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Evaluation of the Xpert HCV Viral Load Finger-Stick Point-of-Care Assay

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Point-of-care hepatitis C virus (HCV) RNA testing is advantageous, enabling diagnosis of active infection in a single visit. This study evaluated the sensitivity and specificity of the Xpert HCV Viral Load Finger-Stick assay (Xpert HCV VL FS) for HCV RNA detection (finger-stick) and the Xpert HCV Viral Load assay (plasma) compared with the Abbott RealTime HCV Viral Load assay by venepuncture. Plasma and finger-stick capillary whole-blood samples were collected from participants in an observational cohort in Australia. Of 223 participants enrolled, HCV RNA was detected in 40% of participants (85 of 210) with available Xpert HCV Viral Load testing. Participants receiving HCV therapy were excluded from subsequent analyses (n = 16). Sensitivity of the Xpert HCV Viral Load assay for HCV RNA quantification in plasma collected by venepuncture was 100.0% (95% confidence interval [CI] 96.9%–100.0%) and specificity was 100.0% (95% CI, 94.4%–100.0%). Sensitivity of the Xpert HCV VL FS assay for HCV RNA quantification in samples collected by finger-stick was 100.0% (95% CI, 93.9%–100.0%) and specificity was 100.0% (95% CI, 96.6%–100.0%). The Xpert HCV VL FS test can accurately detect active infection from a finger-stick sample in 1 hour allowing single-visit HCV diagnosis.

Keywords. testing; diagnostics; rapid; PWID; HCV.

Globally, 71 million people are living with hepatitis C virus (HCV) infection [1, 2]. Despite the availability of tolerable and effective direct-acting antiviral therapies (DAA), only 20% of this population has been diagnosed [3–8]. The World Health Organization (WHO) has set a goal of eliminating HCV as a major global public health threat by 2030, including increasing diagnoses to 90% and the proportion of eligible persons receiving treatment from <10% to 80% [9]. However, gaps remain in the availability of simple, reliable and affordable HCV testing strategies [8].

The current HCV testing algorithm involves detection of HCV antibodies to confirm previous exposure, followed by HCV RNA testing to detect active infection. This 2-step diagnostic pathway requires multiple visits to a practitioner (and off-site phlebotomists) leading to a drop-off in those who receive a HCV RNA diagnosis [10–15]. Simple, accurate, and cost-effective testing strategies are needed to improve HCV screening and diagnosis.

Point-of-care HCV testing has been shown to increase testing [16–19] and linkage to care [18–20] and can include oral fluid rapid diagnostic testing [21–24], finger-stick whole-blood rapid diagnostic testing [21–23], dried blood spot testing [17, 25, 26], and on-site venepuncture-based testing [27, 28]. Many currently available point-of-care tests only measure HCV antibodies, not HCV RNA. Approved point-of-care HCV RNA assays require venepuncture, which is challenging in settings without access to phlebotomists or among people who inject drugs (PWID), due to poor venous access [29]. Further work is needed to evaluate point-of-care tests allowing HCV testing, diagnosis, and treatment to occur in a single clinical encounter.

Previously, a research-use-only (RUO) version of the Xpert HCV Viral Load assay was evaluated using whole blood collected by finger-stick, which was diluted (1 mL buffer) and then loaded into a Conformité Européene certification (CE-marked) plasma cartridge [30]. The sensitivity and specificity in the RUO protocol for HCV RNA quantification was 97.7% (95% confidence interval [CI] 87.7%–99.9%) and 99.1% (95% CI, 94.9%–100.0%), respectively [30]. However, finger-stick whole-blood samples were tested using the existing Xpert HCV Viral Load assay and the time from sample collection to result using this cartridge and protocol was 2 hours, which is not ideal for a single-visit diagnosis.

Recently, Cepheid, in collaboration with the Foundation for Innovative New Diagnostics, developed a new dedicated and redesigned Xpert HCV Viral Load Finger-stick (Xpert HCV VL FS) point-of-care test that can be performed with 100 µL of capillary whole blood and provide test results in 1 hour. This study

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determined the sensitivity and specificity of the Xpert HCV VL FS point-of-care test for HCV RNA quantification from capillary whole blood collected by finger-stick compared with the Abbott RealTime HCV Viral Load assay by venepuncture among participants attending drug treatment and homelessness services in Australia.

METHODS

Study Participants

LiveRLife is an observational cohort study evaluating the effectiveness of an intervention integrating noninvasive liver disease screening on HCV assessment and treatment uptake [31]. Between 3 August 2016 and 13 December 2016 participants were enrolled at 3 drug treatment clinics and 1 homelessness service in Australia. The study protocol is provided in the Supplementary Material.

Inclusion criteria were ≥18 years of age, written informed consent, and a history of injecting drug use (participants recruited from the homelessness service were exempt from this criteria). Participants received a AU$20 voucher for their participation. The study protocol was approved by St. Vincent's Hospital, Sydney Human Research Ethics Committee.

Study Design, Intervention, and Study Assessments

Participants were provided information about the study while accessing services and consecutively enrolled into the study. Each clinic site held 4 campaign days. At enrollment, the following data and samples were collected: a finger-stick capillary whole-blood sample (100 µL for Xpert HCV VL FS assay), a venepuncture blood sample (standard of care clinical testing and storage for HCV RNA testing), a self-administered survey on tablet computer (sociodemographic characteristics, drug use, liver and HCV knowledge), liver stiffness measurement by transient elastography (FibroScan), and a clinical HCV assessment (performed by a nurse).

A capillary whole-blood sample was collected from participants via a finger-stick (Safety Lancet, Super Blade [Order Number 85.1018], Sarstedt, Nümbrecht, Germany) using procedures recommended by the WHO [32] and collected into a 100-µL minivette collection tube (Minivette POCT 100µl K3E [Order number 17.2113.101], Sarstedt, Nümbrecht, Germany).

Immediately following collection, 100 µL of capillary whole blood was placed directly into the Xpert HCV VL FS assay prototype cartridge (research use only, lower limit of quantification of 100 IU/mL; Cepheid, Sunnyvale) for on-site HCV RNA testing. The cartridge was loaded into the GeneXpert instrument. The time to result for Xpert HCV VL FS testing was 60 minutes. For samples collected via venepuncture, 10 mL ethylenediaminetetraacetic acid (EDTA) venous blood was centrifuged for 20 minutes at 1500*g*, at room temperature, plasma collected, and aliquoted into 1.2 mL fractions. All subsequent Xpert HCV Viral Load assay (GXHCV-VL-CE-10) and Abbott RealTime HCV Viral Load testing was performed on aliquots from the same plasma sample.

Plasma (1 mL) was placed into the Xpert HCV Viral Load cartridge (GXHCV-VL-CE-10; Cepheid, Sunnyvale) and loaded into the GeneXpert instrument the following day. All Xpert HCV Viral Load assay testing were performed on a clinic-based GeneXpert R2 6-colour, 4-module machine (GXIV-4-L System, 900-0513, GeneXpert Dx software v4.6a) operated by a trained member of the clinical research team as per the manufacturer's instructions [33]. The time to result for Xpert HCV Viral Load testing is 108 minutes. Participants were not provided the result of their Xpert HCV test results, given that the Xpert HCV Viral Load assay is not approved in Australia. Results were provided to clinic staff to inform subsequent clinical follow-up.

HCV RNA levels were also measured on 0.5 mL stored EDTA plasma samples (stored from the time of collection at −80°C), which were batch tested centrally with the Abbott RealTime HCV Viral Load assay (Abbott Molecular, kit insert reference 4J86, 51-602124/R9, lower limit of quantification of 12 IU/mL) performed on the Abbott RealTime System (Abbott Molecular, assay application v7).

Statistical Analysis

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 was assessed using both detectable and quantifiable thresholds (limit of quantification >10 IU/mL and limit of detection >4 IU/mL for Xpert HCV Viral Load assay, and limit of quantification >100 IU/mL and limit of detection >40 IU/mL for Xpert HCV VL FS) compared to Abbott RealTime HCV Viral Load assay in plasma as the reference standard (limit of quantification >12 IU/mL). Assuming a chronic HCV prevalence of 30% and a sensitivity/specificity of 100%, 150 samples would provide a 95% CI of ±8% for the prevalence estimate and ±4% for the estimates of sensitivity/specificity. Any discordant results were included in all calculations of sensitivity and specificity. A Bland-Altman difference plot was generated to assess bias and agreement measurements, including limits of agreement, between the quantification of HCV by Xpert HCV Viral Load assay and the Xpert HCV VL FS assay, compared to the Abbott RealTime HCV Viral Load assay in plasma. All data are reported in $\log_{10}$ units. In the Bland–Altman plot, the midpoint between zero and the lower limit of quantification was used for unquantifiable HCV RNA, while those with undetectable HCV RNA were excluded. Differences were reported for each Xpert assay result minus the Abbott result.

RESULTS

Among 223 participants enrolled between 3 August and 13 December 2016 all participants had a finger-stick whole-blood sample available (Figure 1). Among those with a finger-stick whole-blood sample (n = 223), 205 had Xpert HCV VL FS testing and 18 (8%) had no valid result (n = 7, errors due to low sample volume; n = 11, errors due to the internal control being out of range).

Among those enrolled (n = 223), 212 had a venepuncture sample, while 11 (4%) participants did not have a venepuncture

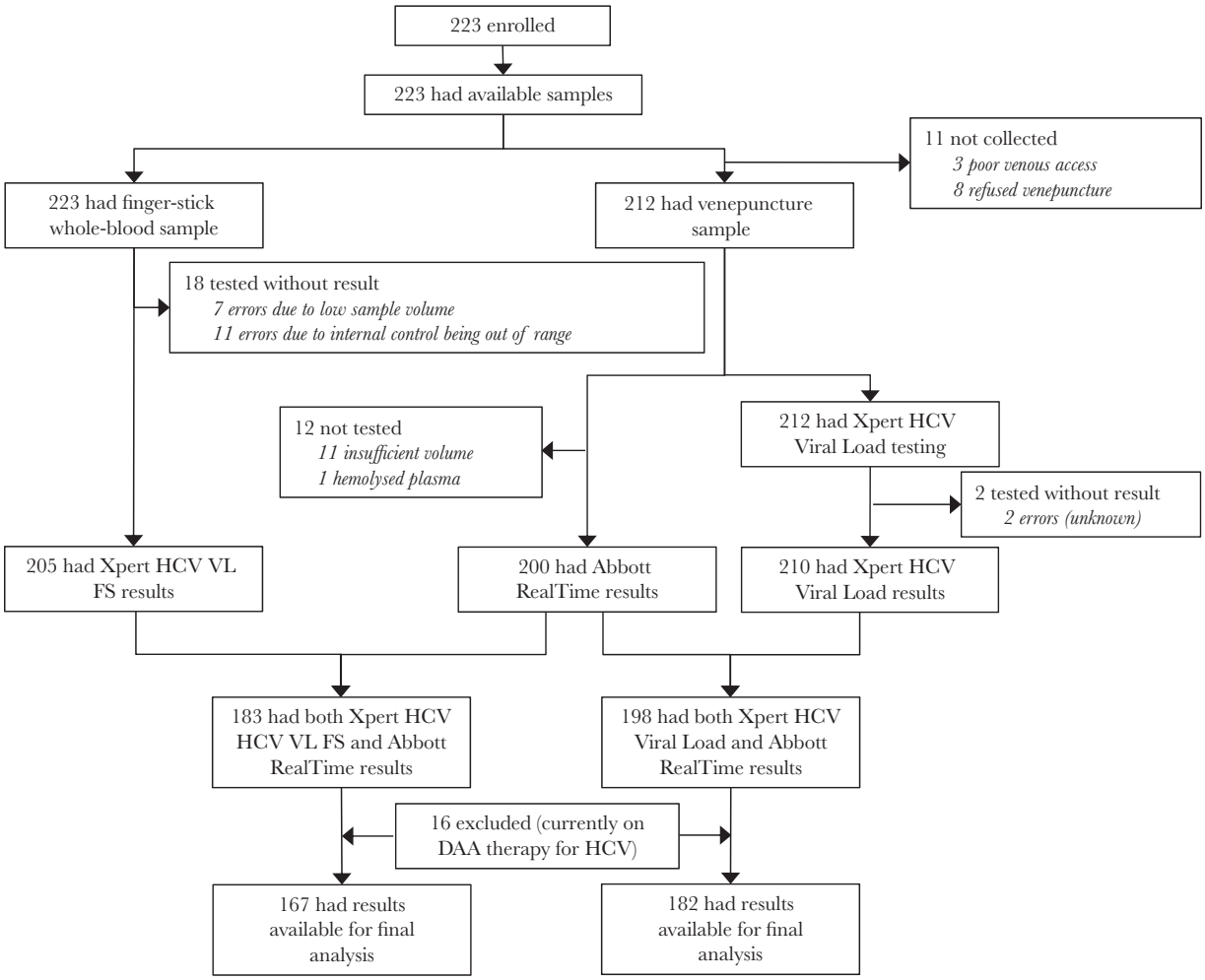

Figure 1. Participant disposition. Abbreviations: DAA, direct-acting antiviral therapy; HCV, hepatitis C virus; Xpert HCV VL FS, Xpert HCV Viral Load Finger-Stick.

sample collected (n = 3, poor venous access; n = 8 refused venepuncture). Among 212 with a venepuncture sample, 210 had Xpert HCV Viral Load testing results available (2 tests with unknown errors, 1% error) and 200 had Abbott RealTime testing results available (12 not tested, including 11 samples with insufficient volume for testing and 1 hemolysed plasma sample). Among 10 participants who did not have any plasma test result, but had available finger-stick whole-blood test results, 5 (50%) were detectable by Xpert HCV VL FS testing.

Among all enrolled participants (n = 223), the median age was 44 years, 80% (n = 178) were male, 72% (n = 160) had a history of injecting drug use, and 46% (n = 102) had injected drugs in the last month (Table 1). HCV RNA was detected in 40% (85 of 210) of participants with available Xpert HCV Viral Load testing. Overall, 16 participants (n = 7%) were receiving DAA therapy and were excluded from subsequent analyses.

Among 167 participants with samples available for a comparison of the Xpert HCV VL FS and Abbott RealTime HCV Viral Load assays, the sensitivity of the Xpert HCV VL FS assay for HCV RNA quantification in capillary whole-blood samples collected by finger-stick was 100.0% (95% CI, 93.9%–100.0%) and specificity was 100.0% (95% CI, 96.6%–100.0%; Table 2). The sensitivity of the Xpert HCV VL FS assay for HCV RNA detection in capillary whole-blood samples collected by finger-stick was 98.3% (95% CI, 91.1%–100.0%) and the specificity was 100.0% (95% CI, 96.6%–100.0%; Table 3). One sample demonstrated a discrepant result: HCV RNA was not detected by Xpert HCV VL FS assay while it was detected but below the limit of quantification (<12 IU/mL) by the Abbott RealTime assay.

Among 182 participants with samples available for a comparison of the Xpert HCV Viral Load assay and the Abbott RealTime HCV Viral Load assay, the sensitivity of the Xpert HCV Viral Load assay for HCV RNA quantification in plasma was 100.0% (95% CI, 96.9%–100.0%) and specificity was 100.0% (95% CI, 94.4%–100.0%; Table 2). The sensitivity of the Xpert HCV Viral Load assay for HCV RNA detection in plasma samples was 100.0% (95% CI, 94.5%–100.0%) and the specificity was 98.3% (95% CI, 94.0%–99.8%; Table 3). Two samples showed a discrepant test result: both samples had

Table 1. Enrolment Characteristics (n = 223)

Characteristic	N (%)
Age, median (25%, 75%)	44 (38, 52)
Gender	
Male	178 (80)
Female	34 (15)
Transgender	2 (1)
Unknown[a]	9 (4)
History of ever injecting drugs	
No	46 (21)
Yes	160 (72)
Unknown[a]	17 (7)
Injecting drug use in the last month	
No	85 (38)
Yes	102 (46)
Unknown[a]	36 (16)
Frequency of drug use in the last month[b]	
None	39 (24)
Less than weekly	28 (18)
More than weekly, but not daily	41 (26)
Daily or more	33 (21)
Unknown[a]	19 (16)
Opioid substitution therapy	
No	91 (40)
Yes, previously	17 (8)
Yes, currently	98 (44)
Unknown[a]	17 (8)
FibroScan liver disease stage	
F0–1	148 (66)
F2	35 (16)
F3	7 (3)
F4	13 (6)
Invalid score	15 (7)
Not performed	5 (2)
Receiving HCV treatment	16 (7)

[a]Missing due to loss of data during data transfer from tablet computer and 1 stolen tablet.
[b]Among participants with a history of injection drug use (n = 160).

detectable HCV RNA but below the lower limit of quantification (<10 IU/mL) in the plasma Xpert HCV Load assay, which was not detected.

As shown by the Bland-Altman plot analysis (Figure 2), HCV RNA concentrations detected by the Xpert HCV Viral Load assay in venepuncture-collected plasma were a mean of 0.02 (standard deviation [SD] 0.15) $\log_{10}$ 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 −0.27 and 0.30 $\log_{10}$ IU/mL. The HCV RNA concentrations detected by the Xpert HCV VL FS assay in finger-stick capillary whole blood were a mean of −0.07 (SD 0.25) $\log_{10}$ IU/mL lower than those measured by the Abbott RealTime Viral Load assay. The limits of agreement indicate that 95% of the differences between Xpert HCV VL assay and the Abbott RealTime Viral Load assay are between −0.56 and 0.42 $\log_{10}$ IU/mL.

The sensitivity and specificity of the Xpert HCV VL FS assay and Xpert HCV Viral Load assay compared to the Abbott RealTime Viral Load assay for HCV RNA quantification and detection in the total population (not including those receiving DAA therapy) are shown in Supplementary Tables 1 and 2. Among the 16 participants currently receiving HCV therapy, the sensitivity of the Xpert HCV VL FS assay for HCV RNA quantification was 100.0% (95% CI, 66.4%–100.0%; Supplementary Table 3) and the specificity was 100% (95% CI, 59.0%–100.0%; Supplementary Table 3). The sensitivity of the Xpert HCV VL FS assay for HCV RNA detection in capillary whole-blood samples collected by finger-stick was 81.8% (95% CI, 48.2%–97.7%) and the specificity was 100.0% (95% CI, 47.8%–100.0%; Supplementary Table 4). Among the 16 participants currently receiving HCV therapy, the sensitivity of the Xpert HCV Viral Load assay for HCV RNA quantification in plasma was 100.0% (95% CI, 66.4%–100.0%; Supplementary Table 3) and the specificity was 100% (95% CI, 59.0%–100.0%; Supplementary Table 3). The sensitivity of the Xpert HCV Viral Load assay for HCV RNA detection in plasma was 100.0% (95% CI, 71.5%–100.0%) and the specificity was 100.0% (95% CI, 47.8%–100.0%; Supplementary Table 4).

DISCUSSION

This is the first study to evaluate the sensitivity and specificity of a redesigned prototype Xpert Finger-stick HCV Viral Load assay for HCV RNA quantitation in capillary whole blood collected by finger-stick with results in 1 hour. This study demonstrated a high degree of correlation for both qualitative and quantitative detection compared to the Abbott RealTime HCV Viral Load RNA assay. This provides a major advance over antibody-based point-of-care tests, which only indicate HCV exposure. Further, the novel Xpert HCV VL FS assay provides a substantial advance over the Xpert HCV Viral Load assay,

Table 2. Sensitivity and Specificity of the Xpert HCV VL FS and Xpert HCV Viral Load Assays for HCV RNA Quantification Compared to the Abbott RealTime HCV Viral Load Assay

	Abbott RealTime HCV Viral Load		
	No. of Quantifiable	No. of Unquantifiable	No. of Total
Xpert HCV VL FS (finger-stick)			
No. of quantifiable	59	0	59
No. of unquantifiable	0	108	108
No. of total	59	108	167
Xpert HCV Viral Load (plasma)			
No. of quantifiable	64	0	64
No. of unquantifiable	0	118	118
No. of total	64	118	182

Xpert HCV Viral Load assay lower limit of quantification 10 IU/mL; Xpert HCV VL FS assay lower limit of quantification 100 IU/mL; Abbott RealTime HCV Viral Load assay lower limit of quantification 12 IU/mL.

Abbreviation: Xpert HCV VL FS, Xpert hepatitis C virus viral load finger-stick.

Table 3. Sensitivity and Specificity of the Xpert HCV VL FS and Xpert HCV Viral Load Assays for HCV RNA Detection Compared to the Abbott RealTime HCV Viral Load assay

	Abbott RealTime HCV Viral Load		
	No. of Detectable	No. of Undetectable	No. of Total
Xpert HCV VL FS (finger-stick)			
No. of detectable	59	0	59
No. of undetectable	1	107	108
No. of total	60	107	167
Xpert HCV Viral Load (plasma)			
No. of detectable	65	2	67
No. of undetectable	0	115	115
No. of total	65	117	182

Xpert HCV Viral Load assay lower limit of detection IU/mL; Xpert HCV VL FS assay lower limit of detection 40 IU/mL; Abbott RealTime HCV Viral Load assay lower limit of detection 12 IU/mL.

Abbreviation: Xpert HCV VL FS, Xpert hepatitis C virus viral load finger-stick.

avoiding the need for plasma separation and enabling testing and diagnosis in 1 hour as compared to 2 hours, increasing the potential to move towards a single-visit diagnosis. Further work is needed to evaluate the impact and cost-effectiveness of finger-stick point-of-care HCV RNA testing as a strategy to enhance HCV testing, linkage to care, and treatment.

The sensitivity and specificity of the Xpert HCV VL FS test for HCV RNA quantification by finger-stick whole blood was 100%. This is higher than previous studies reported with the Xpert HCV Viral Load assay and plasma samples [28, 34] and diluted finger-stick blood, which provided a time to result of 2 hours [30]. The 98% sensitivity and specificity at 100% of the Xpert HCV VL FS test for HCV RNA detection by finger-stick whole blood was lower than the Xpert HCV Viral Load assay. However, the 1 discrepant sample that was not detected by the Xpert HCV VL FS assay was detected, but below the limit of quantification (<12 IU/mL), by the Abbott RealTime assay and is therefore not likely to be clinically meaningful. The Xpert HCV Viral Load assay for plasma also demonstrated a strong agreement with the Abbott RealTime HCV Viral Load assay with ≤0.02 log IU/mL different between 95% of all measurements across all concentrations tested. Lastly, among the samples tested, 8% failed to provide a result on the Xpert HCV VL FS assay due to errors (sampling issue) and invalid results (internal control out of range mostly due to early prototype). As such, the reported sensitivity and specificity reflects the optimal performance of the assay in the absence of any errors. The current evaluation was conducted on an early prototype of the HCV VL FS cartridge, so efforts are being undertaken to reduce the number of errors and repeats tests. Further evaluation of the most recent prototype of the HCV VL FS cartridge is needed to ensure that the errors and invalid results do not preclude broader implementation of this technology.

The results from this study have the potential to considerably change the clinical management of HCV infection. In studies from Australia, Canada, and the United States, among people testing anti-HCV antibody positive, only 46%–73% of people received confirmatory HCV RNA testing [10–15]. The ability to test for HCV RNA from finger-stick whole blood provides a major advance in HCV diagnostic testing, given that available point-of-care assays that can be performed from capillary whole blood detect HCV antibodies (previous exposure with clearance) [21–23] and not HCV RNA (active infection). Point-of-care testing from capillary blood samples avoids the need for phlebotomy, a major advantage where venous access is difficult (eg, PWID) or where phlebotomy services are unavailable. Point-of-care testing also has the potential to reduce nonattendance to off-site phlebotomy, and provides more immediate results to facilitate enhanced counseling, education, and linkage to care. This is particularly useful for remote/rural and outreach settings, and

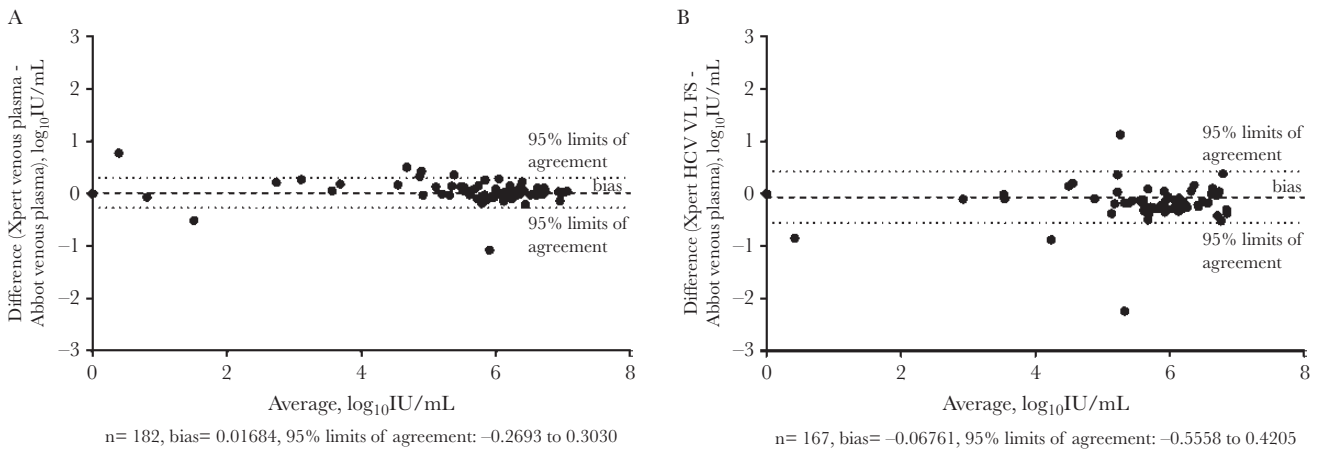

Figure 2. Bland-Altman bias plot of differences. *A,* Xpert HCV Viral Load assay for HCV RNA quantification in plasma samples compared with the Abbott RealTime assay in plasma. *B,* Xpert HCV Viral Load Finger-Stick (Xpert HCV VL FS) assay for HCV RNA quantification in finger-stick whole-blood samples compared with the Abbott RealTime assay in plasma.

in low- and middle-income countries. Furthermore, the evaluation of the novel point-of-care Xpert HCV VL FS assay for blood with a time to result of 1 hour is a substantial improvement over the parent Xpert HCV Viral Load plasma assay, which was in a point-of-care setting to test diluted finger-stick blood with a time to result of 108 minutes [30]. Also, no sample dilution is needed, which simplifies use. This will allow a one-visit diagnosis of HCV infection, addressing the considerable drop-off in the number of people who are HCV antibody positive but are not subsequently tested for HCV RNA. The results from this study are extremely encouraging, given that the performance of rapid diagnostic tests in the field is poorer than in the laboratory [21–23]. As such, this study is novel and adds to the literature in this area.

Finger-stick HCV RNA testing will be particularly useful for enhancing testing in PWID. PWID may have poor venous access from injecting drug use [29], so finger-stick testing is highly acceptable to both the patient and health care provider [35, 36]. In this study, 5% (n = 11) participants either refused to have venepuncture or venepuncture was unsuccessful due to poor venous access. Among those who were tested on whole blood collected by finger-stick, 35% had detectable HCV RNA. Further, data have demonstrated that point-of-care testing increased uptake of HCV testing [16–19] and linkage to HCV care [18–20]. Globally, there is low testing and diagnosis [5], and novel strategies are needed to improve testing in PWID and other marginalized populations.

This study has several limitations. First, this is a prototype cartridge and further validation studies using this final design are needed. A larger sample size would have provided further confidence in the reported sensitivity and specificity. In particular, the data on the sensitivity and specificity among people receiving DAA therapy should be interpreted with caution, given the very small sample size (16 participants). Validation studies in larger sample sizes are needed to include populations from different geographic regions with different HCV genotypes, those with HIV/HBV coinfection, and those receiving DAA therapy. As is common with observational cohort studies, it is possible that there was a selection bias among participants enrolled in this study (persons more engaged in health services and more likely to be HCV RNA negative). This may have led to greater sensitivity and specificity than might be observed in a population with a higher HCV RNA prevalence. In this present study, the time to result was 60 minutes. Reducing this timeframe may further simplify HCV testing. Also, testing was performed by a trained laboratory technician with expertise using the Xpert HCV VL FS assay. Efforts to expand the implementation of this point-of-care finger-stick HCV RNA assay will require the provision of appropriate education and training for the use of the GeneXpert platform and the Xpert HCV VL FS assay for a diverse mix of health care providers. Lastly, research is needed to evaluate the cost-effectiveness of Xpert HCV VL FS testing in different settings, including a consideration of situations where there may be errors and invalid results.

The integration of point-of-care testing as part of HCV "test and treat" strategies in high-prevalence settings may provide an opportunity to enhance diagnosis, linkage to care, and HCV treatment. This may be particularly useful outside the tertiary care setting, in settings where people who are at risk of infection (eg, PWID) already access health services, such as drug treatment clinics, community health centers, prisons, needle and syringe programs, and supervised consumption rooms. In the period following successful treatment, finger-stick point-of-care HCV RNA testing may be useful tool for monitoring of HCV reinfection among populations at high risk of re-exposure. Further research is needed to explore potential applications of point-of-care HCV RNA testing to improve HCV care.

In conclusion, these data demonstrate a good sensitivity and specificity of the Xpert HCV VL Fingerstick test for HCV RNA quantification among people attending drug health and homelessness services. The Xpert HCV VL FS test should be further evaluated as a screening tool for HCV RNA detection in high-prevalence settings, particularly in services for PWID. In addition to broad DAA uptake, efforts to eliminate HCV as a global public health threat will require strategies to enhance HCV testing and diagnosis globally, including the development of assays for rapid detection of HCV RNA.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. J. G., F. L., B. H., G. J. D., and T. L. A. contributed to the study design. J. G., G. J. D., and T. L. A. were the study investigators. J. G., F. L., B. H., Y. M., A. D. M., S. B., J. S., M. E., C. G., N. E., G. J. D., M. M., and T. L. A. contributed to the study implementation and study conduct. F. L., P. C., B. C., and T. L. A. contributed to the laboratory work. J. G., F. L., S. B., B. H., J. A., G. J. D., and T. L. A. contributed to the data interpretation. All authors contributed to the writing and review of the report.

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