage	Index test (in DBS)	Reference standard (in serum or plasma)
Bennett 2012, UK	CC	Whatman® 903	NA	Air-dried at rt for 1 hour, stored at 4°C.	In-house HCV/IC duplex real time RT-PCR assay	Abbott Real-time RT-PCR: m2000 rt
Bibi 2020, Pakistan	CC	Whatman® 903	NA	Packed in zip-locked plastic bag with two silica gel desiccants, kept in an airtight box stored at -20°C.	Qiagen QIAmp DSP	
Catlett 2019a, Australia	CS	Munktell TFN	70 µl	Air-dried in biological safety cabinet for 24 hours, stored in sealable gas impermeable bags with 2 desiccants, stored at −80 °C.	Hologic Aptima HCV Quant Dx assay	Roche CAP/CTM HCV assay on Roche Cobas TaqMan
Catlett 2020, Australia	CS	Munktell TFN	70 µl	Air-dried in biological safety cabinet for 24 hours, stored in sealable gas impermeable bags with 2 desiccants, stored at −80 °C.	Hologic Aptima HCV Quant Dx assay	Multiple assays evaluated: Hologic Aptima HCV Quant assay; Cepheid Cepheid Xpert HCV Viral Load assay
De Crignis 2010, Italy	CC	Whatman® 903	50 µL	Air-dried at 37 ◦C for 1 hour prior to nucleic acid extraction.	Qiagen Multiplex SYBR Green real-time RT-PCR	Siemens Versant HCV RNA 3.0 b-DNA Assay
Dokobu 2014, USA	CC	Whatman® 903	0.5 ml	Air-dried for two hours, packaged and stored at −70◦C.	Novartis dHCV TMA	Novartis Procleix Ultrio TMA Assay
Fouad 2013, Saudi Arabia	CC	Whatman® 903	50 µl	Air-dried at rt, placed in plastic locked bag, stored at -20°C.	Qiagen Artus HCV RG RT-PCR Kit	
Gomez 2020, Spain	CS	Whatman® 903	80 µL	Transferred at rt.	Roche Roche Cobas 6800	
Mahajan 2018, India	CS	Munktell TFN	70 µl	Air-dried overnight at rt, stored in a ziplock sachet with desiccant at 2–8°C.	Abbott Abbott Real-Time HCV assay	
Mössner 2016, Denmark	CC	Whatman® 903	75 µL	Air dried for 24 hours at rt.	Grifols Diagnostic Solutions Procleix Ultrio Elite assay	
Nguyen 2018,	CS	Whatman®	50 µl	Air-dried at rt, stored in sealed	Biocentric HCV Generic assay	Sacace Biotechnologies HCV

Study, country	Study design	Filter paper brand	DBS volume	Storage	Index test (in DBS)	Reference standard (in serum or plasma)
Vietnam		903		plastic bags with desiccant at – 20 °C.		Real-time Quant
Ross 2013, Germany	CS	Whatman® 903	100 µL	Dried and eluated overnight at rt.	Siemens Healthcare Diagnostics VERSANT HCV RNA	
Saludes 2019, Spain	CS	Whatman® 903	50 µl	Air-dried for at 1 hour, stored with desiccant and humidity indicator in individual pouches, shipped weekly to laboratory at rt .	In-house single-step RT real-time PCR HCV-RNA assay	Abbott Abbott Real-Time HCV Assay
Saludes 2020, Spain	CS	Whatman® 903	50 µl	Air-dried and stored at rt, then shipped to central laboratory at rt the following day.	In-house single-step RT real-time PCR HCV-RNA assay	Cepheid Xpert VL assay
Shepherd 2019, UK	CC	Whatman® 903	50µl	Air-dried overnight at rt, stored at −80 °C.	Abbott Abbott RealTime HCV assay	
Solmone 2002, Italy	CC	Whatman® 903	50 µL	Stored at rt.	Multiple assays evaluated: In-house TMA (unspecified); In-house RT-PCR (unspecified)	Multiple assays evaluated: Bayer Diagnostic Versant HCV RNA Qualitative Assay; Roche Amplicor HCV Monitor
Soulier 2016, France	CC	Whatman® 903	50 µL	Air-dried at rt for 1 hour, placed in individual sealed plastic bag with desiccant, stored at −80°C.	Multiple assays evaluated: Roche Cobas AmpliPrep TaqMan HCV Assay Version 2; Abbott Abbott RealTime HCV assay	
Tran 2020, Vietnam	CS	Munktell TFN	70 µL	Air-dried at rt for 3 hours, individually packed in ziplock bag with three desiccants.	Abbott Abbott RealTime HCV assay	
Vazquez-Moron 2018, Spain	CC	Whatman® 903	NA	Air-dried at rt for 4 hours, kept in zip-locked plastic bags with a drierite desiccant, stored at 4°C.	Qiagen Quantitec SYBR Green RT-PCR One Step kit	Siemens VERSANT HCV RNA 1.0 Assay
Wlassow 2019, France	CC	Whatman® 903	50 µL	Air-dried for 1 hour, stored in zip-locked plastic bag with desiccant	Cepheid Xpert HCV Viral Load Assay	Abbott RealTime HCV Viral Load assay/Roche Cobas

Study, country	Study design	Filter paper brand	DBS volume	Storage	Index test (in DBS)	Reference standard (in serum or plasma)
				−80 °C.		AmpliPrep TaqMan HCV Assay Version 2.0

Key: CC – case control; CS – cross sectional; DBS – dried blood spot; HCV – hepatitis C virus; NA – not available; rt – room temperature; RT-PCR – reverse transcription polymerase chain reaction; TMA – transcription-mediated amplification.

Table A2.3. Summary of study characteristics of included studies assessing HCV-core antigen in DBS

Study, country	Study design	Filter paper brand	DBS volume	Storage	Index test (in DBS)	Reference standard (in serum or plasma)
Biondi (2019), Canada	CC	Munktell TFN	50 µL	Air-dried overnight at rt, placed in plastic bag with desiccant pack. Stored at multiple temperatures (−80°C , 4°C, 21°C, 37°C, alternating 37/4°C) for one week during transportation to evaluate affect on diagnostic accuracy.	Abbott HCV ARCHITECT core antigen assay	Roche Cobas AmpliPrep TaqMan HCV Assay
Catlett (2019b), Australia	CS	Whatman® 903	NA	Air-dried overnight at rt, stored in foil bag with dessicant, transported at rt to laboratory and stored at -80°C.	Abbott HCV ARCHITECT core antigen assay	Abbott RealTime HCV Viral Load assay
Lamoury (2018), Australia	CS	Whatman® 903	50 µl	Air-dried at rt, stored at 80°C for 1 to 3 years.	Abbott HCV ARCHITECT core antigen assay	Roche Cobas AmpliPrep TaqMan HCV Assay Version 2.0
Mohamed (2017), Tanzania	CS	Whatman® 903	NA	Air-dried for 1 hour, placed in sealed plastic bag, and stored at −80°C.	Abbott HCV ARCHITECT core antigen assay	Roche Cobas AmpliPrep TaqMan HCV Assay Version 2.0
Nguyen (2018), Vietnam	CS	Whatman® 903	50 µl	Air-dried at rt, stored in sealed plastic bags with desiccant at −20 °C. Samples were defrosted and transported at rt (72 hours), and stored again at − 20°C for a mean duration of 18 months.	Abbott HCV ARCHITECT core antigen assay	Roche Cobas AmpliPrep TaqMan HCV Assay Version 2.0
Soulier (2016), France	CC	Whatman® 903	50 µL	Air-dried at rt for at least 1 hour, placed in sealed plastic bag with desiccant, stored at −80°C.	Abbott HCV ARCHITECT core antigen assay	

Key: CC – case control; CS – cross sectional; DBS – dried blood spot; HCV – hepatitis C virus; NA – not available; rt – room temperature; RT-PCR – reverse transcription polymerase chain reaction; TMA – transcription-mediated amplification.