Evaluation of the Xpert HCV Viral Load point-of-care assay from venepuncture-collected and finger-stick capillary whole-blood samples: a cohort study

Jason Grebely, Francois M J Lamoury, Behzad Hajarizadeh, Yasmin Mowat, Alison D Marshall, Sahar Bajis, Philippa Marks, Janaki Amin, Julie Smith, Michael Edwards, Carla Gorton, Nadine Ezard, David Persing, Marika Kleman, Philip Cunningham, Beth Catlett, Gregory J Dore, Tanya L Applegate, on behalf of the LiveRLife Study Group

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The Kirby Institute (J Grebely PhD, F M J Lamoury EiCNAM, B Hajarizadeh PhD, Y Mowat BSc, A D Marshall MA, S Bajis MIPH, P Marks MPH, J Amin PhD, Prof G J Dore PhD, T L Applegate PhD), **and Faculty of Medicine** (N Ezard PhD), **UNSW Sydney, Sydney, NSW, Australia; Department of Health Systems and Populations, Macquarie University, Sydney, NSW, Australia** (J Amin); **Matthew Talbot Hostel, St Vincent de Paul Society NSW Support Services, Sydney, NSW, Australia** (J Smith RN); **South Western Sydney Local Health District, Cabramatta, NSW, Australia** (M Edwards MBBS); **Cairns Sexual Health Service, Cairns, QLD, Australia** (C Gorton MPH); **Alcohol and Drug Service, St Vincent's Hospital, Sydney, NSW, Australia** (N Ezard); **Cepheid, Sunnyvale, CA, USA** (D Persing PhD); **Cepheid AB, Solna, Sweden** (M Kleman PhD); **and St Vincent's Applied Medical Research, Darlinghurst, Sydney, NSW, Australia** (P Cunningham PhD, B Catlett BSc)

Correspondence to: Dr Jason Grebely, The Kirby Institute, UNSW Sydney, Sydney, NSW, Australia **jgrebely@kirby.unsw.edu.au**

Summary

Background **Point-of-care hepatitis C virus (HCV) RNA testing offers an advantage over antibody testing (which only indicates previous exposure), enabling diagnosis of active infection in a single visit. In this study, we evaluated the performance of the Xpert HCV Viral Load assay with venepuncture and finger-stick capillary whole-blood samples.**

Methods **Plasma and finger-stick capillary whole-blood samples were collected from participants in an observational cohort enrolled at five sites in Australia (three drug and alcohol clinics, one homelessness service, and one needle and syringe programme). We compared the sensitivity and specificity of the Xpert HCV Viral Load test for HCV RNA detection by venepuncture and finger-stick collection with the Abbott RealTime HCV Viral Load assay (gold standard).**

Findings **Of 210 participants enrolled between Feb 8, 2016, and July 27, 2016, 150 participants had viral load testing results for the three assays tested. HCV RNA was detected in 45 (30% [95% CI 23–38]) of 150 participants based on Abbott RealTime. Sensitivity of the Xpert HCV Viral Load assay for HCV RNA detection in plasma collected by venepuncture was 100·0% (95% CI 92·0–100·0) and specificity was 99·1% (95% CI 94·9–100·0). Sensitivity of the Xpert HCV Viral Load assay for HCV RNA detection in samples collected by finger-stick was 95·5% (95% CI 84·5–99·4) and specificity was 98·1% (95% CI 93·4–99·8). No adverse events caused by the index test or the reference standard were observed.**

Implications **The Xpert HCV Viral Load test can detect active infection from a finger-stick sample, which represents an advance over antibody-based tests that only indicate past or previous exposure.**

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Introduction

Despite a growing burden of hepatitis C virus (HCV) infection worldwide,[1,2] testing and diagnosis remain inadequate.[3–5] As highlighted by the 2016 WHO guidance on HCV testing,[6] strategies to improve testing and diagnosis of HCV infection are essential to improve linkage to HCV care and treatment with direct-acting antivirals (DAAs).

Strategies to improve HCV testing and diagnosis include on-site HCV testing (via venepuncture),[7–12] dried blood spot testing,[7,12–17] and point-of-care HCV testing.[18–20] Dried blood spot testing from whole blood collected via finger-stick (also referred to as capillary testing) can enhance HCV testing,[6,12] but requires specialised testing to be done at centralised diagnostic laboratories and people to return for a second visit to receive their result, which is a potential barrier in remote areas and in marginalised populations. Finger-stick[21–24] or oral fluid[22–25] rapid diagnostic HCV tests are available, but many of these tests are restricted—ie, they only measure HCV antibodies (previous exposure), not HCV RNA (active infection).[6] Given that 25% of individuals spontaneously clear HCV infection,[26] efforts to enhance diagnosis of chronic HCV infection and improve the HCV care cascade requires enhanced uptake of HCV RNA testing. Point-of-care HCV RNA platforms enabling detection of HCV RNA and diagnosis of active infection in a single visit would be important for clinical use. As highlighted in the WHO guidance on HCV testing,[6] nucleic acid tests to detect HCV RNA that can be used at or near the point of care have become commercially available, and could improve access to early diagnosis, monitoring, and linkage to care and treatment services. However, no previous evaluation of a finger-stick HCV RNA point-of-care test has been published.

In this study, we aimed to establish the sensitivity and specificity of the Xpert HCV Viral Load point-of-care test for detection of HCV RNA by plasma samples collected by venepuncture and capillary whole-blood samples collected by finger-stick in participants attending drug health and homelessness services in Australia.

Methods

Study design and participants

LiveRLife is an open observational cohort study evaluating the effectiveness of an intervention integrating non-invasive liver disease screening on HCV assessment and treatment uptake.[27] Participants were enrolled at five sites in Australia (three drug and alcohol clinics,

Research in context

Evidence before this study

We searched PubMed and Scopus with the search terms "hepatitis C" or "HCV" and "RNA" in combination with "point-of-care" and "point of care" for articles published in English only on Nov 11, 2016, which revealed 54 articles. No date restrictions were used. None of the articles were directly applicable to our research background because they dealt with the development of methods for the detection of hepatitis C virus (HCV) RNA, not the evaluation of an HCV RNA point-of-care test in a cohort study. A systematic review providing guidance on HCV testing published by WHO in October, 2016, highlights that there are currently no field-based evaluations of platforms for point-of-care HCV RNA testing.

Added value of this study

The findings from this study showed good sensitivity and specificity of the Xpert HCV Viral Load test for HCV RNA detection in capillary whole blood collected by finger-stick and plasma collected by venepuncture compared with the Abbott RealTime HCV Viral Load RNA assay in people attending drug health and homelessness services in Australia. To our knowledge, this is the first evaluation of an assay for HCV RNA detection by finger-stick whole-blood collection in a clinical setting.

Implications of all the available evidence

The manufacturer of the assay is using this study to optimise the assay so that the results of the assay will be provided in 60 min, which should lead to the development of a commercially available Xpert HCV Viral Load test for HCV RNA detection in capillary whole blood collected by finger-stick. WHO guidance on HCV testing highlights the importance of such work by stating that nucleic acid test assays to detect HCV RNA that could be used at or near the point of care have become commercially available, and are expected to greatly improve access to early diagnosis, monitoring, and linkage to care and treatment services, as well as reduce loss to follow-up.

one homelessness service, and one needle and syringe programme). The detailed study protocol is provided in the appendix.

Inclusion criteria were age of 18 years or older, written informed consent, and history of injecting drug use in patients attending drug health services (participants recruited from the homelessness service were exempt from this criterion). Current pregnancy was the only exclusion criterion. Participants received an educational resource package at enrolment (eg, LiveRLife campaign coffee mug, liver health promotion and education booklet; appendix) and an AUS$20 voucher at study completion. All participants provided written, informed consent before study procedures began. The study protocol was approved by the Human Research Ethics Committee at St. Vincent's Hospital, Sydney.

Procedures

Participants were provided information about the study while accessing services and consecutively enrolled into the study. Each clinic site had 4 campaign days with typically 1 campaign day per week over 4 weeks. On each LiveRLife campaign day, participants provided informed consent to be enrolled in the study and completed a self-administered survey on tablet computer, received transient elastography assessment (eg, FibroScan), attended a clinical nurse visit for HCV assessment, and provided venepuncture blood samples (for standard-of-care clinical testing and storage for HCV RNA testing).

Finger-stick capillary whole-blood point-of-care HCV RNA testing was added to the study procedures after a protocol amendment approved on Jan 14, 2016. After informed consent was obtained, a capillary whole-blood sample was collected from participants via a finger-stick (MiniCollect Safety Lancet; Greiner Bio-One, Monroe, Frickenhausen, Germany) using procedures recommended by WHO[28] and collected into a 100 µL minivette collection tube (Minivette POCT 100 µL; Sarstedt, Nümbrech, Germany).

See Online for appendix

Immediately after collection, 100 µL of capillary whole blood was placed directly into the Xpert HCV Viral Load cartridge (GXHCV-VL-CE-10; Cepheid, Sunnyvale, CA, USA; lower limit of quantification of 10 IU/mL) followed by the addition of 1 mL buffer (Cepheid, Sunnyvale, CA, USA) without mixing, for on-site HCV RNA testing. The cartridge was then loaded into the GeneXpert instrument, and capillary whole-blood sample volumes of less than 100 µL were recorded. For samples collected via venepuncture, 10 mL venous blood collected in an K_2EDTA (edetic acid) spray-coated collection tube was centrifuged for 20 min at 1500×g and plasma was collected and aliquoted into 1·2 mL fractions. All subsequent Xpert HCV Viral Load and Abbott RealTime HCV Viral Load testing was done on aliquots from the same plasma sample (ie, same storage conditions and no freeze-thaws).

1 mL plasma was placed into the Xpert HCV Viral Load cartridge and loaded into the GeneXpert instrument. Xpert HCV Viral Load testing of capillary whole blood and venous plasma specimens was done on a clinic-based GeneXpert R2 6-colour, four module machine (GXIV-4-L System; Cepheid, Sunnyvale, CA, USA) operated by a trained member of the clinical research team as per the manufacturer's instructions. Data were analysed with GeneXpert Dx software (version 4.6a). The time taken to obtain a result from Xpert HCV Viral Load testing is 108 min. Participants were not provided with the result of their Xpert HCV test results, because the Xpert HCV Viral load test is not approved for clinical use in Australia. Results were provided to clinic staff to inform subsequent clinical follow-up.

HCV RNA viral load was also measured in 0·5 mL stored EDTA plasma samples tested centrally with the

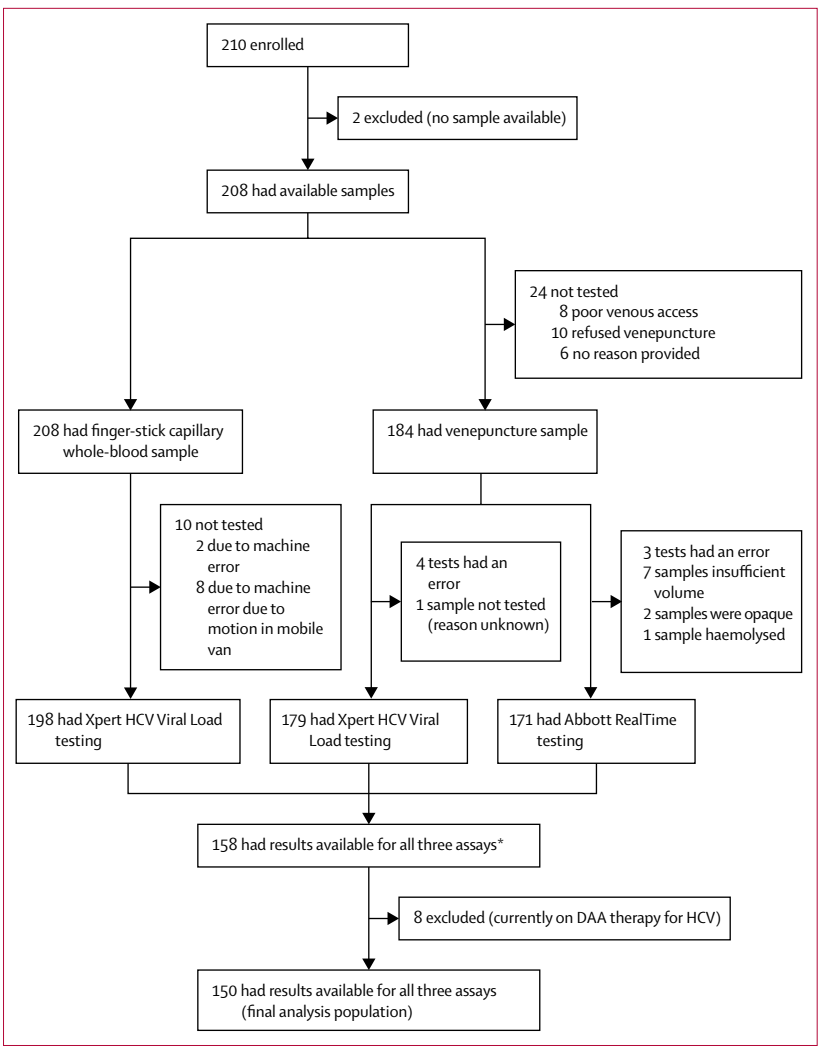

***Figure 1*: Study profile**
DAA=direct-acting antivirals. HCV=hepatitis C virus. *Analysis was restricted to participants in which all three assays were available.

Abbott RealTime HCV Viral Load assay (Abbott Molecular; Des Plaines, IL, USA; kit insert reference 4J86; lower limit of quantification of 12 IU/mL) using the Abbott RealTime System (Abbott Molecular, assay application [version 7]). Samples with discordant HCV RNA results (eg, negative results by Abbott RealTime HCV Viral Load assay and positive by Xpert HCV Viral Load assay) were assessed by in-house TaqMan real-time (rt)PCR assay, as described previously,[29] with modifications as described in the appendix (p 67).

Statistical analysis

We assessed the sensitivity and specificity of the Xpert HCV Viral Load point-of-care test for detection of HCV RNA in plasma samples collected via venepuncture and capillary whole-blood samples collected by finger-stick using both detectable and quantifiable thresholds (limit of quantification >10 IU/mL) for each assay compared with Abbott RealTime HCV Viral Load assay in plasma as the gold standard (limit of quantification >12 IU/mL). Assuming a prevalence of chronic HCV of 30% and a sensitivity and specificity of 100%, 150 samples would provide a 95% CI of 23–38% for the prevalence estimate and a 95% CI of 92·1–100·0% for the estimates of sensitivity and 96·5–100·0% for specificity. We included any discordant results in all calculations of sensitivity and specificity. We generated a Bland–Altman difference plot to assess bias and agreement measurements, including limits of agreement, between the quantification of HCV by Xpert HCV Viral Load with both sample types, compared with the Abbott RealTime HCV Viral Load assay in plasma. All data are reported in $\log_{10}$ units. In the Bland–Altman plot, we used the midpoint between zero and the lower limit of quantification for unquantifiable HCV RNA, whereas those with undetectable HCV RNA were excluded. We reported differences for the Xpert assay results minus the Abbott result. Data were analysed with STATA (version 12.0) and GraphPad Prism (version 7.03).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, or data interpretation. The funders contributed to the writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 210 participants enrolled between Feb 8, 2016, and July 27, 2016, two participants were excluded because they did not have a finger-stick capillary whole-blood or plasma sample available (figure 1). Of 208 participants with an available sample, 208 participants had capillary whole-blood samples collected via finger-stick and 184 participants had plasma samples collected via venepuncture (figures 1, 2). Only the 158 participants who had samples available for all three assays were included in the analysis (figure 1). Of 208 participants with finger-stick capillary whole-blood test results available, 24 (12%) participants had no plasma samples available. 21 plasma samples could not be collected via venepuncture (eight due to poor venous access, ten patients refused to have venepuncture, three for other or not reported reasons) and six were not tested for reasons not provided. In participants who did not have a plasma test, but had available finger-stick capillary whole-blood test, 14 (58%) of 24 were detectable by Xpert HCV Viral Load testing. Of 158 participants with results for all three assays, eight participants were currently on HCV DAA therapy and were excluded from further analyses (figure 1).

In the final analysis population (n=150), the median age was 44 years (IQR 36–52), 130 (87%) were men, 98 (65%) had a history of injecting drug use, and 58 (39%) had injected drugs in the past month (table 1). Overall, 73 (53%) self-reported being HCV-negative and 38 (26%) had

unknown HCV status (table 1). Overall, HCV RNA was detected by Xpert HCV Viral Load in 45 (30% [95% CI 23–38]) of 150 participants.

Of the plasma samples tested, four (2%) of 184 samples did not provide a result on the Xpert HCV Viral Load assay due to error, and three (2%) of 174 samples did not provide a result on the Abbott RealTime HCV Viral Load assay due to error. In the 150 participants with samples available from all three assays, the sensitivity of the Xpert HCV Viral Load assay for HCV RNA detection in plasma collected by venepuncture was 100·0% (95% CI 92·0–100·0) and the specificity was 99·1% (95% CI 94·9–100·0; table 2). In the one plasma sample with a discrepant result for HCV RNA detection, the HCV RNA concentration was 3 380 000 IU/mL when tested by the Xpert HCV Viral Load assay and undetectable when tested by the Abbott RealTime HCV Viral Load assay (table 2). When assessed in-house by a semi-quantitative TaqMan rtPCR assay, this plasma sample was confirmed to contain high concentrations of HCV RNA (indicating a false-negative result by the Abbott RealTime HCV Viral Load assay).

Of the 198 finger-stick capillary samples tested, two (1%) did not provide a result on the Xpert HCV Viral Load assay because of low sample volume in the cartridge. In the 150 participants with samples available from all three assays, the sensitivity of the Xpert HCV Viral Load assay for HCV RNA detection in samples collected by finger-stick capillary whole blood was 95·5% (95% CI 84·5–99·4) and the specificity was 98·1% (95% CI 93·4–99·8; table 2). In the four capillary whole-blood samples with discrepant results, two were detectable when tested by the Abbott RealTime HCV Viral Load assay (<12 IU/mL and 38 IU/mL), but undetectable when tested with the Xpert HCV Viral Load assay, and two were detectable when tested by the Xpert HCV Viral Load assay (<10 IU/mL and 7 686 000 IU/mL), but undetectable when tested with the Abbott RealTime assay.

The sensitivity of the Xpert HCV Viral Load assay for HCV RNA quantification in plasma collected by venepuncture was 97·7% (95% CI 87·7–99·9) and the specificity was 99·1% (95% CI 94·9–100·0; table 3). In the one plasma sample with a discrepant result for HCV RNA quantification, the HCV RNA concentration was less than 10 IU/mL (target detected, but not quantifiable) when tested by the Xpert HCV Viral Load assay and 38 IU/mL when tested by the Abbott RealTime Viral Load assay.

The sensitivity of the Xpert HCV Viral Load assay for HCV RNA quantification in samples collected by finger-stick capillary whole blood was 97·7% (95% CI 87·7–99·9) and the specificity was 99·1% (95% CI 94·9–100·0; table 3). Two samples had discrepant results for HCV RNA quantification with finger-stick capillary whole blood. In the first sample with a discrepant result, the HCV RNA concentration was 7 686 000 IU/mL when tested by the Xpert HCV Viral Load assay and undetectable when tested by the Abbott RealTime assay. In the second sample with a discrepant result, the HCV RNA concentration was undetectable when tested by the Xpert HCV Viral Load assay and 38 IU/mL when tested by the Abbott RealTime assay.

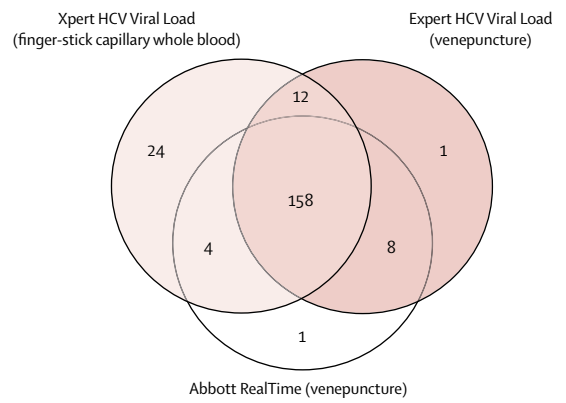

Figure 2: **Number of samples tested with each assay for detection of HCV RNA (n=208)**
Overlap of circles indicates common samples that were tested using the three methods. HCV=hepatitis C virus.

	Participants (n=150)
Gender	
Male	130 (87%)
Female	19 (13%)
Transgender	1 (1%)
Age (years)	44 (36–52)
History of ever injecting drugs	
No	52 (35%)
Yes	98 (65%)
Injecting drug use in the past month	
No	83 (55%)
Yes (less than weekly)	22 (15%)
Yes (more than weekly, but not daily)	20 (13%)
Yes (daily or more)	16 (11%)
Unknown	9 (6%)
Opioid substitution therapy	
No	92 (61%)
Yes (previously)	17 (11%)
Yes (currently)	41 (27%)
Self-reported HCV status	
Negative	73 (53%)
Positive	39 (28%)
Unknown	38 (26%)
Fibrosis stage	
F0–1	104 (69%)
F2	22 (15%)
F3	6 (4%)
F4	11 (7%)
Not available	7 (5%)

Data are n (%) or median (IQR). HCV=hepatitis C virus.

Table 1: **Participant characteristics**

	Detected	Undetected	Total
Xpert HCV Viral Load (plasma)			
Detected	44	1	45
Undetected	0	105	105
Total	44	106	150
Xpert HCV Viral Load (finger-stick capillary whole blood)			
Detected	42	2	44
Undetected	2	104	106
Total	44	106	150

Xpert HCV Viral Load assay lower limit of detection 10 IU/mL; Abbott RealTime lower limit of detection 12 IU/mL. HCV=hepatitis C virus.

Table 2: **Sensitivity and specificity of the Xpert HCV Viral Load assay for HCV RNA detection compared with the Abbott RealTime assay**

	Quantifiable	Unquantifiable	Total
Xpert HCV Viral Load (plasma)			
Quantifiable	42	1	43
Unquantifiable	1	106	107
Total	43	107	150
Xpert HCV Viral Load (finger-stick capillary whole blood)			
Quantifiable	42	1	43
Unquantifiable	1	106	107
Total	43	107	150

Xpert HCV Viral Load assay lower limit of detection 10 IU/mL; Abbott RealTime lower limit of detection 12 IU/mL. HCV=hepatitis C virus.

Table 3: **Sensitivity and specificity of the Xpert HCV Viral Load assay for HCV RNA quantification compared with the Abbott RealTime assay**

As shown by the Bland–Altman plot analysis (figure 3A), HCV RNA concentrations detected by the Xpert HCV Viral Load assay in venepuncture-collected plasma were a mean of 0·04 (SD 0·16) $\log_{10}$ IU/mL higher than those measured by the Abbott RealTime Viral Load assay. The limits of agreement indicate that 95% of the differences between Xpert HCV Viral Load assay and the Abbott RealTime Viral Load assay are between –0·28 and 0·35 $\log_{10}$ IU/mL. The HCV RNA concentrations detected by the Xpert HCV Viral Load assay in finger-stick capillary whole blood were a mean 0·03 (SD 0·27) $\log_{10}$ IU/mL lower than those measured by the Abbott RealTime HCV Viral Load assay (figure 3B). The limits of agreement indicate that 95% of the differences between Xpert HCV Viral Load assay and the Abbott RealTime Viral Load assay are between –0·57 and 0·51 $\log_{10}$ IU/mL.

In the eight participants currently on HCV therapy, the sensitivity of the Xpert HCV Viral Load assay in plasma collected by venepuncture for HCV RNA detection was 100·0% (95% CI 47·8–100·0) and the specificity was 100·0% (95% CI 29·2–100·0). The sensitivity for HCV RNA quantification in those patients was 100·0% (95% CI 39·8–100·0) and the specificity was 100·0% (95% CI 39·8–100·0). In patients on DAA therapy, the sensitivity of the Xpert HCV Viral Load assay in samples collected by finger-stick capillary whole blood for HCV RNA detection was 100·0% (95% CI 47·8–100·0) and specificity was 100·0% (95% CI 29·2–100·0), and for HCV RNA quantification sensitivity was 80·0% (95% CI 28·4–99·5) and the specificity was 100·0% (95% CI 29·2–100·0). In the only sample with a discrepant result in these patients, the HCV RNA concentration was 220 IU/mL when tested by the Xpert HCV Viral Load assay by finger-stick capillary whole blood, and detectable but unquantifiable (<12 IU/mL), when tested by the Abbott RealTime assay.

No adverse events caused by the index test or the reference standard were observed.

Discussion

In this study, we showed good sensitivity and specificity of the Xpert HCV Viral Load test for HCV RNA detection in capillary whole blood collected by finger-stick and plasma collected by venepuncture compared with the Abbott RealTime HCV Viral Load RNA assay in people attending drug health and homelessness services in Australia. The major advance of this point-of-care assay over previous antibody tests, which only indicate HCV exposure, is the ability to detect active HCV infection. These findings also provide support for further evaluation of the Xpert HCV Viral Load test for HCV RNA detection by finger-stick whole-blood collection as a strategy to improve linkage to on-site HCV testing, care, and treatment, by simplifying sample collection.

Although there were four discrepant results when comparing the Xpert HCV Viral Load test for HCV RNA detection by finger-stick whole-blood collection with the Abbott RealTime HCV Viral Load assay, most discrepancies would not have been clinically meaningful. In the discordant result with undetectable HCV by Abbott RealTime HCV Viral Load and high HCV RNA levels by the Xpert HCV Viral Load test, high plasma HCV RNA levels were confirmed using an in-house semi-quantitative RNA assay, suggesting a false-negative result. Also, with the three other discrepant results, RNA was undetectable by one assay and detectable, but not quantifiable in two cases or detectable at very low levels (38 IU/mL) and undetectable. Our results are consistent with previous studies comparing the Xpert HCV Viral Load test for HCV RNA detection by whole-blood collection with the Abbott RealTime HCV Viral Load assay.[30,31] Additionally, only 1% of samples tested did not provide a result on the Xpert HCV Viral Load assay because of low sample volume in the cartridge (all sample volumes <100 µL were recorded). Although several finger-stick and oral fluid rapid diagnostic tests for HCV antibody testing are available, these tests only measure HCV antibodies (previous exposure) and not HCV RNA (active infection), and vary in sensitivity (79–97%) and specificity (80–100%).[21–25] The future role of quantitative HCV RNA data in clinical management remains uncertain; however, the Xpert HCV Viral Load assay showed strong agreement with the Abbott RealTime HCV Viral Load assay with 0·3 $\log_{10}$ IU/mL or lower

difference between 95% limits of agreement of all measurements across all concentrations tested. However, more data are needed to establish the degree of agreement at low concentrations to assess the implications for patients who are on treatment or who have been recently infected. To our knowledge, this is the first evaluation of an on-site point-of-care finger-stick capillary whole-blood collection test for HCV RNA detection in a clinical setting. The results from this study are encouraging, given that the performance of rapid diagnostic tests in clinical settings is poorer than in the laboratory.[21–25] As such, this study is novel and adds considerably to the literature in this area.

Sensitive HCV RNA testing of whole blood collected by finger-stick is particularly appropriate for populations with a high prevalence of HCV infection, such as people attending drug-related health services (eg, drug and alcohol clinics, needle and syringe programmes), and homelessness services. First, people who inject drugs often have poor venous access as a result of injecting, making the collection of blood via venepuncture very difficult. In this study, 24 (12%) participants either refused to have a venepuncture blood sample collected or could not undergo venepuncture because of poor venous access. Of those who were tested for HCV RNA on whole blood collected by finger-stick, 58% had detectable HCV RNA. Second, data have shown that on-site HCV testing with integrated care improves linkage to HCV care.[10] Given that HCV testing and diagnosis remains inadequate in many countries worldwide,[5] novel strategies to improve testing are needed, particularly in people who inject drugs and marginalised populations.

This study has several limitations. Although we acknowledge that the sample size is a limitation, the sensitivity and specificity in this study was good. However, validation studies are needed to further evaluate the performance of this assay in different settings and populations (eg, patients given DAA therapy, those with a sustained virological response, or those with HIV/HCV co-infection). Further studies to assess the reproducibility of the outcomes observed in this study are crucial. As is common with observational cohort studies, a selection bias in participants enrolled in this study is possible (particularly those more engaged in health services and perhaps those more likely to be HCV RNA negative). This bias could have led to a greater sensitivity and specificity than might be observed in a population with a higher HCV RNA prevalence. The time to result was 108 min, which is not ideal. However, a modified assay Xpert HCV Viral Load assay is under development with a time to result of 60 min. This shorter time to result should improve the use of this assay by allowing testing and diagnosis in a single visit. Further research is also needed to evaluate the cost-effectiveness of Xpert HCV Viral Load testing in different settings. However, given the paucity of data on point-of-care assays for the detection of HCV RNA, a finger-stick HCV RNA test might prove to be an important tool for improving HCV testing or diagnosis, particularly in people who inject drugs.

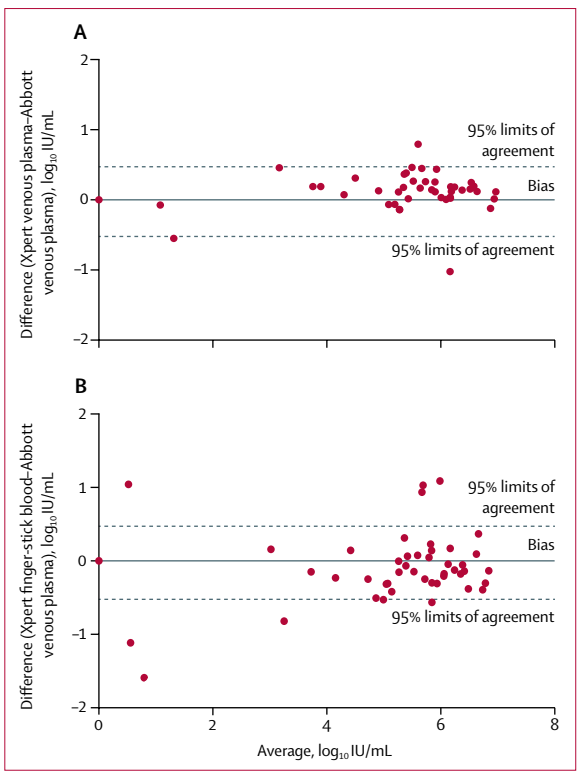

Figure 3: **Bland–Altman bias plot of differences**
(A) Xpert HCV Viral Load assay for HCV RNA detection in plasma samples compared with the Abbott RealTime assay in plasma (one discrepant undetectable result excluded from analysis); n=149, bias −0·0357, 95% limits of agreement −0·28 to 0·35. (B) Xpert HCV Viral Load assay for HCV RNA detection in finger-stick capillary whole-blood samples compared with the Abbott RealTime assay in plasma (one discrepant undetectable result excluded from analysis); n=149, bias −0·0028, 95% limits of agreement −0·57 to 0·51. HCV=hepatitis C virus.

In conclusion, our data show good sensitivity and specificity of the Xpert HCV Viral Load test for HCV RNA detection by finger-stick capillary whole-blood-collection assay in people attending drug health and homelessness services. The Xpert HCV Viral Load test with finger-stick capillary whole-blood collection should be further evaluated as a potential assay to screen for HCV RNA detection, especially in settings with high HCV prevalence or in services for people who inject drugs (eg, drug and alcohol clinics and needle and syringe programmes). This study highlights the importance of further assay development for the rapid detection of HCV RNA to improve testing, diagnosis, linkage to care, and DAA therapy in people living with HCV worldwide, particularly in people who inject drugs.

Contributors
JG, FMJL, BH, GJD, and TLA contributed to the study design. JG, GJD, and TLA were the study investigators. JG, FMJL, BH, YM, ADM, SB, JS, ME, CG, NE, GJD, and TLA contributed to the study implementation and study conduct. FMJL, PC, BC, and TLA contributed to the laboratory work. JG, FMJL, BH, JA, GJD, and TLA contributed to the data interpretation. PM contributed to the study implementation and study conduct. DP and MK provided technical assistance relating to the development of the cartridges and in the application of the cartridges in this study. All authors contributed to the writing and review of the report.

Declaration of interests
JG is a consultant and adviser and has received research grants from AbbVie, Bristol-Myers Squibb, Cepheid, Gilead Sciences, and Merck MSD. GJD is a consultant and adviser and has received research grants from Abbvie, Abbot Diagnostics, Bristol-Myers Squibb, Cepheid, Gilead Sciences, GlaxoSmithKline, Merck, Janssen, and Roche. TLA is a consultant and adviser for Cepheid and has received research grants from Abbott Diagnostics and Cepheid. DP and MK are employees and have equity interests in Cepheid. All other authors declare no competing interests.

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