Systematic review of the accuracy of antibody tests used to screen asymptomatic adults for hepatitis C infection

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Funding statement:
No funding was received for this research.

Competing interests:
The authors have no competing interests.

Word count (max 2,500): 2,789

ABSTRACT

Background: Several expert groups, including the United States Preventive Services Task Force and the Canadian Task Force on Preventive Health Care, have recently examined or are currently examining whether or not primary care physicians should screen asymptomatic adults for hepatitis C virus (HCV) infection~~The Canadian Task Force on Preventive Health Care is examining whether or not Canadian primary care physicians should screen asymptomatic adults for hepatitis C virus (HCV) infection~~. To inform decision-making on HCV screening, we performed a systematic review of the accuracy of antibody tests, as compared to other immunoassays and RNA detection, for screening asymptomatic adults for HCV infection.

Methods: MEDLINE and EMBASE databases were searched from 1990-2016; resulting citations were uploaded into DistillerSR and independently screened by 2 reviewers. Risk of bias was assessed using the QUADAS-2 tool; the quality of the 'body of evidence' was assessed using GRADE methodology.

Results: Of 1,537 articles identified, 81 underwent full-text review, and 9 studies met inclusion criteria. Compared to RNA detection, the sensitivity of ELISA v3.0 was variable (61.0%-81.8%) and the specificity was high (97.5%-99.7%). As expected, there were more false-positive results when comparing antibody tests to RNA detection than to other immunoassays; in fact, there were more false-positives than true-positives. Our GRADE assessment suggested that there was a high concern for risk of bias, particularly verification bias, and substantial inconsistency between studies in terms of their design.

Interpretation: More research is needed to better characterize the accuracy of antibody tests used to screen for HCV infection in the general population. Jurisdictions that recently adopted birth cohort screening for HCV infection are encouraged to evaluate and report on HCV screening test accuracy, and screening benefits and harms.

PROSPERO registration: #CRD42016039710

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INTRODUCTION

The incidence of hepatitis C virus (HCV) infection in Canada has declined in recent years (1;2), and the population prevalence of chronic HCV infection is estimated at 0.64-0.71% (2), which is approximately half of that of the United States (3). An estimated 21-44% of those Canadians with chronic HCV infection are unaware of their infection (1;2). In low-prevalence countries like Canada and the United Kingdom, the approach to HCV prevention and control has focused on case-finding (4;5), i.e., testing persons with risk factors for HCV infection, such as intravenous drug users ~~(IVDU)~~ and refugees from endemic countries. The recent development of costly but effective treatment for chronic hepatitis C (6) has led some to re-evaluate the evidence for/against population screening for HCV infection (7). In 2013, the United States Preventive Services Task Force ~~(USPSTF)~~ revised their 2004 recommendation against screening asymptomatic adults for HCV infection (8); it now recommends one-time screening for all adults born between 1945 and 1965 (7). The Canadian Task Force on Preventive Health Care ~~(CTFPHC)~~ is currently examining whether or not primary care physicians should screen asymptomatic adults for HCV infection (9).

The World Health Organization (10;11) and the UK National Screening Committee's (12) guidance on when screening should be performed emphasizes the fundamental importance of having a 'safe, valid, and reliable' screening test. Screening for HCV infection typically relies on antibody testing. Because approximately 25% of HCV-infected persons spontaneously clear the infection (13) but antibodies may persist (14), antibody testing cannot discriminate current from resolved infections, leading to false-positive results (15). False-positive results can also occur from other antibodies interacting non-specifically with the test (16). False-positive results can cause harm (e.g., through labeling, anxiety). Therefore, individuals who screen positive typically undergo further testing, which has resource implications and may carry additional inherent risk. To inform decision-making on screening for chronic HCV infection, we performed a systematic review of the evidence on the accuracy of antibody tests used to screen asymptomatic adults for HCV infection.

METHODS

Laboratory tests for HCV

Laboratory tests for HCV can be divided into 3 categories based on what they detect: 1) antibodies to HCV, 2) antibodies to HCV and HCV core antigen (i.e., antigen-antibody tests), or 3) HCV RNA (16). Antibody tests include third-generation enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), electro chemiluminescent immunoassay (ECLIA), chemiluminescent microparticle immunoassay (CMIA), and

microparticle enzyme immunoassay (MEIA). Each commercial antibody testing kit uses slightly different sets of HCV antigens to bind and detect host antibodies (Table 1). Antigen-antibody tests include fourth-generation ELISAs; because they detect antibodies to HCV, they would have the same issues with false-positives as 3rd generation ELISAs, but higher sensitivity because they also detect HCV antigen. Molecular techniques that detect HCV RNA, such as polymerase chain reaction (PCR) and nucleic acid testing (NAT), are recommended as confirmatory tests (17), even though immunocompromised and hemodialysis patients may have false-negative results (16). RNA-based testing can detect HCV before antibodies are produced (early infection) and can differentiate between current and resolved infection (i.e., fewer false-positives). It is important to note that there is no perfect reference standard for HCV infection, and the United States Centers for Disease Control and Prevention~~S CDC~~ has recommended a testing strategy where the initial test is an antibody test, and the confirmatory test may be either RNA detection or serological methods similar to the index test (17).

Environmental scan of laboratory testing for HCV in Canada

To scope our systematic review, we first performed an environmental scan of laboratory testing for HCV in Canada. Given that provincial/territorial (P/T) laboratories generally perform the majority of HCV testing (18), one author (GC) searched the grey literature on P/T laboratory and CATIE websites, and contacted P/T laboratories by email in February 2016, on behalf of the Canadian Task Force for Preventive Health Care, to inquire about HCV testing (Appendix A). In a majority of P/T labs, the initial test used was an antibody test, such as CLIA (British Columbia, Northwest Territory, Saskatchewan), CMIA (Alberta, Manitoba, New Brunswick, Newfoundland & Labrador, Nova Scotia, Ontario), or MEIA (Quebec). Confirmatory testing was typically performed using another immunoassay, such as CMIA (British Columbia, Saskatchewan), CLIA (Ontario), fourth-generation ELISA (Alberta), or recombinant immunoblot assay (RIBA) (Manitoba, Quebec), rather than PCR (New Brunswick, Newfoundland & Labrador, Nova Scotia). Use of a different immunoassay to confirm HCV infection (19), rather than RNA detection, is also aligned with current US Centers for Disease Control and Prevention ~~(CDC)~~ recommendations (17).

Research question

Our objective was to carry out a systematic review to estimate the accuracy of antibody tests used in Canada (i.e., CMIA, CLIA, ECLIA, MEIA, and ELISA version 3.0) to screen for HCV infection among asymptomatic, non-pregnant, treatment-naïve adults with unknown liver enzyme values. In the absence of a perfect reference standard, we used two different sets of reference tests: 1) inferior serological reference tests (CMIA, CLIA, ECLIA, MEIA, ELISA version 3.0+, RIBA) commonly used as confirmatory tests in Canada, and 2) superior RNA–based reference tests (PCR, NAT), which are less commonly used. We also sought to assess the accuracy of the

two-step HCV screening procedure (i.e., the combination of the initial and confirmatory tests) currently used in Canada. The research protocol to answer this question was registered with PROSPERO (#CRD42016039710).

Literature search strategy

The literature search strategy was developed with the help of a librarian at the University of Toronto Gerstein Science Information Centre (Appendix B). Ovid MEDLINE® 1946 to present, Ovid MEDLINE® In-Process & Other Non-Indexed Citations, and Ovid EMBASE® were systematically searched using both controlled vocabulary and keywords. Because the oldest immunoassay of interest (ELISA v3.0) was first marketed in 1993 (20), retrieval was limited to articles published between January 1, 1990 and May 6, 2016. Language was restricted to English or French. Conference abstracts were excluded from the search results.

Selection criteria

Original research studies, systematic reviews and meta-analyses were eligible for inclusion. At least 80% of the study population had to be asymptomatic, non-pregnant, treatment-naïve adults with unknown liver enzyme values and unknown HCV status (e.g., general population, blood donors); high-risk groups such as hemodialysis patients, transplant/transfusion recipients, ~~IVDUs~~intravenous drug users, patients co-infected with other blood-borne infections were excluded, as well as blood bank specimens that previously tested negative for HCV and specimen panels. The index test had to be one of CLIA, ECLIA, CMIA, MEIA, or ELISA v3.0; rapid tests, tests performed on specimens other than blood (e.g., saliva), and sero/genotyping tests were excluded. Within a given study, the reference test had to be different from the index test. In the absence of a perfect reference standard, two different sets of reference tests were eligible for inclusion: inferior serological reference tests (CMIA, CLIA, ECLIA, MEIA, ELISA version 3.0+, RIBA) and superior RNA–based reference tests (PCR, NAT). The reference test had to be applied to some subjects who tested positive on the index test as well as some of those who tested negative on the index test, so that a 2x2 table could be filled and sensitivity and/or specificity estimated. The setting had to resemble primary care (e.g., blood donation centre, population-based screening); hospital-based specialty clinics and inpatient hospital settings were excluded.

Selection method

Using DistillerSR software, both reviewers (GC, JC) independently screened all titles and abstracts using the pre-determined selection criteria above. Potentially relevant articles were retrieved, and both reviewers (GC, JC) screened all full-text articles using the same selection criteria as above. Disagreements were resolved through discussion.

Data extraction

One reviewer (GC) extracted data on study characteristics and findings from each included study into Tables 2-4, as appropriate. Raw data was abstracted to create 2x2 tables of index test(s) compared to reference test(s) for each study. The second reviewer (JC) verified the accuracy and completeness of the other's data extraction. Disagreements were resolved through discussion. Authors of one included study were contacted (21) to obtain additional data not reported in the published article (22).

Risk of bias assessment

One reviewer (GC), with previous experience conducting validation studies (23;24) and performing statistical adjustment for verification bias (25), assessed the risk of bias and applicability of each included study using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. QUADAS-2 focuses on four domains: patient selection, index test, reference test, as well as patient flow and timing of testing (26). The second reviewer (JC) verified the accuracy and completeness of the other's assessment. Disagreements were resolved through discussion.

Statistical analyses

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false-positive ~~(FP)~~ rate, false-negative ~~(FN)~~ rate, and 95% confidence intervals (CI) were estimated using the raw data (2x2 tables) extracted from the included studies. For studies that suffered from verification bias as a result of the sampling strategy used, statistical adjustment of sensitivity and specificity estimates (and therefore false-positive~~FP~~ and false-negative~~FN~~ rate) was performed (27). Given the small number of included studies and the heterogeneity of index–reference test pairings between studies, quantitative synthesis was not considered appropriate.

Assessment of the quality of the 'body of evidence' using GRADE

Whereas studies using inferior serological reference tests reflect current laboratory practice in several P/T, they likely underestimate the true number of false-positives. For this reason, we further restricted the 'body of evidence' to studies where the index test was an antibody test (reflecting current laboratory practice) and the reference test detected RNA. Two reviewers (GC, JC) independently used GRADE methodology for diagnostic testing accuracy ~~(DTA)~~ studies (28-31) to assess the body of evidence; disagreements between reviewers were resolved through discussion. The GRADE criteria evaluate the evidence in terms of study design, risk of bias, indirectness, imprecision, and publication bias (31).

RESULTS

Literature search results and characteristics of included studies

1,537 articles were identified, of which 81 underwent full-text review, and 9 were included (Figure 1). A list of all excluded studies and each study's reason for exclusion is available in Appendix C. We did not identify any eligible studies that evaluated the two-step HCV screening procedure. Included studies (Table 2) were conducted in countries with low (15;32;33), moderate (34;35), and high (22;36-38) HCV prevalence (39). ~~Only 2 studies involved routine HCV screening of the general population (21;31); the other 7 studies involved blood donor screening (14;32-37). The 9 included studies reported on 11 different index – reference test pairings: 5 studies compared antibody tests to other antibody tests (14;21;31;32;35), 2 studies compared antigen-antibody tests to antibody tests (33;36), 3 studies compared antibody tests to RNA detection (20;21;35;37), and 1 study compared an antigen-antibody test to RNA detection (34).~~

~~Seven studies applied the index and reference tests to all samples in parallel (14;31;32;34-37); 2 studies (21;33) suffered from verification bias as a result of applying the reference test to a larger proportion of samples that had tested positive on the index test relative to those that had tested negative on the index test (Appendix D – QUADAS-2 assessment).~~

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Accuracy of immunoassays as compared to other immunoassays

The sample size of studies comparing two immunoassays ranged from 106 to 5,208 (Table 3). For antibody tests compared to other antibody tests, sensitivity ranged from 70.4% to 99.5% and specificity ranged from 98.7% to 99.8%. For an antigen-antibody test compared to an antibody test, sensitivity ranged from 52.4% to 95.6% and specificity was over 99%.

Accuracy of immunoassays as compared to RNA detection

The sample size of studies comparing an antibody test to RNA detection ranged from 106 to 21,115 (Table 4). For antibody tests compared to RNA detection, sensitivity ranged from 61.0% to 81.8% and specificity ranged from 97.5% to 99.7%. As expected, the antigen-antibody test performed better against RNA detection than the antibody tests did; its sensitivity was 90.2% and its specificity was 99.8%. Also as expected, the number of false-positive results was higher when comparing antibody tests to RNA detection than to other antibody tests; in fact, there were more false-positives than true-positives.

Assessment of included studies' risk of bias using QUADAS-2

With respect to patient selection, ~~O~~only 2 studies involved routine HCV screening of the general population (22;32); the other 7 studies involved blood donor screening (15;33-38) (Appendix D – QUADAS-2 assessment). T~~T~~he 9 included studies reported on 11 different index—reference test pairings: 5 studies compared antibody tests to other antibody tests (15;22;32;33;36), 2 studies compared antigen-antibody tests to antibody tests (34;37), 3 studies compared antibody tests to RNA detection (21;22;36;38), and 1 study compared an antigen-antibody test to RNA detection (35). With respect to study flow and timing, 7 studies applied the index and reference tests to all samples in parallel (15;32;33;35-38); 2 studies (22;34) suffered from verification bias as a result of applying the reference test to a larger proportion of samples that had tested positive on the index test relative to those that had tested negative on the index test.

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Assessment of the quality of the 'body of evidence' using GRADE

Three studies comparing antibody tests to RNA detection were considered for inclusion in the 'body of evidence'. One study (22) was excluded because, as a result of there being zero false-negatives, the correction of the sensitivity estimate for verification bias was overly conservative (40) and the uncorrected sensitivity was too biased to be meaningful. Another study (36) was excluded because, based on its results, the study prevalence of HCV was 23.6%; this very high prevalence suggests that either the study population was not reflective of the general population or a case-control design was used (in either case, it did not meet our inclusion criteria). Findings from the remaining study (38) were assessed as 'very low' quality of evidence using GRADE (Table 5); this study reported a sensitivity of 81.8%, 95% CI (59.0-100%) and a specificity of 99.7%, 95% CI (99.6-99.8%). Assuming an HCV seroprevalence of 0.96% as in the general Canadian population (2), instead of the 0.1% prevalence among the 17,840 blood donors in the study (38), the PPV would be 72.7%, 95% CI (66.2-78.8%), and the NPV would be 99.8%, 95% CI (99.8-99.9%). ~~-~~Applying this study's findings to 1,000 individuals drawn from the general Canadian population (Table 5), ~~assuming a population HCV seroprevalence of 0.96% (0.61%-1.34%) (2),~~ we would expect 8, 95% CI (6-10) true-positives, 987, 95% CI (986-988) true-negatives, 3, 95% CI (2-4) false-positives, and 2, 95% CI (0-4) false-negatives.

INTERPRETATION

We performed a systematic review of the evidence on the accuracy of antibody tests, as compared to other immunoassays and RNA detection, for screening asymptomatic adults for HCV infection. We found that the sensitivity of antibody tests was highly variable (52.4%-99.5%) and the specificity was high (97.5%-99.8%). The lack of a perfect reference test for HCV raises concerns that these estimates are biased. In particular, when an inferior serological reference test that shares the same risk of false-positives as the index test is used, the

specificity could be overestimated. As expected, we found that there were more false-positive results when comparing antibody tests to superior RNA-based reference tests, than to inferior serological reference tests; in fact, there were more false-positives than true-positives owing to the very low prevalence of HCV. Bias correction of the specificity estimate could make the difference even greater. This finding highlights a potential problem with using an inferior serological reference test as a confirmatory test (17;19) when screening the general population for HCV infection. Our assessment of the 'body of evidence' using GRADE methodology led us to focus on a single 'least biased' study (38), which reported the sensitivity of ELISA v3.0 compared to NAT as 81.8%, 95% CI (59.0%-100%), its specificity as 99.8%, 95% CI (99.7%-99.8%), and its PPV as 13.8%, 95% CI (5.4-22.2).

The only other systematic review of HCV antibody test accuracy was performed in the context of the United States Preventive Services Task Force's 2004 recommendation on HCV screening. However, the populations of the studies included in that review (i.e., hemodialysis patients, patients with histologically-verified hepatitis, patients hospitalized with suspected acute/chronic hepatitis, blood donors with persistently elevated liver enzymes, and blood donors who previously screened positive for HCV) did not reflect the general population, therefore those findings are not directly comparable to ours (8;20). When the United States Preventive Services Task Force revised its recommendation on HCV screening in 2013, it did not re-assess HCV screening test accuracy, despite the introduction of new immunoassays (i.e., CLIA, ECLIA, CMIA, MEIA) since its 2004 systematic review (3). Similarly, when the WHO published its HCV screening guideline in 2014 (41), it did not re-assess screening test accuracy, instead citing a 2001 report (42) on simple/rapid test accuracy and a 2002 report (43) on ELISA v3.0+ immunoassay accuracy, both involving blood panels not reflective of the general population. Because antibody tests have not been adequately evaluated for population-based HCV screening, and because the availability of a 'safe, valid, and reliable' screening test is a fundamental consideration of any screening recommendation or program (10-12), this highlights a knowledge gap and brings into question the evidence basis for these recommendations.

Our findings are limited by the paucity and the low quality of the available evidence published in English or French. In particular, we were unable to locate any studies of the accuracy of CLIA, CMIA, or MEIA (the HCV screening tests most commonly used in Canada) as compared to RNA detection for HCV screening in the general population. The applicability of our findings to the general Canadian population is limited because a majority of included studies were conducted among blood donors, and persons eligible to donate blood are at lower risk of blood-borne infections like HCV than the general population. Rapid and point-of-care tests were beyond the scope of our review; whereas those tests are important for reaching some vulnerable populations,

a majority of HCV testing in Canada is laboratory-based (18); also, a systematic review of the accuracy of rapid tests was recently published (44).

In conclusion, the availability of a 'safe, valid, and reliable' screening test is a primordial consideration for decision-making about screening (10-12), but our study has shown that ~~more~~ further research is needed to adequately characterize the accuracy of antibody tests used to screen the general population for HCV infection. ~~Whereas decision-making about screening involves multiple considerations, such as the availability of safe and effective treatment, having a 'safe, valid, and reliable' screening test is a primordial consideration (10-12)~~. Our study focused on the accuracy of HCV screening tests; however, several other important factors must be considered when making decisions about HCV screening, including: the benefits and harms of screening, the benefits and harms of treatment for screen-detected cases, the cost-effectiveness of screening, as well as patient preferences related to screening. A review of the evidence related to these considerations is beyond the scope of the present study, but such a review is being performed by others in the context of the Canadian Task Force on Preventive Health Care's upcoming guidelines on HCV screening. To help inform decision-making about HCV screening, ~~W~~we encourage jurisdictions that have already adopted population-based (birth cohort) ~~HCV~~ screening for HCV to carefully evaluate and report on the accuracy of antibody tests, as well as screening benefits and harms~~; these findings will help inform future HCV screening policies~~.

ACKNOWLEDGEMENTS

We are grateful to Dr Roland Grad and Alejandra Jaramillo, members of the working group on HCV screening of the Canadian Task Force for Preventive Health Care, for their helpful comments on our study design, findings, and manuscript~~We are grateful to the members of the Canadian Task Force for Preventive Health Care and the Public Health Agency of Canada's Preventive Guidelines Division for enabling us to undertake this work and for their helpful comments on our manuscript~~. We would also like to thank Dr Barry Pakes, ~~our~~ Public Health & Preventive Medicine residency program director at the University of Toronto, ~~Dr Barry Pakes,~~ for his support and editorial suggestions.

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Systematic review of the accuracy of antibody tests used to screen asymptomatic adults for hepatitis C infection

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Funding statement:
No funding was received for this research.

Competing interests:
The authors have no competing interests.

Word count (max 2,500): 2,789

ABSTRACT

Background: Several expert groups, including the United States Preventive Services Task Force and the Canadian Task Force on Preventive Health Care, have recently examined or are currently examining whether or not primary care physicians should screen asymptomatic adults for hepatitis C virus (HCV) infection. To inform decision-making on HCV screening, we performed a systematic review of the accuracy of antibody tests, as compared to other immunoassays and RNA detection, for screening asymptomatic adults for HCV infection.

Methods: MEDLINE and EMBASE databases were searched from 1990-2016; resulting citations were uploaded into DistillerSR and independently screened by 2 reviewers. Risk of bias was assessed using the QUADAS-2 tool; the quality of the 'body of evidence' was assessed using GRADE methodology.

Results: Of 1,537 articles identified, 81 underwent full-text review, and 9 studies met inclusion criteria. Compared to RNA detection, the sensitivity of ELISA v3.0 was variable (61.0%-81.8%) and the specificity was high (97.5%-99.7%). As expected, there were more false-positive results when comparing antibody tests to RNA detection than to other immunoassays; in fact, there were more false-positives than true-positives. Our GRADE assessment suggested that there was a high concern for risk of bias, particularly verification bias, and substantial inconsistency between studies in terms of their design.

Interpretation: More research is needed to better characterize the accuracy of antibody tests used to screen for HCV infection in the general population. Jurisdictions that recently adopted birth cohort screening for HCV infection are encouraged to evaluate and report on HCV screening test accuracy, and screening benefits and harms.

PROSPERO registration: #CRD42016039710

INTRODUCTION

The incidence of hepatitis C virus (HCV) infection in Canada has declined in recent years (1;2), and the population prevalence of chronic HCV infection is estimated at 0.64-0.71% (2), which is approximately half of that of the United States (3). An estimated 21-44% of those Canadians with chronic HCV infection are unaware of their infection (1;2). In low-prevalence countries like Canada and the United Kingdom, the approach to HCV prevention and control has focused on case-finding (4;5), i.e., testing persons with risk factors for HCV infection, such as intravenous drug users and refugees from endemic countries. The recent development of costly but effective treatment for chronic hepatitis C (6) has led some to re-evaluate the evidence for/against population screening for HCV infection (7). In 2013, the United States Preventive Services Task Force revised their 2004 recommendation against screening asymptomatic adults for HCV infection (8); it now recommends one-time screening for all adults born between 1945 and 1965 (7). The Canadian Task Force on Preventive Health Care is currently examining whether or not primary care physicians should screen asymptomatic adults for HCV infection (9).

The World Health Organization (10;11) and the UK National Screening Committee's (12) guidance on when screening should be performed emphasizes the fundamental importance of having a 'safe, valid, and reliable' screening test. Screening for HCV infection typically relies on antibody testing. Because approximately 25% of HCV-infected persons spontaneously clear the infection (13) but antibodies may persist (14), antibody testing cannot discriminate current from resolved infections, leading to false-positive results (15). False-positive results can also occur from other antibodies interacting non-specifically with the test (16). False-positive results can cause harm (e.g., through labeling, anxiety). Therefore, individuals who screen positive typically undergo further testing, which has resource implications and may carry additional inherent risk. To inform decision-making on screening for chronic HCV infection, we performed a systematic review of the evidence on the accuracy of antibody tests used to screen asymptomatic adults for HCV infection.

METHODS

Laboratory tests for HCV

Laboratory tests for HCV can be divided into 3 categories based on what they detect: 1) antibodies to HCV, 2) antibodies to HCV and HCV core antigen (i.e., antigen-antibody tests), or 3) HCV RNA (16). Antibody tests include third-generation enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), electro chemiluminescent immunoassay (ECLIA), chemiluminescent microparticle immunoassay (CMIA), and

microparticle enzyme immunoassay (MEIA). Each commercial antibody testing kit uses slightly different sets of HCV antigens to bind and detect host antibodies (Table 1). Antigen-antibody tests include fourth-generation ELISAs; because they detect antibodies to HCV, they would have the same issues with false-positives as 3rd generation ELISAs, but higher sensitivity because they also detect HCV antigen. Molecular techniques that detect HCV RNA, such as polymerase chain reaction (PCR) and nucleic acid testing (NAT), are recommended as confirmatory tests (17), even though immunocompromised and hemodialysis patients may have false-negative results (16). RNA-based testing can detect HCV before antibodies are produced (early infection) and can differentiate between current and resolved infection (i.e., fewer false-positives). It is important to note that there is no perfect reference standard for HCV infection, and the United States Centers for Disease Control and Prevention has recommended a testing strategy where the initial test is an antibody test, and the confirmatory test may be either RNA detection or serological methods similar to the index test (17).

Environmental scan of laboratory testing for HCV in Canada

To scope our systematic review, we first performed an environmental scan of laboratory testing for HCV in Canada. Given that provincial/territorial (P/T) laboratories generally perform the majority of HCV testing (18), one author (GC) searched the grey literature on P/T laboratory and CATIE websites, and contacted P/T laboratories by email in February 2016, on behalf of the Canadian Task Force for Preventive Health Care, to inquire about HCV testing (Appendix A). In a majority of P/T labs, the initial test used was an antibody test, such as CLIA (British Columbia, Northwest Territory, Saskatchewan), CMIA (Alberta, Manitoba, New Brunswick, Newfoundland & Labrador, Nova Scotia, Ontario), or MEIA (Quebec). Confirmatory testing was typically performed using another immunoassay, such as CMIA (British Columbia, Saskatchewan), CLIA (Ontario), fourth-generation ELISA (Alberta), or recombinant immunoblot assay (RIBA) (Manitoba, Quebec), rather than PCR (New Brunswick, Newfoundland & Labrador, Nova Scotia). Use of a different immunoassay to confirm HCV infection (19), rather than RNA detection, is also aligned with current US Centers for Disease Control and Prevention recommendations (17).

Research question

Our objective was to carry out a systematic review to estimate the accuracy of antibody tests used in Canada (i.e., CMIA, CLIA, ECLIA, MEIA, and ELISA version 3.0) to screen for HCV infection among asymptomatic, non-pregnant, treatment-naïve adults with unknown liver enzyme values. In the absence of a perfect reference standard, we used two different sets of reference tests: 1) inferior serological reference tests (CMIA, CLIA, ECLIA, MEIA, ELISA version 3.0+, RIBA) commonly used as confirmatory tests in Canada, and 2) superior RNA–based reference tests (PCR, NAT), which are less commonly used. We also sought to assess the accuracy of the

two-step HCV screening procedure (i.e., the combination of the initial and confirmatory tests) currently used in Canada. The research protocol to answer this question was registered with PROSPERO (#CRD42016039710).

Literature search strategy

The literature search strategy was developed with the help of a librarian at the University of Toronto Gerstein Science Information Centre (Appendix B). Ovid MEDLINE® 1946 to present, Ovid MEDLINE® In-Process & Other Non-Indexed Citations, and Ovid EMBASE® were systematically searched using both controlled vocabulary and keywords. Because the oldest immunoassay of interest (ELISA v3.0) was first marketed in 1993 (20), retrieval was limited to articles published between January 1, 1990 and May 6, 2016. Language was restricted to English or French. Conference abstracts were excluded from the search results.

Selection criteria

Original research studies, systematic reviews and meta-analyses were eligible for inclusion. At least 80% of the study population had to be asymptomatic, non-pregnant, treatment-naïve adults with unknown liver enzyme values and unknown HCV status (e.g., general population, blood donors); high-risk groups such as hemodialysis patients, transplant/transfusion recipients, intravenous drug users, patients co-infected with other blood-borne infections were excluded, as well as blood bank specimens that previously tested negative for HCV and specimen panels. The index test had to be one of CLIA, ECLIA, CMIA, MEIA, or ELISA v3.0; rapid tests, tests performed on specimens other than blood (e.g., saliva), and sero/genotyping tests were excluded. Within a given study, the reference test had to be different from the index test. In the absence of a perfect reference standard, two different sets of reference tests were eligible for inclusion: inferior serological reference tests (CMIA, CLIA, ECLIA, MEIA, ELISA version 3.0+, RIBA) and superior RNA–based reference tests (PCR, NAT). The reference test had to be applied to some subjects who tested positive on the index test as well as some of those who tested negative on the index test, so that a 2x2 table could be filled and sensitivity and/or specificity estimated. The setting had to resemble primary care (e.g., blood donation centre, population-based screening); hospital-based specialty clinics and inpatient hospital settings were excluded.

Selection method

Using DistillerSR software, both reviewers (GC, JC) independently screened all titles and abstracts using the pre-determined selection criteria above. Potentially relevant articles were retrieved, and both reviewers (GC, JC) screened all full-text articles using the same selection criteria as above. Disagreements were resolved through discussion.

Data extraction

One reviewer (GC) extracted data on study characteristics and findings from each included study into Tables 2-4, as appropriate. Raw data was abstracted to create 2x2 tables of index test(s) compared to reference test(s) for each study. The second reviewer (JC) verified the accuracy and completeness of the other's data extraction. Disagreements were resolved through discussion. Authors of one included study were contacted (21) to obtain additional data not reported in the published article (22).

Risk of bias assessment

One reviewer (GC), with previous experience conducting validation studies (23;24) and performing statistical adjustment for verification bias (25), assessed the risk of bias and applicability of each included study using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. QUADAS-2 focuses on four domains: patient selection, index test, reference test, as well as patient flow and timing of testing (26). The second reviewer (JC) verified the accuracy and completeness of the other's assessment. Disagreements were resolved through discussion.

Statistical analyses

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false-positive rate, false-negative rate, and 95% confidence intervals (CI) were estimated using the raw data (2x2 tables) extracted from the included studies. For studies that suffered from verification bias as a result of the sampling strategy used, statistical adjustment of sensitivity and specificity estimates (and therefore false-positive and false-negative rate) was performed (27). Given the small number of included studies and the heterogeneity of index–reference test pairings between studies, quantitative synthesis was not considered appropriate.

Assessment of the quality of the 'body of evidence' using GRADE

Whereas studies using inferior serological reference tests reflect current laboratory practice in several P/T, they likely underestimate the true number of false-positives. For this reason, we further restricted the 'body of evidence' to studies where the index test was an antibody test (reflecting current laboratory practice) and the reference test detected RNA. Two reviewers (GC, JC) independently used GRADE methodology for diagnostic testing accuracy studies (28-31) to assess the body of evidence; disagreements between reviewers were resolved through discussion. The GRADE criteria evaluate the evidence in terms of study design, risk of bias, indirectness, imprecision, and publication bias (31).

RESULTS

Literature search results and characteristics of included studies

1,537 articles were identified, of which 81 underwent full-text review, and 9 were included (Figure 1). A list of all excluded studies and each study's reason for exclusion is available in Appendix C. We did not identify any eligible studies that evaluated the two-step HCV screening procedure. Included studies (Table 2) were conducted in countries with low (15;32;33), moderate (34;35), and high (22;36-38) HCV prevalence (39).

Accuracy of immunoassays as compared to other immunoassays

The sample size of studies comparing two immunoassays ranged from 106 to 5,208 (Table 3). For antibody tests compared to other antibody tests, sensitivity ranged from 70.4% to 99.5% and specificity ranged from 98.7% to 99.8%. For an antigen-antibody test compared to an antibody test, sensitivity ranged from 52.4% to 95.6% and specificity was over 99%.

Accuracy of immunoassays as compared to RNA detection

The sample size of studies comparing an antibody test to RNA detection ranged from 106 to 21,115 (Table 4). For antibody tests compared to RNA detection, sensitivity ranged from 61.0% to 81.8% and specificity ranged from 97.5% to 99.7%. As expected, the antigen-antibody test performed better against RNA detection than the antibody tests did; its sensitivity was 90.2% and its specificity was 99.8%. Also as expected, the number of false-positive results was higher when comparing antibody tests to RNA detection than to other antibody tests; in fact, there were more false-positives than true-positives.

Assessment of included studies' risk of bias using QUADAS-2

With respect to patient selection, only 2 studies involved routine HCV screening of the general population (22;32); the other 7 studies involved blood donor screening (15;33-38) (Appendix D – QUADAS-2 assessment). The 9 included studies reported on 11 different index—reference test pairings: 5 studies compared antibody tests to other antibody tests (15;22;32;33;36), 2 studies compared antigen-antibody tests to antibody tests (34;37), 3 studies compared antibody tests to RNA detection (21;22;36;38), and 1 study compared an antigen-antibody test to RNA detection (35). With respect to study flow and timing, 7 studies applied the index and reference tests to all samples in parallel (15;32;33;35-38); 2 studies (22;34) suffered from verification bias as a result of applying the reference test to a larger proportion of samples that had tested positive on the index test relative to those that had tested negative on the index test.

Assessment of the quality of the 'body of evidence' using GRADE

Three studies comparing antibody tests to RNA detection were considered for inclusion in the 'body of evidence'. One study (22) was excluded because, as a result of there being zero false-negatives, the correction of the sensitivity estimate for verification bias was overly conservative (40) and the uncorrected sensitivity was too biased to be meaningful. Another study (36) was excluded because, based on its results, the study prevalence of HCV was 23.6%; this very high prevalence suggests that either the study population was not reflective of the general population or a case-control design was used (in either case, it did not meet our inclusion criteria). Findings from the remaining study (38) were assessed as 'very low' quality of evidence using GRADE (Table 5); this study reported a sensitivity of 81.8%, 95% CI (59.0-100%) and a specificity of 99.7%, 95% CI (99.6-99.8%). Assuming an HCV seroprevalence of 0.96% as in the general Canadian population (2), instead of the 0.1% prevalence among the 17,840 blood donors in the study (38), the PPV would be 72.7%, 95% CI (66.2-78.8%), and the NPV would be 99.8%, 95% CI (99.8-99.9%). Applying this study's findings to 1,000 individuals drawn from the general Canadian population (Table 5), we would expect 8, 95% CI (6-10) true-positives, 987, 95% CI (986-988) true-negatives, 3, 95% CI (2-4) false-positives, and 2, 95% CI (0-4) false-negatives.

INTERPRETATION

We performed a systematic review of the evidence on the accuracy of antibody tests, as compared to other immunoassays and RNA detection, for screening asymptomatic adults for HCV infection. We found that the sensitivity of antibody tests was highly variable (52.4%-99.5%) and the specificity was high (97.5%-99.8%). The lack of a perfect reference test for HCV raises concerns that these estimates are biased. In particular, when an inferior serological reference test that shares the same risk of false-positives as the index test is used, the specificity could be overestimated. As expected, we found that there were more false-positive results when comparing antibody tests to superior RNA-based reference tests, than to inferior serological reference tests; in fact, there were more false-positives than true-positives owing to the very low prevalence of HCV. Bias correction of the specificity estimate could make the difference even greater. This finding highlights a potential problem with using an inferior serological reference test as a confirmatory test (17;19) when screening the general population for HCV infection. Our assessment of the 'body of evidence' using GRADE methodology led us to focus on a single 'least biased' study (38), which reported the sensitivity of ELISA v3.0 compared to NAT as 81.8%, 95% CI (59.0%-100%), its specificity as 99.8%, 95% CI (99.7%-99.8%), and its PPV as 13.8%, 95% CI (5.4-22.2).

The only other systematic review of HCV antibody test accuracy was performed in the context of the United States Preventive Services Task Force's 2004 recommendation on HCV screening. However, the populations of the studies included in that review (i.e., hemodialysis patients, patients with histologically-verified hepatitis, patients hospitalized with suspected acute/chronic hepatitis, blood donors with persistently elevated liver enzymes, and blood donors who previously screened positive for HCV) did not reflect the general population, therefore those findings are not directly comparable to ours (8;20). When the United States Preventive Services Task Force revised its recommendation on HCV screening in 2013, it did not re-assess HCV screening test accuracy, despite the introduction of new immunoassays (i.e., CLIA, ECLIA, CMIA, MEIA) since its 2004 systematic review (3). Similarly, when the WHO published its HCV screening guideline in 2014 (41), it did not re-assess screening test accuracy, instead citing a 2001 report (42) on simple/rapid test accuracy and a 2002 report (43) on ELISA v3.0+ immunoassay accuracy, both involving blood panels not reflective of the general population. Because antibody tests have not been adequately evaluated for population-based HCV screening, and because the availability of a 'safe, valid, and reliable' screening test is a fundamental consideration of any screening recommendation or program (10-12), this highlights a knowledge gap and brings into question the evidence basis for these recommendations.

Our findings are limited by the paucity and the low quality of the available evidence published in English or French. In particular, we were unable to locate any studies of the accuracy of CLIA, CMIA, or MEIA (the HCV screening tests most commonly used in Canada) as compared to RNA detection for HCV screening in the general population. The applicability of our findings to the general Canadian population is limited because a majority of included studies were conducted among blood donors, and persons eligible to donate blood are at lower risk of blood-borne infections like HCV than the general population. Rapid and point-of-care tests were beyond the scope of our review; whereas those tests are important for reaching some vulnerable populations, a majority of HCV testing in Canada is laboratory-based (18); also, a systematic review of the accuracy of rapid tests was recently published (44).

In conclusion, the availability of a 'safe, valid, and reliable' screening test is a primordial consideration for decision-making about screening (10-12), but our study has shown that further research is needed to adequately characterize the accuracy of antibody tests used to screen the general population for HCV infection. Our study focused on the accuracy of HCV screening tests; however, several other important factors must be considered when making decisions about HCV screening, including: the benefits and harms of screening, the benefits and harms of treatment for screen-detected cases, the cost-effectiveness of screening, as well as

patient preferences related to screening. A review of the evidence related to these considerations is beyond the scope of the present study, but such a review is being performed by others in the context of the Canadian Task Force on Preventive Health Care's upcoming guidelines on HCV screening. To help inform decision-making about HCV screening, we encourage jurisdictions that have already adopted population-based (birth cohort) screening for HCV to carefully evaluate and report on the accuracy of antibody tests, as well as screening benefits and harms.

ACKNOWLEDGEMENTS

We are grateful to Dr Roland Grad and Alejandra Jaramillo, members of the working group on HCV screening of the Canadian Task Force for Preventive Health Care, for their helpful comments on our study design, findings, and manuscript. We would also like to thank Dr Barry Pakes, Public Health & Preventive Medicine residency program director at the University of Toronto, for his support and editorial suggestions.

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Table 1. Screening tests for HCV based on antibody detection

Antibody test	Examples of assays (manufacturer)	Antigens (region of the genome)
3rd generation ELISA	▪ ORTHO HCV 3.0 ELISA (Ortho)	▪ c100-3 (NS3-NS4) ▪ c33-c (NS3) ▪ c22-3 (core) ▪ NS5
CLIA	▪ ARCHITECT i4000 anti-HCV assay (Abbott) ▪ VITROS Eci anti-HCV assay (Ortho) ▪ ADVIA Centaur, Siemens	▪ c22-3 (core) ▪ c200 (NS3 - NS4) ▪ NS5
ECLIA	▪ Elecsys anti-HCV assay (Roche)	▪ Core ▪ NS3 ▪ NS4
CMIA	▪ ARCHITECT® anti-HCV (Abbott)	▪ HCr43 (core-NS3) ▪ c100-3 (NS3-NS4)
MEIA	▪ AxSYM® HCV 3.0 (Abbott)	▪ HCr43 (Fusion core e NS3) ▪ c200 (NS3 - NS4) ▪ c100-3 (NS3-NS4) ▪ NS5

Source: Villar LM, *et al*. Update on hepatitis B and C virus diagnosis. *World Journal of Virology*. 2015; 4(4): 323-42.

Table 2. Characteristics of included studies

Study	Country (HCV prevalence[1])	Setting & study period	Funding source & conflict(s) of interests	Study population	Study flow & timing	Index test(s)	Reference test(s)
Denoyel, 2004	France (low)	NR	NR	5,228 individuals: 5,015 random blood donors and 213 hospitalized patients	Not specified; based on results, all samples underwent both the index and reference tests	CLIA (ADVIA Centaur® HCV assay)	MEIA (AxSYM® HCV v.3.0 assay, Abbott)
Tashkandy, 2007	Saudi Arabia (high)	Immunology and Serology Department, Al-Noor Specialist Hospital, Makkah; study period: NR	NR	106 male blood donors; samples from patients with diabetes or other endocrine diseases and autoimmune diseases were excluded	All samples were aliquoted into two portions: one for PCR, the other for serological testing	ELISA v3.0 (Murex anti-HCV, Abbott)	LIA (INNo-LIA HCV Ab III Update, Innogenetics) RT-PCR (High Pure Viral Nucleic Acid reagent set, Roche)
Benouda, 2009	Morocco (high)	Workplace blood specimen collection; December 2005 to April 2007	NR	8,326 adults from the general population with unknown HCV serology	A subset of 158/161 (98%) ELISA-positive and 100/8,165 (1%) ELISA-negative adults were called back to undergo the reference standard (verification bias); 3/161 lost to follow-up; time interval between tests not specified	ELISA v3.0 (Murex anti-HCV, Abbott)	MEIA (AxSYM® HCV v.3.0 assay, Abbott) RT-PCR (Amplicor HCV® v2.0, Roche)

Study	Country (HCV prevalence[1])	Setting & study period	Funding source & conflict(s) of interests	Study population	Study flow & timing	Index test(s)	Reference test(s)
Rao, 2009	China (high)	Beijing Red Cross Blood Center and Peking University Hepatology Institute; study period: NR	Grants from the Chinese Basic Research Foundation, National Science and Technology Key Project, and Key Clinical Research Program of Ministry of Health; conflict(s) of interest: NR, but one author affiliated with Aldatis	2,559 individuals: 2,082 blood donors (Beijing Red Cross Blood Center) and 477 (18.6%) patients (Peking University Hepatology Institute; including various HCV genotypes, non-C hepatitis, pregnant women, and lipidemia sera)	All samples underwent both the index and reference tests "side-by-side"	ELISA v4.0 (EIAgen, Adaltis)	ELISA v3.0 (Ortho HCV 3.0 ELISA)
Ol, 2009	Cambodia (moderate)	Rural areas of 2 Cambodian provinces: Battambang and Pailin; May to June 2007	Sponsored by the European Plasma Fraction Foundation and Tromsoe Mine Victim Resource Center, University Hospital, North Norway; conflict(s) of interest: NR	1,200 potential volunteer blood donors: 677 females, 523 males, mean age: 32.8 years, age range 18-52 year; sample stratified by province (600 each)	A subset of 80/176 (45%) ELISA-positive and 40/1,024 (4%) ELISA-negative samples were selected to undergo the reference test (verification bias); all samples underwent both the index and reference tests	ELISA v4.0 (Monolisa™, BioRad)	CMIA (Abbott)

Study	Country (HCV prevalence[1])	Setting & study period	Funding source & conflict(s) of interests	Study population	Study flow & timing	Index test(s)	Reference test(s)
Kosan, 2010	Turkey (high)	Turkish Red Crescent Çapa Blood Centre of Istanbul; intermittently from February 2007 to March 2008	"No role for sponsors in our study"; "there are no financial or personal relationships with other people or organisations that could inappropriately influence (bias) our work"	18,200 volunteer blood donors: 546 (3%) were women, 17,654 (97%) were men, 18,198 (99.9%) were first-time donors, mean age: 40 years, age range: 18-60 years; individuals underwent a mandatory physical exam prior to blood drawing	For each subject, two sets of blood samples were collected: one underwent serological testing and one underwent NAT testing	ELISA v3.0 (Innotest HCV Ab III, Innogenetics)	NAT (Procleix Ultrio kit, Chiron)
Park, 2012	South Korea (low)	"Routine HCV screening"; August 2009 to January 2011	Funding source not reported, but Ortho provided the CLIA assay kits; conflict(s) of interest: NR	1,011 sera from individuals undergoing routine HCV screening	Not specified; based on results, all samples underwent both the index and reference test	CLIA (Vitros Anti-HCV assay)	ECLIA (Elecsys anti-HCV test, Roche)
Sommese, 2014	Italy (low)	Volunteer blood donors, Second University of Naples; January to June 2013	"The authors do not have any conflicts of interest to declare. No funding was received."	840 volunteer blood donors: 275 (32.7%) were women, 564 (67.3%) men, mean age: 37.7 years (SD 12.5 years)	All samples underwent both the index and reference test in parallel	CMIA (Architect i200SR, Abbott)	ECLIA (Cobas e411, Roche)
Arora, 2016	India (moderate)	Blood bank; January 2013 to March 2014	"Financial support and sponsorship: Nil. Conflicts of interest: there are no conflicts of interest."	21,115 blood donors	All samples underwent both the index and reference test in parallel	ELISA v4.0 (Monolisa Ag-Ab Ultra, BioRad)	NAT (Procleix Ultrio kit, Chiron)

[1] Country HCV prevalence is categorized as low (<1.5%), moderate (1.5-3.5%), or high (>3.5%), as per Hanafiah *et al.*, 2013.(38)

Abbreviations: NR: not reported; ELISA: enzyme-linked immunosorbent assay; CLIA: chemiluminescent immunoassay; CMIA: chemiluminescent microparticle immunoassay; MEIA: microparticle enzyme immunoassay; LIA: line immunoassay; RIBA: recombinant immunoblot assay; NAT: nucleic acid amplification test; PCR: polymerase chain reaction; RT-PCR: reverse-transcription polymerase chain reaction.

Table 3. Accuracy of HCV screening immunoassays as compared to serological reference tests

Study	Index test	Serological reference test	TP	FP	FN	TN	Pr (%)	Sn (%) (95% CI)	Sp (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	FP rate (%) (95% CI)	FN rate (%) (95% CI)
Immunoassays that detect only antibodies to HCV													
Denoyel, 2004	CLIA (ADVIA Centaur® HCV assay)	MEIA (AxSYM® HCV v3.0 assay, Abbott)	NR	9	NR	5,199	-	-	99.8 (99.7-99.9)	-	-	0.2 (0.1-0.3)	-
Tashkandy, 2007	ELISA v3.0 (Murex anti-HCV, Abbott)	LIA (INNo-LIA HCVAb III Update, Innogenetics)	19	1	8[†]	78	25.4	70.4 (53.1-87.6)	98.7 (96.3-100)	95.0 (85.4-100)	90.7 (84.6-96.8)	1.3 (0-3.7)	29.6 (12.4-46.9)
Benouda, 2009	ELISA V3.0 (Murex anti-HCV, Abbott)	MEIA (AxSYM® HCV v.3.0 assay, Abbott)	100	58	0	100	38.8	71.6 (13.6-97.6)[‡]	99.3 (99.0-99.5)[‡]	63.3 (55.8-70.8)	100 (100-100)	0.7 (0.5-1.0)[‡]	28.4 (2.4-86.4)[‡]
Park, 2012	CLIA (Vitros Anti-HCV assay)	ECLIA (Elecsys anti-HCV test, Roche)	213	3	1	794	8.8	99.5 (98.6-100)	99.6 (99.2-100)	98.6 (97.1-100)	99.9 (99.6-100)	0.4 (0-0.8)	0.5 (0-1.4)
Sommese, 2014	CMIA (Architect i200SR, Abbott)	ECLIA (Cobas e411, Roche)	17	8	2	813	69.2	89.5 (75.7-100)	99.0 (98.4-99.7)	68.0 (49.7-86.3)	99.8 (99.4-100)	1.0 (0.3-1.6)	10.5 (0-24.3)
Immunoassay that detects both antibodies to HCV and viral antigen													
Rao, 2009	ELISA v4.0 (EIAgen, Adaltis)	ELISA v3.0 (Ortho HCV 3.0 ELISA)	216	4	10	2,329	21.2	95.6 (92.9-98.3)	99.8 (99.7-100)	98.2 (96.4-99.9)	99.6 (99.3-99.8)	0.2 (0-0.3)	4.4 (1.7-7.1)
Ol, 2009	ELISA v4.0 (Monolisa™, BioRad)	CMIA (Abbott)	77	3	6	34	2.3	52.4 (34.1-70.1)[‡]	99.2 (96.4-99.8)[‡]	96.3 (92.1-100)	85.0 (73.9-96.1)	0.8 (0.2-3.6)[‡]	47.6 (29.9-65.9)[‡]

Abbreviations: Pr: prevalence in the study; TP: true-positives; FN: false-negatives; FP: false-positives; TN: true-negatives; Sn: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; FP rate: false-positive rate; FN rate: false-negative rate. ELISA: enzyme-linked immunosorbent assay; CLIA: chemiluminescent immunoassay; ECLIA: electrochemiluminescent immunoassay; CMIA: chemiluminescent microparticle immunoassay; MEIA: microparticle enzyme immunoassay; LIA: line immunoassay; RIBA: recombinant immunoblot assay; NAT: nucleic acid amplification test; PCR: polymerase chain reaction; RT-PCR: reverse-transcription polymerase chain reaction. NR: not reported.

[†]Tashkandy, 2007 report the number of RIBA-positive samples as 22±5 (i.e., 5 borderline-positive samples); whereas they exclude those 5 samples from their analyses (bringing the total sample size to 101 from 106), the preferred and more conservative analysis includes the 5 borderline samples, so that FN=8 instead of FN=3.

[‡]Adjusted for verification bias.(44)

Table 4. Accuracy of HCV screening immunoassays as compared to RNA detection

Study	Index test	RNA-based reference test	TP	FP	FN	TN	Pr (%)	Sn (%) (95% CI)	Sp (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	FP rate (%) (95% CI)	FN rate (%) (95% CI)
Immunoassays that detect only antibodies to HCV													
Tashkandy, 2007	ELISA v3.0 (Murex anti-HCV, Abbott)	RT-PCR (High Pure Viral Nucleic Acid reagent set, Roche)	18	2	7	79	23.6	72.0 (54.4-89.6)	97.5 (94.2-100)	90.0 (76.9-100)	91.9 (86.1-97.6)	2.5 (0-5.8)	28.0 (10.4-45.6)
Benouda, 2009[†]	ELISA V3.0 (Murex anti-HCV, Abbott)	RT-PCR (Amplicor HCV® v2.0, Roche)	62	96	0	100	24.0	61.0 (8.9-96.2)[‡]	98.8 (98.5-99.0)[‡]	39.2 (31.6-46.9)	100 (100-100)	1.2 (1.0-1.5)[‡]	39.0 (3.8-91.1)[‡]
Kosan, 2010	ELISA v3.0 (Innotest HCV Ab III, Innogenetics)	NAT (Procleix Ultrio kit, Chiron)	9	56	2	17,784	0.1	81.8 (59.0-100)	99.7 (99.6-99.8)	13.8 (5.4-22.2)	100 (100-100)	0.3 (0.2-0.4)	18.2 (0-41.0)
Immunoassay that detects both antibodies to HCV and viral antigen													
Arora, 2016	ELISA v4.0 (Monolisa Ag-Ab Ultra, BioRad)	NAT (Procleix Ultrio kit, Chiron)	37	46	4	21,028	0.2	90.2 (81.2-99.3)	99.8 (99.7-99.8)	44.6 (33.9-55.3)	100 (100-100)	0.2 (0.2-0.3)	9.8 (0.7-18.8)

Abbreviations: Pr: prevalence in the study; TP: true-positives; FN: false-negatives; FP: false-positives; TN: true-negatives; Sn: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; FP rate: false-positive rate; FN rate: false-negative rate.ELISA: enzyme-linked immunosorbent assay; CLIA: chemiluminescent immunoassay; ECLIA: electrochemiluminescent immunoassay; CMIA: chemiluminescent microparticle immunoassay; MEIA: microparticle enzyme immunoassay; LIA: line immunoassay; RIBA: recombinant immunoblot assay; NAT: nucleic acid amplification test; PCR: polymerase chain reaction; RT-PCR: reverse-transcription polymerase chain reaction.

[†]The Benouda 2009 article does not report on the comparison of ELISA v3.0 to PCR; these data were obtained through personal communication with the authors.(20)

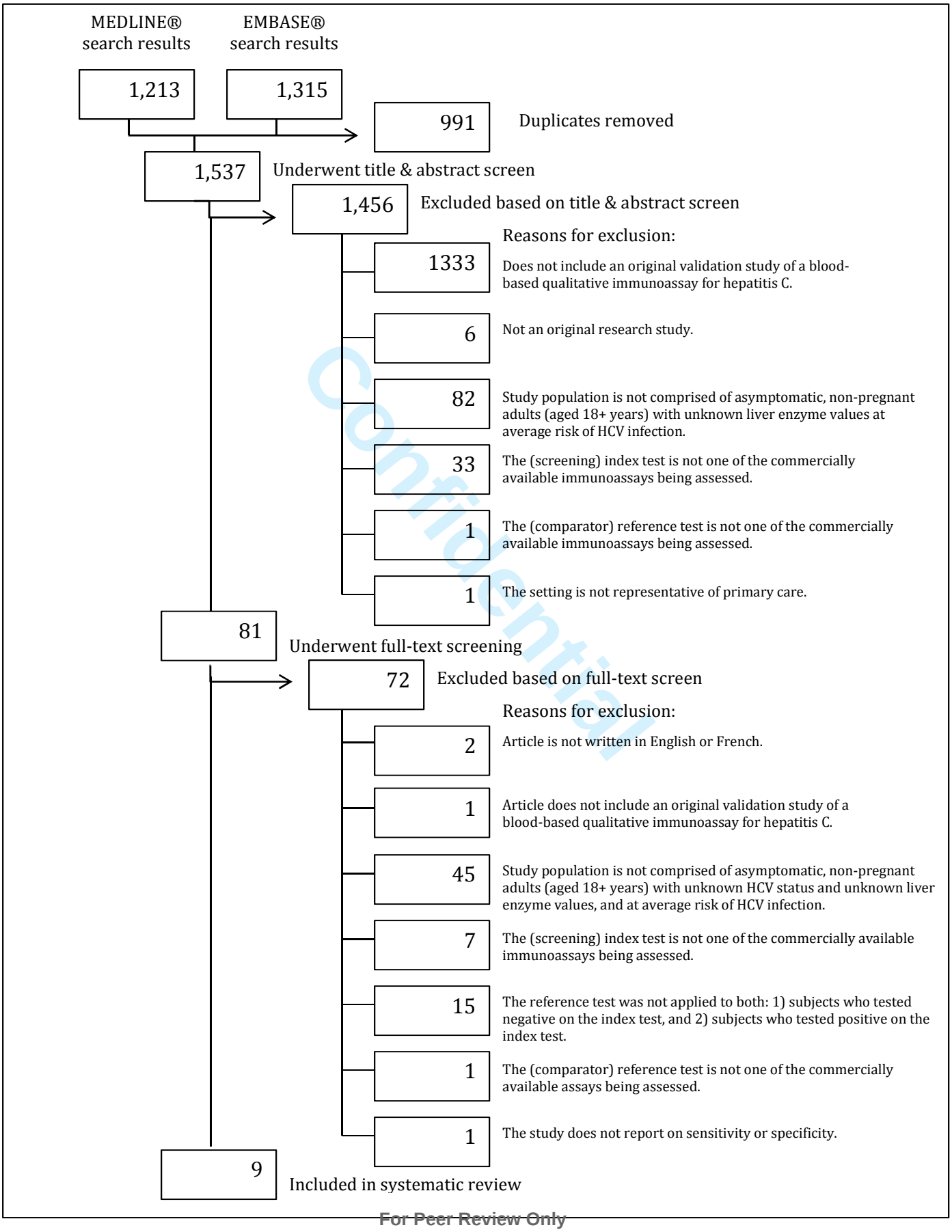
[‡]Adjusted for verification bias.(44)

Table 5. Assessment of the quality of the 'body of evidence' using GRADE

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease quality of evidence					Expected number per 1000 patients tested			Test accuracy QoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability[1] of 0.96%	pre-test probability[1] of 0.61%	pre-test probability[1] of 1.34%	
True positives (patients with HCV infection)	1 study 11 patients [2]	cross-sectional (cohort type accuracy study)	not serious [3]	very serious [4]	serious [5]	very serious [6]	none	8 (6 to 10)	5 (4 to 6)	11 (8 to 13)	⊕◯◯◯ VERY LOW
False negatives (patients incorrectly classified as not having HCV infection)								2 (0 to 4)	1 (0 to 2)	2 (0 to 5)	-
True negatives (patients without HCV infection)	1 study 17.840 patients [2]	cross-sectional (cohort type accuracy study)	not serious [3]	very serious [4]	serious [5]	not serious [7]	none	987 (986 to 988)	991 (990 to 992)	984 (983 to 985)	⊕◯◯◯ VERY LOW
False positives (patients incorrectly classified as having HCV infection)								3 (2 to 4)	3 (2 to 4)	3 (2 to 4)	-

[1] The pre-test probabilities correspond to the HCV seroprevalence estimate in the general Canadian population of 0.96, 95% CI (0.61-1.34) (2)
[2] Kosan *et al*., 2010
[3] These outcomes were not downgraded for risk of bias; based on the QUADAS-2, the risk of bias was assessed to be low (Appendix D).
[4] These outcomes were downgraded by 2 points for indirectness, because 1) the study population is different from our research question (Turkish volunteers who underwent a mandatory physical examination prior to blood donation versus the general Canadian population), and 2) this study answers an indirect question about diagnostic testing accuracy rather than directly assessing the effectiveness of screening the general population for HCV infection.
[5] These outcomes were downgraded by 1 point for inconsistency, because we expect that other studies may have different estimates.
[6] The outcomes were downgraded by 1 point for imprecision, because the very low number of true-positives and false-negatives led to a very wide 95% confidence interval for the sensitivity estimate.
[7] These outcomes were not downgraded for imprecision, because the large number of true negatives led to a very narrow 95% confidence interval for the specificity estimate.

Figure 1. PRISMA flow diagram of study selection



Appendix A: Hepatitis C testing at provincial/territorial labs: environmental scan

OBJECTIVE

The objective of this environmental scan was to obtain information about the tests and testing sequences used to diagnose HCV infection in Canada.

METHODS

Given that provincial and territorial (P/T) laboratories would be expected to perform a majority of HCV testing (and nearly all HCV genotyping), I focused on HCV testing performed by the P/T laboratories. I first performed a grey literature search of all P/T laboratory websites, as well as websites from reputable HCV-related non-profit organization such as CATIE.

Next, to supplement the grey literature search, I contacted each P/T laboratory by email and/or telephone and asked to speak to the person most directly responsible for HCV testing. I asked the following 6 questions for each P/T laboratory:

> *The Canadian Task Force on Preventive Health Care (CTFPHC) is currently working on a guideline re: screening for hepatitis C in primary care. To help with this process, we would like more information about how each of the provincial labs conducts testing for hepatitis C.*
>
> *Could you please provide us with the following information:*
>
> *1) What is the initial test for hepatitis C used by your laboratory (e.g., ELISA)?*
> *2) What is the confirmatory test for hepatitis C used by your laboratory (e.g., PCR)?*
> *3) Are other tests done routinely (other than viral load)?*
> *4) Is the confirmatory test applied to all specimens that are positive on the initial test automatically?*
> *5) Is additional specimen collection required in order to apply the confirmatory test?*
> 6) *When is the ordering physician notified of the test result (i.e., after the initial test, or only after the confirmatory test)?*

For ambiguous answers, I obtained clarification by email or telephone as needed. Data collection for this environmental scan took place from February 25 to March 2, 2016.

RESULTS

The grey literature search identified useful but potentially outdated information about each province's HCV testing on the CATIE website (http://www.catie.ca/en/practical-guides/hepc-in-depth/testing/testing-your-region). A search of individual P/T laboratory websites similarly identified some useful but potentially outdated information (Table 1). Information identified through the grey literature search was verified and supplemented by surveying each P/T laboratory (Table 1).

Initial screening test
9 provinces and 1 territory performed the initial screening test for HCV locally; 3 referred it to other P/T labs (NU, PE, YT). For the initial HCV screening test, 6/10 laboratories used a CMIA (AB, MB, NB, NL, NS, ON), 3/10 used a CLIA (BC, NT, SK), and 1/10 used an MEIA (QC).

Confirmatory testing
9 provinces performed confirmatory testing for HCV locally; 4 referred it to other P/T laboratories (NT, NU, PE, YT). For the confirmatory HCV test, 6/9 laboratories used an immunoassay (AB, BC, MB, ON, QC, SK), and 3/9 used PCR (NB, NL, NS).

Testing sequence
In all P/T laboratories, the confirmatory test is usually performed on the same blood specimen as the initial test, provided that the specimen is sufficient; the patient is not typically required to provide an additional blood specimen for the confirmatory test to be applied. Some laboratories release the results of the initial test to the ordering physician immediately, whereas others wait until the confirmatory test result is available.

DISCUSSION

This rapid environmental scan revealed that a large majority of P/T laboratories use immunoassays for both their initial screening test and their confirmatory test for HCV. The initial screening tests for HCV included CMIA, CLIA, and MEIA; ELISA was not used by any P/T laboratory as an initial screening test. Only a small minority of P/T laboratories use PCR as their confirmatory test for HCV.

A limitation of this study is that not all testing for HCV is done by the P/T laboratories; this study did not ascertain the tests used by non-P/T (typically, commercial) laboratories. This study does not account for the use of 'rapid tests' (based on finger prick blood) that may be conducted in some healthcare settings; however, in Canada, 'rapid tests' would typically be used to reach high-risk and/or vulnerable populations, and not the general population targeted by the CTFPHC clinical practice guideline.

Table 1. Hepatitis C testing by provincial labs: results from the environmental scan

Province/ territory	First test	Second test	Third test	Notification of ordering physician	Testing site (laboratory)	Information source
Alberta (AB)	Architect Anti-HCV CMIA	BioRad Monolisa HCV Ag-Ab ULTRA Assay (EIA)	We do not currently have a reflex to viral load testing, although for first time positives and for indeterminate results (i.e. when 1/2 EIAs are positive), we recommend to the physician that they send in a sample for viral load testing.	The result is reported after the confirmatory EIA is completed.	Most Hep C tests in Alberta are sent to the Provincial Laboratory of Public Health (ProvLab).	Carmen L Charlton Clinical Microbiologist Provincial Laboratory of Public Health (ProvLab) (responded by email on 2016-02-25)
British Columbia (BC)	Advia Centaur HCV CLIA	Architect Anti-HCV CMIA	For all seropositive or equivocal samples we automatically request a new sample to be drawn in an EDTA blood for HCV RNA testing.	After the confirmatory (second) test, which is done automatically if the initial test is positives or equivocal.	Most Hep C tests in British Columbia are sent to the BC Centre for Disease Control (BCCDC).	Mel Krajden BC Public Health Microbiology & Reference Laboratory (responded by email on 2016-02-28)
Manitoba (MB)	Architect Anti-HCV CMIA	Chiron RIBA HCV test 3.0	COBAS AmpliPrep/COBAS AMPLICOR HCV test		Most Hep C tests in Manitoba are sent to the Cadham Provincial Lab.	CATIE, 2010. http://www.catie.ca/en/practical-guides/hepc-in-depth/testing/testing-your-region/manitoba No response was obtained from the Cadham Provincial Laboratory.

Province/ territory	First test	Second test	Third test	Notification of ordering physician	Testing site (laboratory)	Information source
New Brunswick (NB)	Architect Anti-HCV CMIA	COBAS AmpliPrep/COBAS AMPLICOR HCV test	INNO-LIA HCV Score v2	Depends on the hospital policy. All tests are run automatically if the sample is sufficient.	"*Les tests de dépistage sont faits dans les 7 hôpitaux régionaux du NB. Nous utilisons tous le même test de dépistage. Les tests supplémentaires référés à mon laboratoire, qui sert de laboratoire provincial en la matière.*"	Richard Garceau Microbiology Laboratory at George Dumont Hospital (responded by email 2016-02-29)
Newfoundland & Labrabor (NL)	Architect Anti-HCV CMIA	Roche Diagnostics. Cobas Amplicor (PCR); if reactive anti-HCV (auto-reflex testing by laboratory)	If PCR is negative, then INNO-LIA HCV test		All Hep C tests in the province are sent to the Public Health Laboratory in St. John's.	Newfoundland and Labrador Public Health Laboratory Testing algorithm on lab website (2012): http://publichealthlab.ca/wp-content/uploads/2012/10/Hepatitis-Algorithm.pdf and http://publichealthlab.ca/service/anti-hcv-anti-hepatitis-c-virus-antibodies/
Northwest Territories (NT)	VITROS Anti-HCV CLIA	See AB	See AB	See AB	Hep C antibody tests from Yellowknife HSS area are sent to the Stanton Territorial Hospital in Yellowknife. All other Health authorities in the Northwest Territories send their Hep C antibody tests directly to Alberta.	CATIE, 2010. http://www.catie.ca/en/practical-guides/hepc-in-depth/testing/testing-your-region/northwest-territories

Province/ territory	First test	Second test	Third test	Notification of ordering physician	Testing site (laboratory)	Information source
Nova Scotia (NS)	Architect Anti-HCV CMIA	Roche Taqman HCV viral load assay. We do not ask for a second specimen for confirmation.	If the Viral load assay is negative we perform an InnoLIa immunoblot assay to see if the positive screen was due to a previous infection that has resolved or a false positive screening result. These are reflexively added.	We release the result as a screening test only and highlight that a confirmatory test is to follow.	Most Hep C tests in Nova Scotia are sent to the Queen Elizabeth II Health Sciences Centre in Halifax.	Dr Todd F. Hatchette Chief, Division of Microbiology Department of Pathology and Laboratory Medicine Nova Scotia Health Authority (responded by email on 2016-02-25)
Nunavut (NU)	See AB	See AB	See AB	See AB	Most Hep C tests in Nunavut are sent to the Provincial Laboratory of Public Health (ProvLab) in Alberta	CATIE, 2010. http://www.catie.ca/en/practical-guides/hepc-in-depth/testing/testing-your-region/nunavut
Ontario (ON)	Architect Anti-HCV CMIA	Advia Centaur HCV CLIA	For reactive (positive) or inconclusive anti-HCV results, submission of a 2.5 mL frozen serum or frozen plasma is recommended for HCV RNA viral load (Roche Assay) +/- HCV genotyping (Abbott Assay)	CMIA and CLIA results are reported together, turnaround time is up to 5 days	Most Hep C tests in Ontario are sent to the Public Health Ontario Laboratory (PHOL).	Public Health Ontario Laboratory (PHOL) Jocelyn Maregmen Supplemental Virology (responded by email on 2016-02-25)
Prince Edward Island (PE)	See NS	See NS	See NS	See NS	All Hep C tests in Prince Edward Island are sent to the lab at the Queen Elizabeth II Health Sciences Centre in Halifax, Nova Scotia	CATIE, 2010. http://www.catie.ca/en/practical-guides/hepc-in-depth/testing/testing-your-region/prince-edward-island

Province/ territory	First test	Second test	Third test	Notification of ordering physician	Testing site (laboratory)	Information source
Quebec (QC)	AxSym Anti-HCV MEIA	Chiron RIBA HCV test 3.0	COBAS AmpliPrep/COBAS AMPLICOR HCV test (PCR)		Most Hep C tests in Québec are sent to Le Laboratoire de Santé Publique du Quebec (LSPQ).	CATIE, 2010. http://www.catie.ca/en/practical-guides/hepc-in-depth/testing/testing-your-region/quebec No response was obtained from the Laboratoire de sante publique du Québec (LSPQ)
Saskatchewan (SK)	Advia Centaur anti-HCV CLIA	Architect HCV-Ag CMIA	if the Architect HCV-Ag CMIA is negative then a INNO-LIA HCV Score test is run	Only after the confirmatory test	Most Hep C tests in the province are sent to the Saskatchewan Disease Control Laboratory in Regina.	Jim Putz, Saskatchewan Disease Control Laboratory (responded by email 2016-02-26)
Yukon (YT)	See BC	See BC	See BC	See BC	Most Hep C tests in Yukon are sent to the BCCDC Provincial Lab in British Columbia	CATIE, 2010. http://www.catie.ca/en/practical-guides/hepc-in-depth/testing/testing-your-region/yukon

CLIA: chemiluminescent immunoassay; CMIA: chemiluminescent microparticle immunoassay; MEIA: microparticle enzyme immunoassay; ELISA : enzyme-linked immunosorbent assay; RIBA : recombinant immunoblot assay; PCR: polymerase chain reaction

Appendix B: literature search strategies

Search strategy for Ovid MEDLINE® 1946-present and Ovid MEDLINE® In-Process & Other Non-Indexed Citations; run on 2016-MAY-06, yielding 1,213 results.

#	Search
1	Hepatitis C/ or Hepatitis C, Chronic/ or Hepacivirus/ or Hepatitis C Antibodies/ or exp Hepatitis C Antigens/
2	(hepatitis C or hepC or hep C or hepacivirus or HCV).ti,ab,kf
3	1 or 2
4	exp Enzyme-Linked Immunosorbent Assay/
5	(ELISA or EIA or enzyme immunoassa* or enzyme linked immunosorben* or enzyme linked immunoassa* or enzyme linked immuno-sorben* or enzyme linked immunoblot*).ti,ab,kf
6	((immunosorb* or immuno-sorb*) adj2 enzyme* adj2 (assay or assays)).ti,ab,kf
7	(CMIA or CLIA or CIA or MEIA).ti,ab,kf
8	((chemilumin* or chemi-lumin*) adj2 (immunoassa* or immuno-assa* or assa*)).ti,ab,kf
9	((micropart* or micro-part*) adj2 (immunoassa* or immuno-assa* or assa*)).ti,ab,kf
10	(Architect or Vitros or ADVIA or Centaur or AxSYM or Monolisa or Murex).ti,ab,kf
11	4 or 5 or 6 or 7 or 8 or 9 or 10
12	3 and 11
13	exp Mass Screening/
14	(detect* or screen*).ti,ab,kf
15	13 or 14
16	12 and 15
17	exp "Sensitivity and Specificity"/ or Diagnostic errors/ or exp False Positive Reactions/ or exp "Predictive Value of Tests"/
18	(false adj2 (positive* or negative*)).ti,ab,kf
19	(predict* adj2 (positive* or negative*)).ti,ab,kf
20	(sensitiv* or specific*).ti,ab,kf
21	((test* or diagnos*) adj2 (performance or accura* or characteristic*)).ti,ab,kf
22	DTA.ti,ab,kf
23	17 or 18 or 19 or 20 or 21 or 22
24	3 and 11 and 15 and 23
25	24 not conference abstract.pt
26	limit 25 to English language
27	limit 25 to French
28	26 or 27
29	limit 28 to yr="1990-current"
30	remove duplicates from 29

Search strategy for EMBASE®; run on 2016-MAY-06, yielding 1,315 results.

#	Search
1	exp hepatitis C/ or exp Hepatitis C virus/ or exp hepatitis C antibody/ or exp hepatitis C antigen/
2	(hepatitis C or hepC or hep C or hepacivirus or HCV).ti,ab
3	1 or 2
4	exp enzyme linked immunosorbent assay/ or exp chemiluminescence immunoassay/ or exp microparticle enzyme immunoassay/
5	(ELISA or EIA or enzyme immunoassa* or enzyme linked immunosorben* or enzyme linked immunoassa* or enzyme linked immuno-sorben* or enzyme linked immunoblot*).ti,ab
6	((immunosorb* or immuno-sorb*) adj2 enzyme* adj2 (assay or assays)).ti,ab
7	(CMIA or CLIA or CIA or MEIA).ti,ab
8	((chemilumin* or chemi-lumin*) adj2 (immunoassa* or immuno-assa* or assa*)).ti,ab
9	((micropart* or micro-part*) adj2 (immunoassa* or immuno-assa* or assa*)).ti,ab
10	(Architect or Vitros or ADVIA or Centaur or AxSYM or Monolisa or Murex).ti,ab
11	4 or 5 or 6 or 7 or 8 or 9 or 10
12	3 and 11
13	exp antibody screening/ or exp mass screening/ or exp screening/ or exp screening test/
14	(detect* or screen*).ti,ab
15	13 or 14
16	12 and 15
17	exp diagnostic accuracy/ or exp "sensitivity and specificity"/ or exp diagnostic error/ or exp false positive result/ or exp false negative result/ or exp predictive value/
18	(false adj2 (positive* or negative*)).ti,ab
19	(predict* adj2 (positive* or negative*)).ti,ab
20	(sensitiv* or specific*).ti,ab
21	((test* or diagnos*) adj2 (performance or accura* or characteristic*)).ti,ab
22	DTA.ti,ab
23	17 or 18 or 19 or 20 or 21 or 22
24	3 and 11 and 15 and 23
25	24 not conference abstract.pt
26	limit 25 to English language
27	limit 25 to French
28	26 or 27
29	limit 28 to yr="1990-current"
30	remove duplicates from 29

Appendix C: Excluded articles by level and question

Level 1: Title and Abstract Screening

1. Is it or does it include an original validation study of a blood-based qualitative immunoassay for hepatitis C? (Exclude rapid/point-of-care tests, geno/serotyping tests, tests applied to dried blood or saliva.)

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S R Lee, C L Wood, M J Lane, B Francis, C Gust, C M Higgs, M J Nelles, A Polito, R DiNello, D Achord (1995//). Increased detection of hepatitis C virus infection in commercial plasma donors by a third-generation screening assay. *Transfusion*, 35(10), 845

T J Tucker, M Voigt, A Bird, S Robson, B Gibbs, J

Kannemeyer, M Galloway, R E Kirsch, H Smuts (1997//). Hepatitis C virus infection rate in volunteer blood donors from the Western Cape--comparison of screening tests and PCR. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde*, 87(5), 603

T Kodama, S Ichiyama, K Sato, T Nada, N Nakashima (1998//). Evaluation of a membrane filter assay system, Ortho HCV Ab Quick Pack, for detection of anti-hepatitis C virus antibody. *Journal of clinical microbiology*, 36(5), 1439

T Tanaka, J Y Lau, M Mizokami, E Orito, E Tanaka, K Kiyosawa, K Yasui, Y Ohta, A Hasegawa, S Tanaka (1995//). Simple fluorescent enzyme immunoassay for detection and quantification of hepatitis C viremia. *Journal of hepatology*, 23(6), 742

Y Lazizi (1994//). Discordance between four second-generation enzyme immunoassay kits in anti-hepatitis C virus screening. The Study Group of the Laboratoires de Virologie des Centres Hospitaliers Universitaires francais. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology*, 13(3), 280

Weise W.,Backer U.,Seidl S.,Flacke H.,Sondag D.,Pirenne H.,Schroeter E.,Troonen H.,Broeksma C.,Heyermann H. (1992//). Abbott HCV EIA 2nd generation: a new screening assay *Beitrage zur Infusionstherapie = Contributions to infusion therapy*, 30(#issue#), 34

5. Is the (comparator) REFERENCE TEST one of the following?

Nahed Ismail, Geoffrey E Fish, Michael B Smith (2004//). Laboratory evaluation of a fully automated chemiluminescence immunoassay for rapid detection of HBsAg, antibodies to HBsAg, and antibodies to hepatitis C virus. *Journal of clinical microbiology*, 42(2), 610

6. Does the study report on the following OUTCOMES? (Tick all that apply)

7. Is the SETTING representative of primary care?

Pierre Zachary, Murielle Ullmann, Saadi Djeddi, Nicolas Meyer, Marie-Josee Wendling, Evelyne Schvoerer, Francoise Stoll-Keller, Jean-Pierre Gut (2005//). Evaluation of three commercially available hepatitis C virus antibody detection assays under the conditions of a clinical virology laboratory. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*, 34(3), 207

8. Does the study take place in a country with a LOW OR MODERATE (<3.5%) PREVALENCE of chronic HCV infection?

Level 2: Full Text Review

1. Is the full-text article available for review?

2. What language is this article written in?

Ecemis T.,Akcali S.,Erbay Dundar P.,Sanlidag T. (2012//). The threshold value of anti-HCV test in the diagnosis of HCV infection *Turkiye Klinikleri Journal of Medical Sciences*, 32(6), 1648

Suslov A.P.,Kuzin S.N.,Golosova T.V.,Shalunova N.V.,Malyshev N.A.,Sadikova N.V.,Vavilova L.M.,Somova A.V.,Musina E.E.,Ivanova M.V.,Kipor T.T.,Timonin I.M.,Kuzina L.E.,Godkov M.A.,Bajenov A.I.,Nesterenko V.G. (2002//). Limits of diagnostic accuracy of anti-hepatitis C virus antibodies detection by ELISA and immunoblot assay *Russian journal of immunology : RJI : official journal of Russian Society of Immunology*, 7(2), 175

3. Is it or does it include an original validation study of a blood-based qualitative immunoassay for hepatitis C?

Kaur H.,Manjari M.,Thaman R.G.,Singh G. (2012//). Prevalence of markers of Hepatitis C virus among the blood donors *Journal of Clinical and Diagnostic Research*, 6(6), 959

4. What TYPE OF PUBLICATION is it?

5. Is the STUDY POPULATION comprised of asymptomatic, non-pregnant adults (aged 18+ years) with unknown HCV status and

unknown liver enzyme values, and at average risk of HCV infection?

A D Kitchen, N V Tucker (1995//). The specificity of anti-HCV supplementary assays. *Vox sanguinis*, 69(2), 100

Annemarie Berger, Holger Rabenau, Regina Allwinn, Hans Wilhelm Doerr (2008//). Evaluation of the new ARCHITECT anti-HCV screening test under routine laboratory conditions. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*, 43(2), 158

B Seigneres, F Descamps, R Croise, V Barlet, M Bouvier-Alias, S Chevaliez, J M Pawlotsky, W Abdelhady, M Rafik, A M Avellon, J M Echevarria, M Hausmann, J-M Dugua (2016//). Multicenter clinical evaluation of the new 3rd generation assay for detection of antibodies against hepatitis C virus on the VIDAS() system. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*, 78(#issue#), 20

Benjamin Maasoumy, Birgit Bremer, Regina Raupach, Patrick Lehmann, Michael P Manns, Markus Cornberg, Heiner Wedemeyer (2014//). How to interpret borderline HCV antibody test results: a comparative study investigating four different anti-HCV assays. *Viral immunology*, 27(1), 7

C Berasain, M Garcia-Granero, J I Riezu-Boj, M P Civeira, J Prieto, F Borras-Cuesta (1993//). Detection of anti-hepatitis C virus antibodies by ELISA using synthetic peptides. *Journal of hepatology*, 18(1), 80

C Colin, D Lanoir, S Touzet, L Meyaud-Kraemer, F Bailly, C Trepo,HEPATITIS Group (2001//). Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. *Journal of viral hepatitis*, 8(2), 87

D Lavanchy, C Mayerat, B Morel, P Schneider, C Zufferey, J J Gonvers, A Pecoud, P C Frei (1994//). Evaluation of third-generation assays for detection of anti-hepatitis C virus (HCV) antibodies and comparison with presence of HCV RNA in blood donors reactive to c100-3 antigen. *Journal of clinical microbiology*, 32(9), 2272

D Lavanchy, J Steinmann, A Moritz, P C Frei (1996//). Evaluation of a new automated third-generation anti-HCV enzyme immunoassay. *Journal of clinical laboratory analysis*, 10(5), 269

D Robert Dufour, Mageli Talastas, Maria D A Fernandez, Barbara Harris (2003//). Chemiluminescence assay improves specificity of hepatitis C antibody detection. *Clinical chemistry*, 49(6 Pt 1), 940

Dinesh O Shah, Chi D Chang, Lily X Jiang, Kevin Y Cheng, A Scott Muerhoff, Robin A Gutierrez, Thomas P Leary, Suresh M Desai, Irenea V Batac-Herman, Vince A Salbilla, Alla S Haller, James L Stewart, George J Dawson (2003//). Combination HCV core antigen and antibody assay on a fully automated chemiluminescence analyzer. *Transfusion*, 43(8), 1067

Elliot Eli Dogbe, Fareed Arthur (2015//). Diagnostic accuracy of blood centers in the screening of blood donors for viral markers. *The Pan African medical journal*, 20(#issue#), 119

F Ansaldi, B Bruzzone, G Testino, M Bassetti, R Gasparini, P Crovari, G Icardi (2006//). Combination hepatitis C virus antigen and antibody immunoassay as a new tool for early diagnosis of infection. *Journal of viral hepatitis*, 13(1), 5

F Ferrer, M J Candela, C Garcia, L Martinez, J Rivera, V Vicente (1997//). A comparative study of two third-generation anti-hepatitis C virus ELISAs. *Haematologica*, 82(6), 690

F Hmaied, M Ben Mamou, Z Arrouji, A Slim, S Ben Redjeb (2007//). [Use of combined detection of hepatitis C virus core antigen and antibodies to reduce the serological window-phase]. *Pathologie-biologie*, 55(2), 121

G Icardi, F Ansaldi, B M Bruzzone, P Durando, S Lee, C de Luigi, P Crovari (2001//). Novel approach to reduce the hepatitis C virus (HCV) window period: clinical evaluation of a new enzyme-linked immunosorbent assay for HCV core antigen. *Journal of clinical microbiology*, 39(9), 3110

H Cano, M J Candela, M L Lozano, V Vicente (2003//). Application of a new enzyme-linked immunosorbent assay for detection of total hepatitis C virus core antigen in blood donors. *Transfusion medicine (Oxford, England)*, 13(5), 259

H Hennig, P Schlenke, H Kirchner, I Bauer, B Schulte-Kellinghaus, H Bludau (2000//). Evaluation of newly developed microparticle enzyme immunoassays for the detection of HCV antibodies. *Journal of virological methods*, 84(2), 181

H Vrielink, H L Zaaijer, H W Reesink, C L van der Poel, H T Cuypers, P N Lelie (1995//). Sensitivity and specificity of three third-generation anti-

hepatitis C virus ELISAs. *Vox sanguinis*, 69(1), 14

K Aoyagi, C Ohue, K Iida, T Kimura, E Tanaka, K Kiyosawa, S Yagi (1999//). Development of a simple and highly sensitive enzyme immunoassay for hepatitis C virus core antigen. *Journal of clinical microbiology*, 37(6), 1802

L H Tobler, S R Lee, S L Stramer, J Peterson, R Kochesky, K Watanabe, S Quan, A Polito, M P Busch (2000//). Performance of second- and third-generation RIBAs for confirmation of third-generation HCV EIA-reactive blood donations. Retrovirus Epidemiology Donor Study. *Transfusion*, 40(8), 917

M Tsopanomichalou, M Ergazaki, D A Spandidos (1997//). Evaluation of western blot in routine diagnosis of hepatitis C virus. *The International journal of biological markers*, 12(1), 35

M da Silva Cardoso, K Koerner, S Epple, B Kubanek (1993//). Prevalence of HCV-RNA-positive blood donors and correlation to ELISA and RIBA status. *Annals of hematology*, 66(3), 147

M Schroter, H H Feucht, P Schafer, B Zollner, S Polywka, R Laufs (1999//). Definition of false-positive reactions in screening for hepatitis C virus antibodies. *Journal of clinical microbiology*, 37(1), 233

Menha Swellam, Magda Sayed Mahmoud, Adel Abdel-Fatah Ali (2011//). Diagnosis of hepatitis C virus infection by enzyme-linked immunosorbent assay and reverse transcriptase-nested polymerase chain reaction: a comparative evaluation. *IUBMB life*, 63(6), 430

Mohsan Saeed, Ryosuke Suzuki, Madoka Kondo, Hideki Aizaki, Takanobu Kato, Toshiaki Mizuochi, Takaji Wakita, Haruo Watanabe, Tetsuro Suzuki (2009//). Evaluation of hepatitis C virus core antigen assays in detecting recombinant viral antigens of various genotypes. *Journal of clinical microbiology*, 47(12), 4141

Par A.,Kantor I.,Barcsay E.,Hollos I.,Mezey I.,Brojnas J.,Takacs M.,Hejjas M.,Illes M.,Szontagh L. (1991//). Prevalence of antibody to hepatitis C virus in blood donors, high-risk groups and patients with liver diseases in Hungary. A multicentre study using ABBOTT EIA test and a comparison with an ORTHO ELISA test system *Acta medica Hungarica*, 48(3-4), 167

P Bonanni, G C Icardi, A M Raffo, M Ferrari Bravo, A Roccatagliata, P Crovari (1996//). Analytical and laboratory evaluation of a new fully-automated third generation enzyme immunoassay for the detection of antibodies to the hepatitis C virus. *Journal of virological methods*, 62(2), 113

P Kiely, D Wilson (2000//). Results of HCV screening of volunteer blood donors with a chemiluminescent immunoassay and a second- or third-generation EIA: overlap of false-positive reactivity and its impact on donor management. *Transfusion*, 40(5), 580

P Vermeersch, M Van Ranst, K Lagrou (2010//). Evaluation of the use of a combined HCV antigen/antibody assay in routine laboratory practice. *Acta clinica Belgica*, 65(4), 245

Philip Kiely, Kyall Walker, Susan Parker, Anthea Cheng (2010//). Analysis of sample-to-cutoff ratios on chemiluminescent immunoassays used for blood donor screening highlights the need for serologic confirmatory testing. *Transfusion*, 50(6), 1344

Pierpaolo Valcavi, Maria Cristina Medici, Francesca Casula, Maria Cristina Arcangeletti, Flora De Conto, Federica Pinardi, Adriana Calderaro, Carlo Chezzi, Giuseppe Dettori (2004//). Evaluation of a total hepatitis C virus (HCV) core antigen assay for the detection of antigenaemia in anti-HCV positive individuals. *Journal of medical virology*, 73(3), 397

S K Dhaliwal, L E Prescott, B C Dow, F Davidson, H Brown, P L Yap, E A Follett, P Simmonds (1996//). Influence of viraemia and genotype upon serological reactivity in screening assays for antibody to hepatitis C virus. *Journal of medical virology*, 48(2), 184

S R Lee, J Peterson, P Niven, C Bahl, E Page, R DeLeys, D Giordano-Schmidt, D Baggett, G Green (2001//). Efficacy of a hepatitis C virus core antigen enzyme-linked immunosorbent assay for the identification of 'window-phase' blood donations. *Vox sanguinis*, 80(1), 19

Son H.C.,Yoon M.S.,Kim Y.J.,Kim I.H.,Kim Y.S. (2002//). Type-specific antibody for hepatitis C virus detected by use of NS-4 peptide and hepatitis C virus genome in Korea *Rinsho byori. The Japanese journal of clinical pathology*, 50(5), 533

Susan L Stramer, Roger Y Dodd, Jaye P Brodsky (2013//). The value of screening signal-to-cutoff ratios for hepatitis C virus antibody confirmation. *Transfusion*, 53(7), 1497

T P Leary, R A Gutierrez, A S Muerhoff, L G Birkenmeyer, S M Desai, G J Dawson (2006//). A chemiluminescent, magnetic particle-based immunoassay for the detection of hepatitis C virus core antigen in human serum or plasma. *Journal of*

medical virology, 78(11), 1436

Dow B.C.,Follett E.A.C.,Munro H.,Buchanan I.,Roy K.,McOmish F.,Yap P.L.,Simmonds P. (1994//). Failure of 2nd- and 3rd-generation HCV ELISA and RIBA to detect HCV polymerase chain reaction - Positive donations [2] *Vox Sanguinis*, 67(2), 236

Hofmann H.,Konczer A. (1993//). Commercial test kits in diagnosis of hepatitis C *Serodiagnosis and Immunotherapy in Infectious Disease*, 5(2), 76

Mixson-Hayden T.,Dawson G.J.,Teshale E.,Le T.,Cheng K.,Drobeniuc J.,Ward J.,Kamili S. (2015//). Performance of ARCHITECT HCV core antigen test with specimens from US plasma donors and injecting drug users *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*, 66(#issue#), 15

Muerhoff A.S.,Jiang L.,Shah D.O.,Gutierrez R.A.,Patel J.,Garolis C.,Kyrk C.R.,Leckie G.,Frank A.,Stewart J.L.,Dawson G.J. (2002//). Detection of HCV core antigen in human serum and plasma with an automated chemiluminescent immunoassay *Transfusion*, 42(3), 349

Oh E.-J.,Chang J.,Yang J.-Y.,Kim Y.,Park Y.-J.,Han K. (2013//). Different signal-to-cut-off ratios from three automated anti-hepatitis C virus chemiluminescence immunoassays in relation to results of recombinant immunoblot assays and nucleic acid testing *Blood Transfusion*, 11(3), 471

Re V.,Gallego S.,Trevino E.,Barbas G.,Dominguez C.,Elbarcha O.,Bepre H.,Contigiani M. (2005//). Evaluation of five screening tests licensed in Argentina for detection of hepatitis C virus antibodies *Memorias do Instituto Oswaldo Cruz*, 100(3), 303

Shirachi M.,Sata M.,Suzuki H.,Fukuizumi K.,Tanikawa K.,Itoh Y.,Mizumoto T.,Kondoh S.,Kasahara M.,Manabe S. (1998//). Evaluation of third generation anti-HCV test kit (SYNPEP HCV-EIA II) using sera of inhabitants from HCV hyperendemic area *The Kurume medical journal*, 45(1), 81

Uyttendaele S.,Claeys H.,Mertens W.,Verhuert H.,Vermylen C. (1994//). Evaluation of third-generation screening and confirmatory assays for HCV antibodies *Vox Sanguinis*, 66(2), 122

6. Is the (screening) INDEX TEST one of the following commercially-available immunoassays?

Alter H.J.,Tegtmeier G.E.,Jett B.W.,Quan S.,Shih J.W.,Bayer W.L.,Polito A. (1991//). The use of a recombinant immunoblot assay in the interpretation of anti-hepatitis C virus reactivity among prospectively followed patients, implicated donors, and random donors *Transfusion*, 31(8), 771

B Betlach, S Anderson, R Rodriguez, K Kuramoto, K Sazama, P V Holland (1992//). Comparison of two approved enzyme immunoassays for the detection of antibodies to the hepatitis C virus in 5216 United States blood donors. *Transfusion*, 32(2), 191

Diallo A.,Lazizi Y.,Le Guenno B.,Pillot J. (1991//). Non-specific reactions of the ELISA kit for the detection of blood donors infected with hepatitis C virus *Presse Medicale*, 20(8), 383

C J Tibbs, S J Palmer, R Coker, S K Clark, G M Parsons, S Hojvat, D Peterson, J E Banatvala (1991//). Prevalence of hepatitis C in tropical communities: the importance of confirmatory assays. *Journal of medical virology*, 34(3), 143

Galel S.A.,Strong D.M.,Tegtmeier G.E.,Holland P.V.,Kuramoto I.K.,Kemper M.,Pietrelli L.,Gallarda J. (2002//). Comparative yield of HCV RNA testing in blood donors screened by 2.0 versus 3.0 antibody assays *Transfusion*, 42(11), 1507

J P Allain, A Kitchen, S Aloysius, I Reeves, J Petrik, J A Barbara, L M Williamson (1996//). Safety and efficacy of hepatitis C virus antibody screening of blood donors with two sequential screening assays. *Transfusion*, 36(5), 401

Ornopia G.L.,Kuramoto K. (1995//). Detection of anti-hepatitis C virus using chemiluminescence *Journal of Viral Hepatitis*, 2(4), 215

W Prohaska, C Wolff, E Lechler, K Kleesiek (1991//). High rate of false positives in blood donor screening for antibodies to hepatitis C virus. Cause of underestimation of virus transmission rate?. *Klinische Wochenschrift*, 69(7), 294

7. STUDY DESIGN: was the REFERENCE TEST applied to both: 1) subjects who tested NEGATIVE on the INDEX TEST, AND 2) subjects who tested POSITIVE on the INDEX TEST?

Ali Acar, Sabri Kemahli, Husnu Altunay, Erdogan Kosan, Oral Oncul, Levent Gorenek, Saban Cavuslu (2010//). The significance of repeat testing in Turkish blood donors screened with HBV, HCV and HIV immunoassays and the importance of S/CO ratios in the interpretation of

HCV/HIV screening test results and as a determinant for further confirmatory testing. *Transfusion medicine (Oxford, England)*, 20(3), 152

Bruhn R.,Lelie N.,Busch M.,Kleinman S.,Vermeulen M.,Reddy R.,Bird A.,Cable R.,Goubran H.,Moftah F.,El Ekiaby M.,Ghiazza P.,Manzini P.,Favilli F.,Peduzzi C.,Roig R.,Alvarez M.,Sauleda S.,Niederhauser C.,Levicnik S.,Nograsek P.,Wessberg S.,Elkblom S.,Lankinen M.,Ulm H.,Harritshoj L.,Nielsen C.,Jorgensen S.,Erikstrup C.,O'Riordan J.,Brojer E.,Grabarczyk P.,Gdowska J.,Piotrowski D.,Lam S.,Teo D.,Chua S.S.,Lin C.K.,Tsoi W.C.,Bon A.H.,Peng S.L.T.,Flanagan P.,Brown S.,Kiely P.,Margaritis A. (2015//). Relative efficacy of nucleic acid amplification testing and serologic screening in preventing hepatitis C virus transmission risk in seven international regions *Transfusion*, 55(6), 1195

Clive R Seed, Angelo R Margaritis, Wayne V Bolton, Philip Kiely, Susan Parker, Lisa Piscitelli, Australian Red Cross Blood Service Virology Subcommittee of the National Donor and Product Safety Committee (2003//). Improved efficiency of national HIV, HCV, and HTLV antibody testing algorithms based on sequential screening immunoassays. *Transfusion*, 43(2), 226

F Alborino, A Burighel, F-W Tiller, J van Helden, C Gabriel, A Raineri, R Catapano, H Stekel (2011//). Multicenter evaluation of a fully automated third-generation anti-HCV antibody screening test with excellent sensitivity and specificity. *Medical microbiology and immunology*, 200(2), 77

Gesa Jonas, Claudia Pelzer, Christian Beckert, Michael Hausmann, Hans-Peter Kapprell (2005//). Performance characteristics of the ARCHITECT anti-HCV assay. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*, 34(2), 97

J Petitjean Lecherbonnier, S Gouarin, J Dina, A Vabret, F Freymuth (2007//). [Hepatitis C virus screening: performances characteristics of a commercial automated chemiluminescent microparticle immunoassay (CMIA-ARCHITECT anti-HCV)]. *Pathologie-biologie*, 55(10), 512

Lei Li, Pei-Jer Chen, Ming-Hung Chen, Kin-Fu Chak, Kuo-Sin Lin, Su-Jen Lin Tsai (2008//). A pilot study for screening blood donors in Taiwan by nucleic acid amplification technology: detecting occult hepatitis B virus infections and closing the serologic window period for hepatitis C virus. *Transfusion*, 48(6), 1198

Linda Sommese, Carmela Iannone, Francesco Cacciatore, Gustavo De Iorio, Claudio Napoli (2014//). Comparison between screening and confirmatory serological assays in blood donors in a region of South Italy. *Journal of clinical laboratory analysis*, 28(3), 198

Mohamed Abdel-Hamid, Mai El-Daly, Sherif El-Kafrawy, Nabiel Mikhail, G Thomas Strickland, Alan D Fix (2002//). Comparison of second- and third-generation enzyme immunoassays for detecting antibodies to hepatitis C virus. *Journal of clinical microbiology*, 40(5), 1656

P Zachary, M Ullmann, S Djeddi, M-J Wendling, E Schvoerer, F Stoll-Keller, J-P Gut (2004//). [Evaluation of two commercial enzyme immunoassays for diagnosis of hepatitis C in the conditions of a virology laboratory]. *Pathologie-biologie*, 52(9), 511

Pieter Vermeersch, Marc Van Ranst, Katrien Lagrou (2008//). Validation of a strategy for HCV antibody testing with two enzyme immunoassays in a routine clinical laboratory. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*, 42(4), 394

Recep Kesli, M Ozdemir, M G Kurtoglu, M Baykan, B Baysal (2009//). Evaluation and comparison of three different anti-hepatitis C virus antibody tests based on chemiluminescence and enzyme-linked immunosorbent assay methods used in the diagnosis of hepatitis C infections in Turkey. *The Journal of international medical research*, 37(5), 1420

Ruifeng Yang, Wenli Guan, Qian Wang, Yan Liu, Lai Wei (2013//). Performance evaluation and comparison of the newly developed Elecsys anti-HCV II assay with other widely used assays. *Clinica chimica acta; international journal of clinical chemistry*, 426(#issue#), 95

Steven H Kleinman, Susan L Stramer, Jaye P Brodsky, Sally Caglioti, Michael P Busch (2006//). Integration of nucleic acid amplification test results into hepatitis C virus supplemental serologic testing algorithms: implications for donor counseling and revision of existing algorithms. *Transfusion*, 46(5), 695

Yuan-Hung Kuo, Kuo-Chin Chang, Jing-Houng Wang, Pei-Shan Tsai, Shu-Feng Hung, Chao-Hung Hung, Chien-Hung Chen, Sheng-Nan Lu (2012//). Is hepatitis C virus core antigen an adequate marker for community screening?. *Journal of clinical microbiology*, 50(6), 1989

8. Is the (comparator) REFERENCE TEST one of the following commercially-available assays?

O'Flynn N.,Jilg W.,McQuillan T.,Bauer I.,Heyermann M.,Schulte-Kellinghaus B.,Moller D. (1997//). New HCV assay on the Abbott AxSYM random access analyzer *Clinical Laboratory*, 43(5),403

9. Does the study report on the following OUTCOMES?

M Schmidt, A Jimenez, A Muhlbacher, S Oota, L Blanco, T Sakuldamrongpanich, H Schennach, E Seifried (2015//). Head-to-head comparison between two screening systems for HBsAG, anti-HBc, anti-HCV and HIV combination immunoassays in an international, multicentre evaluation study. *Vox sanguinis*, 109(2), 114

10. Is the SETTING representative of primary care?

11. Does the study take place in a country with a LOW OR MODERATE (<3.5%) PREVALENCE of chronic HCV infection?

QUADAS-2	Denoyel, 2004	Tashkandy, 2007	Benouda, 2009	Rao, 2009	Ol, 2009	Kosan, 2010	Park, 2012	Sommese, 2014	Arora, 2016
Study population	5,228 individuals: 5,015 random blood donors and 213 hospitalized patients	106 male blood donors	8,326 adults from the general population with unknown HCV serology underwent initial screening with ELISA v3.0	2,559 individuals: 2,082 blood donors (Beijing Red Cross Blood Center) and 477 patients (Peking University Hepatology Institute; including various HCV genotypes, non-C hepatitis, pregnant women, and lipidemia sera)	1,200 potential volunteer blood donors underwent screening with ELISA v4.0: 677 females, 523 males, mean age: 32.8 years, age range 18-52 year; sample stratified by two Cambodian provinces (600 each)	18,200 volunteer blood donors: 546 (3%) were women, 17,654 (97%) were men, 18,198 (99.9%) were first-time donors, mean age: 40 years, age range: 18-60 years	1,011 sera from individuals undergoing routine HCV screening	840 volunteer blood donors; Second University of Naples; January to June 2013	21,115 blood donors; blood bank; January 2013 to March 2014
Index test(s)	CLIA (ADVIA Centaur® HCV assay)	ELISA v3.0 (Abbott Murex anti-HCV)	ELISA V3.0 (Abbott Murex anti-HCV)	ELISA v4.0 (EIAgen, Adaltis)	ELISA v4.0 (Monolisa™, BioRad)	ELISA v3.0 (Innotest HCV Ab III, Innogenetics, Belgium)	CLIA (Vitros Anti-HCV assay, UK)	CMIA (Architect i200SR, Abbott, Germany)	ELISA v4.0 (Monolisa BioRad Ag-Ab Ultra)
Reference test(s)	MEIA (Abbott AxSYM® HCV v.3.0 assay)	LIA (INNo-LIA HCVAb III Update, INNOGENTICS, Belgium) and RT-PCR (High Pure Viral Nucleic Acid reagent set, Roche Molecular Biochemicals)	MEIA (Abbott AxSYM® HCV v.3.0 assay) and RT-PCR (Roche Amplicor HCV® v2.0)	ELISA v3.0 (Ortho HCV 3.0 ELISA)	CMIA (Abbott)	NAT (Procleix Ultrio kit, Chiron, USA)	ECLIA (Elecsys anti-HCV test, Roche, Germany)	ECLIA (Cobas e411, Roche, Germany)	NAT (Procleix Ultrio kit, Chiron, USA)
DOMAIN 1: PATIENT SELECTION									
Describe methods of patient selection:	Not described	All samples were collected at the Immunology and Serology Department at the Al-Noor Specialist Hospital, Makkah, Saudi Arabia. We excluded all samples from patients with diabetes or other endocrine diseases and autoimmune diseases.	8,326 adults from the general population with unknown HCV serology underwent initial screening with ELISA v3.0. Initial blood specimen collection took place in work settings, from December 2005 to April 2007 (16 months). A subset of 161 ELISA-positives and 100 ELISA-negatives were called back to undergo the reference standard (verification bias); 3/161 lost to follow-up.	2,559 individuals: 2,082 blood donors (Beijing Red Cross Blood Center) and 477 patients (Peking University Hepatology Institute; including various HCV genotypes, non-C hepatitis, pregnant women, and lipidemia sera)	1,200 potential volunteer blood donors underwent screening with ELISA. A subset of 80 ELISA-positive and 40 ELISA-negative were selected to undergo the reference test (verification bias)	Turkish Red Crescent Çapa Blood Centre of Istanbul; intermittently from February 2007 to March 2008; individuals underwent a mandatory physical exam (no exclusion criteria specified) prior to blood drawing;	Individuals undergoing "routine HCV screening"; sera collected between August 2009 and January 2011	"we selected a group of 840 samples from volunteer blood donors"	"we reviewed the donor screening data for anti-HCV from January 2013 to May 2014" Presumably, all donors during this time period are included.
Was a consecutive or random sample of patients enrolled:	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Was a case-control design avoided?	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes
Did the study avoid inappropriate exclusions?	Yes	No	Yes	Yes	Yes	Yes	Yes	Unclear	Yes
Could the selection of patients have introduced bias?	Unclear risk	Unclear risk	High risk; due to verification bias, but easily correctable statistically	Low risk	High risk; due to verification bias, but easily correctable statistically	Low risk	Low risk	Low risk	Low risk
Describe included patients (prior testing, presentation, intended use of index test and setting):	5,015 random blood donors and 213 hospitalized patients; the 213 (4.1%) hospitalized patients do not reflect the setting or population of the review question.	106 male blood donors	8,326 adults from the general population with unknown HCV serology	477 / 2,559 (18.6%) patients did not meet our inclusion criteria in that they were sampled from a hepatology clinic and their HCV status was known at the outset	1,200 potential volunteer blood donors underwent screening with ELISA	18,200 volunteer blood donors: 546 (3%) were women, 17,654 (97%) were men, 18,198 (99.9%) were first-time donors, mean age: 40 years, age range: 18-60 years; individuals underwent a mandatory physical exam (no exclusion criteria specified) prior to blood drawing	1,011 sera from individuals undergoing routine HCV screening	840 volunteer blood donors: 275 (32.7%) were women, 564 (67.3%) men, mean age: 37.7 years (SD 12.5 years)	21,115 blood donors; presumably all consecutive blood donors during the study period
Is there concern that the included patients do not match the review question?	Low concern	Low concern	Low concern	High concern	Low concern	Low concern	Low concern	Low concern	Low concern
DOMAIN 2: INDEX TEST(S)									
Describe the index test and how it was conducted and interpreted:	CLIA (ADVIA Centaur® HCV assay); no further description	ELISA v3.0 (Abbott Murex anti-HCV)	ELISA v3.0 (Abbott Murex anti-HCV)	ELISA v4.0 (EIAgen, Adaltis)	ELISA v4.0 (Monolisa™, BioRad)	ELISA v3.0 (Innotest HCV Ab III, Innogenetics, Belgium); done "in parallel" with the reference test	CLIA (Vitros Anti-HCV assay, UK)	CMIA (Architect i200SR, Abbott, Germany)	ELISA v4.0 (Monolisa BioRad Ag-Ab Ultra)
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear	Unclear	Unclear	Yes; the EIAgen and Ortho tests were always run "side-by-side"	Yes	Unclear	Unclear	Unclear	Unclear
If a threshold was used, was it pre-specified?	Yes. "The presence or absence of antibodies to HCV is determined by comparing the sample index to the cut-off. Samples with an index value greater than 1.0 are considered to be reactive for anti-HCV."	Unclear; presumably used as per the kit manufacturer's instructions.	Unclear; presumably used as per the kit manufacturer's instructions.	Yes. "Both EIAs yield their final results as ratios of the specimen signal (in relative light units) to the cut-off value (signal-to-cut-off ratio, S/CO). S/CO ratios ≥1.0 were considered reactive for anti-HCV antibodies while those <1.0 were considered nonreactive. Specimen preparation and testing were carried out according to the manufacturers' instructions."	Yes. "The CMIA analysis of anti-HCV is based on the signal to cut-off ration (S/CO). An S/CO value less than 1.00 is classified as negative, and a value higher than 1.00 is classified as positive. Units with ratios in the range of 0.90-1.00 are classified as equivocal and re-analyzed twice."	Unclear; presumably used as per the kit manufacturer's instructions.	Yes. "A signal to cut-off ration (S/CO) greater than 1.0 was regarded as positive."	Yes. "For all assays, S/CO ratios ≥1 were considered as initial reactive (IR)."	Yes. "Samples with an S/CO ratio of ≥1.0 are defined by the manufacturer as positive."
Could the conduct or interpretation of the index test have introduced bias?	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Is there concern that the index test, its conduct, or interpretation differ from the review question?	Low concern	Low concern	Low concern	Low concern	Low concern	Low concern	Low concern	Low concern	Low concern
DOMAIN 3: REFERENCE STANDARD(S)									
Describe the reference standard and how it was conducted and interpreted:	MEIA (Abbott AxSYM® HCV v.3.0 assay) -- detects antibodies only, cannot distinguish between acute, chronic or resolved (~15%) HCV infection	1) LIA (INNo-LIA HCVAb III Update, INNOGENTICS, Belgium) -- cannot differentiate between current and resolved infection; and 2) RT-PCR (High Pure Viral Nucleic Acid reagent set, Roche Molecular Biochemicals) -- can differentiate between current and resolved infection	1) MEIA (Abbott AxSYM® HCV v.3.0 assay) -- cannot differentiate between current and resolved infection; 2) RT-PCR (Roche Amplicor HCV® v2.0) -- able to differentiate between current and resolved infection	ELISA v3.0 (Ortho HCV 3.0 ELISA) -- Cannot differentiate between current and resolved infection	CMIA (Abbott)	NAT (Procleix Ultrio kit, Chiron, USA); done "in parallel" with the index test	ECLIA (Elecsys anti-HCV test, Roche, Germany)	ECLIA (Cobas e411, Roche, Germany)	NAT (Procleix Ultrio kit, Chiron, USA)
Is the reference standard likely to correctly classify the target condition (i.e., HCV infection)?	Yes; but unlike PCR/NAT this reference standard would fail to identify false-positives due to resolved infection	Yes (for PCR)	Yes (for PCR)	Yes; but unlike PCR/NAT this reference standard would fail to identify false-positives due to resolved infection	Yes; but unlike PCR/NAT this reference standard would fail to identify false-positives due to resolved infection	Yes	Yes; but unlike PCR/NAT this reference standard would fail to identify false-positives due to resolved infection	Yes; but unlike PCR/NAT this reference standard would fail to identify false-positives due to resolved infection	Yes
Were the reference standard results interpreted without knowledge of the results of the index test?	Unclear	Unclear	Unclear	Yes; the EIAgen and Ortho tests were always run "side-by-side"	Yes; the subsample of blood units was "blindly re-analyzed" in Norway	Unclear	Unclear	Unclear	Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?	High risk	Unclear risk	Unclear risk	High risk	High risk	Low risk	High risk	High risk	Low risk
Is there concern that the target condition as defined by the reference standard does not match the review question?	Serious concern	Low concern (for PCR)	Low concern (for PCR)	Serious concern	Serious concern	Low concern	Serious concern	Serious concern	Low concern
DOMAIN 4: FLOW AND TIMING									
Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):	2 patients with equivocal positive results were excluded from the authors' specificity calculation, but we will include them	All patients received ELISA, LIA, and RT-PCR.	A subset of 161 ELISA-positives and 100 ELISA-negatives were called back to undergo the reference standard (verification bias); 3/161 lost to follow-up	None	A subset of 80 ELISA-positive and 40 ELISA-negative were selected to undergo the reference test (verification bias)	None	None	None	None
Describe the time interval and any interventions between index test(s) and reference standard:	Not specified; presumably, both tests were applied to the same blood samples	All samples were aliquated into two portions; one was kept at 70°C until processing for RT-PCR and the other was subjected to HCV antibody detection by ELISA and LIA methods	time interval between tests not specified	The EIAgen and Ortho tests were always run "side-by-side"	Both tests were applied to the same blood samples	For each subject, two sets of blood samples were collected: one for serological testing and one for NAT testing	Not specified; presumably, all sera underwent both the index and reference test	Serum samples of the 840 blood donors were tested in parallel using the index and reference tests	All samples were screened with the index and reference tests in parallel; 3 pilot tube samples were collected with each donation - one was used for NAT, another for ELISA
Was there an appropriate interval between index test(s) and reference standard?	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes
Did all patients receive a reference standard?	Yes	Yes	No; verification bias is present	Yes	No; verification bias is present	Yes	Yes	Yes	Yes
Did patients receive the same reference standard?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were all patients included in the analysis?	Yes	Yes	No; 3/161 lost to follow-up	Yes	Yes	Yes	Yes	Yes	Yes
Could the patient flow have introduced bias?	Low risk	Low risk	High risk; the differential sampling of 158/161 (98%) ELISA-positive and 100/8,165 (1%) ELISA-negative persons; Sn and Sp reported in the article are not adjusted for differential sampling leading to large verification bias; this is easily correctable statistically	Low risk	High risk; the differential sampling of 80/176 (45%) ELISA-positive and 40/1,024 (4%) ELISA-negative persons; Sn and Sp reported in the article are not adjusted for differential sampling leading to large verification bias; this is easily correctable statistically	Low risk	Low risk	Low risk	Low risk

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix B
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Appendix D
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Table 5
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	9
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	9

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097