




# Field evaluation of GeneXpert<sup>®</sup> (Cepheid) HCV performance for RNA quantification in a genotype 1 and 6 predominant patient population in Cambodia

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## Abstract

GeneXpert<sup>®</sup> (Cepheid) is the only WHO prequalified platform for hepatitis C virus (HCV) nucleic acid amplification testing that is suitable for point-of-care use in resource-limited contexts. However, its application is constrained by the lack of evidence on genotype 6 (GT6) HCV. We evaluated its field performance among a patient population in Cambodia predominantly infected with GT6. Between August and September 2017, we tested plasma samples obtained from consenting patients at Médecins Sans Frontières' HCV clinic at Preah Kossamak Hospital for HCV viral load (VL) using GeneXpert<sup>®</sup> and compared its results to those obtained using COBAS<sup>®</sup> AmpliPrep/Cobas<sup>®</sup> TaqMan<sup>®</sup> HCV Quantitative Test, v2.0 (Roche) at the Institut Pasteur du Cambodge. Among 769 patients, 77% of the seropositive patients (n = 454/590) had detectable and quantifiable VL using Roche and 43% (n = 195/454) were GT6. The sensitivity and specificity of GeneXpert<sup>®</sup> against Roche were 100% (95% CI 99.2, 100.0) and 98.5% (95% CI 94.8, 99.8). The mean VL difference was -0.01 (95% CI -0.05, 0.02) log<sub>10</sub> IU/mL for 454 samples quantifiable on Roche and -0.07 (95% CI -0.12, -0.02) log<sub>10</sub> IU/mL for GT6 (n = 195). The limit of agreement (LOA) was -0.76 to 0.73 log<sub>10</sub> IU/mL for all GTs and -0.76 to 0.62 log<sub>10</sub> IU/mL for GT6. Twenty-nine GeneXpert<sup>®</sup> results were outside the LOA. Frequency of error and the median turnaround time (TAT) for GeneXpert<sup>®</sup> were 1% and 0 days (4 days using Roche). We demonstrated that the GeneXpert<sup>®</sup> HCV assay has good sensitivity, specificity, quantitative agreement, and TAT in a real-world, resource-limited clinical setting among GT6 HCV patients.

## KEYWORDS

Cambodia, HCV genotype 6, Hepatitis C, Point-of-care testing, RNA viral load

**Abbreviations:** CE-IVD, Conformité Européenne In Vitro Diagnostic Medical Device; DBS, dried-blood spots; EDTA, ethylenediaminetetraacetic acid; GT, genotype; HCV, hepatitis C virus; HCVc-Ag, hepatitis C virus core antigen; HIV, human immunodeficiency virus; IQR, interquartile range; IU/mL, international units per millilitre; LOA, limit of agreement; LOD, limit of detection; LOQ, limit of quantification; NAT, nucleic acid test; MSF, Médecins Sans Frontières; POC, point of care; qRT-PCR, real-time quantitative reverse transcription-polymerase chain reaction; RNA, ribonucleic acid; SD, standard deviation; TAT, turnaround time; VL, viral load; WHO, World Health Organization; 95% CI, 95% confidence interval.

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## 1 | INTRODUCTION

The emergence of novel therapeutics demonstrating high cure rates from chronic hepatitis C virus (HCV) infection has allowed public health leaders to envision the possibility of its global elimination as a public health threat by year 2030.<sup>1</sup> However, the majority of the estimated 71 million infected worldwide are unaware of their status, which constitutes a major challenge to elimination.<sup>2</sup>

The diagnosis of HCV infection is performed by a qualitative or quantitative RNA nucleic acid test (NAT); the latter is commonly referred to as the viral load (VL) test.<sup>3</sup> Most of the laboratory-based NAT platforms, such as Abbott RealTime<sup>®</sup> and Roche Cobas<sup>®</sup> TaqMan<sup>®</sup>, require specialized, centralized laboratories with skilled laboratory technicians<sup>4</sup>; this impedes the decentralization and scale-up of HCV programmes. The requirement of cold-chain transport of blood samples and the need to batch samples before performing the tests mean patients must wait up to a few weeks to receive their results.<sup>5</sup> At-risk patients, especially people who inject drugs, may easily become lost to follow-up during this time. To deliver HCV care to such patients and to support the scale-up of HCV programmes, there is an urgent need for an alternative, reliable point-of-care (POC) HCV RNA test with a short turnaround time (TAT).

GeneXpert<sup>®</sup> HCV VL (Cepheid, hereafter called Xpert) is an automated real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) VL test for HCV that is intended to be used as a POC test for patient management (ie, outside of a central laboratory, near the point of patient care, for the diagnosis and monitoring of treatment response). It has a limit of detection (LOD) of 4.91 (95% confidence interval [CI] 3.17, 10.69) or 0.69 log<sub>10</sub> IU/mL and reports across a range of 10–100 000 000 (1.0–8.0 log<sub>10</sub>) IU/mL.<sup>6</sup> Xpert is a more robust platform than conventional platforms and implementable with minimal laboratory set-up. It allows patients to receive their HCV diagnosis within two hours at the price of \$17.10 per cartridge, which is the preferential pricing for low- and middle-income countries. Although this is an ex-works price, it is nonetheless lower than conventional platforms which cost \$60 to \$95 per VL test in commercial laboratories across Cambodia.<sup>7</sup> Moreover, as part of Cepheid's High Burden Developing Countries Program, the purchase of significant volumes of Xpert cartridges further reduces their price.<sup>4</sup> For example, the price can be reduced from \$17.10 to \$12.45 per cartridge if four million tests are purchased annually, an attainable volume when bundling purchases with other Xpert virological cartridges such as the human immunodeficiency virus (HIV) VL, HIV early infant diagnosis, and human papillomavirus (bundle purchases cannot be made with bacteriological cartridges such as the Xpert MTB/RIF [mycobacterium tuberculosis/rifampicin resistance]). As HCV VL testing may cost up to \$200 in public and even up to \$290 in private sectors in some countries, the Xpert HCV test is a more affordable alternative.<sup>4,7,8</sup>

However, the implementation of Xpert in Southeast Asia is constrained by the lack of evidence on its field performance where genotype 6 (GT6) predominates.<sup>9–11</sup> Evidence used to demonstrate clinical performance in the WHO Prequalification Programme relied predominantly on results from GT1 patients, with the inclusion of only 23 non-GT1 samples.<sup>6</sup> Previous studies evaluating the performance of the Xpert assay have also been conducted in resource-rich countries where the majority of the samples tested were GT1/GT3.<sup>5,12</sup> Non-GT1/GT3 HCV is prevalent in low- and middle-income countries in East and Southeast Asia, North Africa, the Middle East, and Southern sub-Saharan Africa and is disproportionately under-represented in the literature.<sup>5,6,9–12</sup> GT6 is one of the most common genotypes in Southeast Asia and is also the most variable with high genetic heterogeneity compared to other genotypes which may affect the reliability of HCV RNA quantification.<sup>13</sup> This underscores the importance of assessing the field performance of Xpert in resource-limited contexts among GT6 patients prior to using the platform in regions with a high prevalence of this genotype.

We aimed to compare the performance of HCV VL testing using Xpert at a clinic against the Roche HCV VL test performed at a reference laboratory among a GT6 predominant patient population in Cambodia.

## 2 | MATERIALS AND METHODS

### 2.1 | Study setting

The study was conducted at the Médecins Sans Frontières' (MSF) (Doctors Without Borders) HCV clinic located in the Preah Kossamak Hospital in Phnom Penh, Cambodia. One week prior to participant recruitment, a laboratory was set up in a room of the clinic previously used for administrative purposes. The minimal laboratory set-up included a water supply, air conditioner, two-four-module Xpert machines with power backup and voltage stabilizers, one desktop computer, two centrifuge machines, one vortex, and one 200-litre fridge to store samples. Prior to study launch, four laboratory technicians received a two-day training provided by Cepheid and an experienced laboratory manager.

### 2.2 | Sample size

We calculated the sample size for the sensitivity and specificity of diagnostic studies using the methods described by Naing et al<sup>14</sup> At an expected sensitivity of 98% and prevalence (proportion of viremic patients among seropositive patients) of 72% based on clinic operational data, the desired sample size for the qualitative assessment was calculated as 263 seropositive patients. Considering that 45% of patients would be GT6 from previous clinic data, the total sample size required to make the same conclusion for GT6 patients was calculated as 585 seropositive patients (=263/0.45). This sample size was also adequate for the Bland-Altman analysis at 80% power and an alpha level of 0.05

using calculation methods presented by Lu et al<sup>15</sup> with an expected mean of differences at  $\mu = 0.03$ , standard deviation (SD) of differences at  $\sigma = 0.2$ , and a predefined clinical agreement limit for the maximum allowed difference between two methods set at  $0.5 \log_{10}$  IU/mL from the previous literature.<sup>5</sup> We determined that 585 seropositive patients were adequate to obtain the 156 GT6 patients calculated for the quantitative analysis.

## 2.3 | Clinical samples

Adult patients 18 years of age and older seeking assessment of HCV serostatus at MSF's HCV clinic, willing and able to provide informed consent for the study, were consecutively recruited between 1 August and 6 September 2017. Patients excluded from the study comprised those testing HCV seronegative by SD Bioline<sup>®</sup> HCV (Standard Diagnostics, Inc., Rest of World regulatory version, sensitivity 98.8% [95% CI 95.6, 99.7] and specificity 100% [95% CI 98.9, 100],<sup>16</sup>), those on HCV treatment, or those for whom sufficient blood samples could not be obtained. For each eligible, consenting patient, 20 mL of venous whole blood was collected in five 4-mL ethylenediaminetetraacetic acid (EDTA) tubes at the same time—three tubes were kept for VL testing using Xpert and for backup in the event that additional blood for retesting was needed, and two tubes were transported to the reference laboratory within 18 hours. Samples were transported to the reference laboratory twice each day in a cooler box kept between 2 and 22°C (one-hour transport).

## 2.4 | Xpert<sup>®</sup> (Cepheid) HCV RNA viral load testing at the clinic

The Xpert HCV VL test was performed at MSF's HCV clinic, according to the manufacturer's protocols.<sup>6</sup> We extracted fresh plasma from three EDTA tubes into a homogenized sample and aliquoted them for storage and VL testing. The fresh plasma samples were maintained at 2–8°C and (almost all) tested on the same day as the blood draw using the Xpert platform; on rare occasions, when the number of patients recruited in the study exceeded the laboratory capacity during the week, a few plasma samples were frozen over the weekend at –20°C and tested the following week according to the manufacturer's recommendations. Results were considered valid when VL was undetected, detected below  $1.0 \log_{10}$  IU/mL or above  $8.0 \log_{10}$  IU/mL—which are the lower and upper limits of quantification (LOQ), or detected within the LOQ (numeric value between 1.0 and  $8.0 \log_{10}$  IU/mL). When VL results were detectable within the LOQ, we defined the result as quantifiable. In the event of absent (no result), invalid, or erroneous results, VL testing was repeated until a valid test result was obtained. The Xpert assay reports no results when tests are prematurely terminated during the PCR. Invalid results are obtained when the internal quality control fails. Results display errors when the assay is aborted due to reasons such as insufficient samples or when the integrity of the cartridge is undermined. Technicians at the reference laboratory and at MSF's HCV clinic were blinded to the VL test results each obtained.

## 2.5 | Cobas<sup>®</sup> TaqMan<sup>®</sup> (Roche) HCV RNA viral load and genotype testing at the reference laboratory

The comparison VL test was performed at the reference laboratory (Institut Pasteur du Cambodge [IPC], Phnom Penh, Cambodia) using the COBAS<sup>®</sup> AmpliPrep/Cobas<sup>®</sup> TaqMan<sup>®</sup> HCV Quantitative Test, v2.0 platform (hereafter called Roche) according to manufacturer's instructions. The two venous whole blood samples obtained in EDTA tubes for VL and genotype testing at IPC were maintained between 2 and 8°C and shipped to IPC within 18 hours of the blood draw. Samples for VL testing using the Roche assay were prepared at IPC by their laboratory technicians. External quality control was routinely performed. Results were recorded as undetected, detected under ( $1.2 \log_{10}$  IU/mL) or above ( $8.0 \log_{10}$  IU/mL) the LOQ, or detected within the LOQ (numeric value between 1.2 and  $8.0 \log_{10}$  IU/mL). When VL results were detectable within these boundaries, we defined the result as a quantifiable VL result. The amplification and detection were done using the Cobas<sup>®</sup>Taqman<sup>®</sup> 48 machine, 3.3 software.

All samples with detectable HCV RNA results using the Roche platform were further tested for genotype. HCV genotype was determined based on the phylogenetic analysis of the HCV nonstructural 5B (NS5B) genome region (371 bp) that was amplified using a semi-nested RT-PCR.<sup>17</sup> PCR amplified fragments were sent to the MacroGen Company (MacroGen Inc., Seoul, Republic of Korea) for sequencing using the Big Dye<sup>®</sup> Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Chromatograms were sent back to IPC for verification by visual inspection using Ceq2000 software (Beckman Coulter, California, USA). Viral sequences were aligned with reference sequences for HCV subtype available in the GenBank database. A phylogenetic tree was constructed according to the maximum likelihood method using the MEGA6 software<sup>18</sup> to determine the HCV genotype.

## 2.6 | Data collection

We prospectively collected data on patient age, sex, previous HCV treatment history, results of transient elastography using FibroScan<sup>®</sup> as well as date of blood sampling for HCV VL, date of performance of the laboratory tests, VL results, and dates on which results were received by the healthcare workers at MSF's HCV clinic. Cirrhosis was defined as having a value of 14.1 kPa or above (fibrosis stage 4) on the FibroScan<sup>®</sup>. TAT was defined as the number of days from sample collection to receipt of laboratory results by the healthcare workers at MSF's HCV clinic. Anonymized data were prospectively collected using the REDCap electronic database (Research Electronic Data Capture; Vanderbilt University, USA) hosted at Epicentre, Médecins Sans Frontières (Paris, France).<sup>19</sup> The VL and genotype data were entered in REDCap using printed results obtained from MSF and IPC laboratories. All data were double entered by two independent data entry operators and cleaned for any discrepant entries prior to analysis. The study was approved by the Cambodian National Ethics Committee

for Health Research (Reference: 148 NECHR). Samples and data were only collected from patients providing written informed consent for participation in the study.

## 2.7 | Statistical analysis

We used descriptive statistics to report aggregated demographic and baseline characteristics of the patients included in the study. Qualitative analysis included calculating the sensitivity and specificity of the Xpert HCV VL compared to the Roche HCV RNA assay when considering undetectable or detectable VL under the lower LOQ on respective platforms, as negative results, and considering quantifiable VL between the boundaries of LOQ as positive results. Discrepancy between the results of VL from the two assays was defined as quantifiable VL or above the upper LOQ on one platform (positive), but undetectable or lower than the LOQ on the other platform (negative).

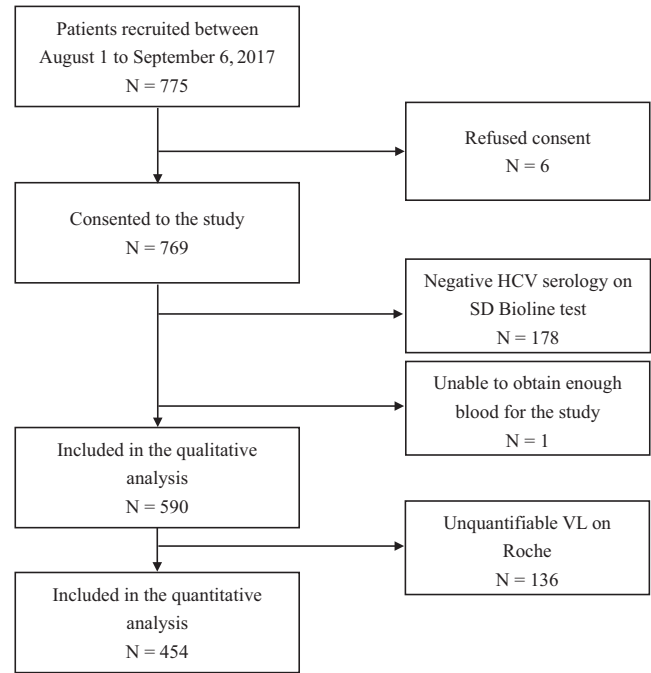
For the quantitative analysis, we assessed the level of agreement for the quantitative VL results by calculating the Pearson's correlation coefficients, Deming regression, and Bland-Altman method using the  $\log_{10}$ -transformed VL values in IU/mL. Samples with unquantifiable VL (undetectable or detectable above or below the LOQ) on either platform were excluded from the quantitative analysis. The mean difference between the platforms, lower and upper limits of agreement (LOA), and their 95% CIs were calculated. Statistical analyses were stratified by genotype to assess the performance of the Xpert HCV VL test for GT6 patients. Data were cleaned and analysed using Stata (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP), and images were exported using MedCalc (v17.9.7, Ostend, Belgium).

## 3 | RESULTS

Between 1 August and 6 September 2017, 775 patients seeking evaluation of HCV serostatus at MSF's HCV clinic were recruited to participate, and six non-consenting, 178 consenting but seronegative, and one consenting patient with inadequate blood samples for the study were excluded. None of the patients recruited were on treatment. This left 590 patients in the qualitative analysis, of whom 454 with quantifiable VL on Roche were included in the quantitative analysis (Figure 1).

The median age and interquartile range (IQR) for the 590 patients were 57 years (IQR 49-62 years), 355 (60.2%) were female, and a quarter of the patients ( $n = 150$ ) had cirrhosis. Nineteen patients (3%) had a previous history of HCV treatment. Among the 454 patients with quantifiable VL on Roche, GT1 (44%,  $n = 200/454$ ) and GT6 (43%,  $n = 195/454$ ) were the most commonly found genotypes (Table 1).

Of the 590 seropositive samples, 454 (77%) had a detectable and quantifiable VL above the lower LOQ on both platforms, and 125 (21%) had undetectable VL on both platforms (Table 2). The



**FIGURE 1** Flow chart of patient enrolment

VL values of the two samples detectable and unquantifiable under the lower LOQ on Roche, but quantifiable on Xpert, were 1.23 and 1.28  $\log_{10}$  IU/mL. These samples were ungenotyped as they had unquantifiable VL results on Roche. There were no samples with VL above the LOQ on Roche but below the LOQ on Xpert. The median and IQR VL values for samples quantifiable on Roche were 6.2 (IQR 5.5-6.6)  $\log_{10}$  IU/mL and 6.2 (IQR 5.3-6.8)  $\log_{10}$  IU/mL for Xpert. Considering results of VL below the lower LOQ as undetectable, the sensitivity and specificity of the Xpert HCV VL assay compared to Roche were 100% (95% CI 99.2, 100.0) and 98.5% (95% CI 94.8, 99.8), respectively.

Deming regression for the 454 samples that had detectable and quantifiable VL on Roche showed a strong correlation between the two platforms ( $r = 0.94$ , Deming regression equation  $Y = 0.92X + 0.48$ , 95% CI for slope 0.87, 0.96) (Figure 2A). Correlation for GT6 samples ( $n = 195$ ) was also strong ( $r = 0.93$ , Deming regression equation  $Y = 0.85X + 0.84$ , 95% CI for slope 0.79, 0.91) (Figure 3E) and similarly strong for all other genotypes (Figure 3A, C, and G).

As demonstrated in the Bland-Altman plot in Figure 2B assessing 454 samples quantifiable on Roche, the mean difference between the two platforms was  $-0.01 \log_{10}$  IU/mL (95% CI  $-0.05$ ,  $0.02$ ) with differences between the platforms ranging from  $-1.32$  to  $1.48 \log_{10}$  IU/mL. The lower and upper LOAs were  $-0.76 \log_{10}$  IU/mL (95% CI  $-0.82$ ,  $-0.70$ ) and  $0.73 \log_{10}$  IU/mL (95% CI  $0.67$ ,  $0.79$ ), respectively. Twenty-nine (6.4%) samples fell outside of the LOA (16 were GT1b, seven GT6, two GT2a, and four indeterminate genotype). Sixty-three (13.9%) samples fell outside the predefined clinical agreement limit for the maximum allowed difference between two methods which were set at  $0.5 \log_{10}$  IU/mL. Stratified

**TABLE 1** Characteristics of patients in the study (N = 590)

Patient characteristics	Number <sup>a</sup>
Age, y	57 (49-62)
Female	355 (60.2%)
Fibrosis stage	
F0 (<5.1 kPa)	98 (16.6%)
F1 (≥5.1 and <7.2 kPa)	126 (21.4%)
F2 (≥7.2 and <9.6 kPa)	91 (15.4%)
F3 (≥9.6 and <14.1 kPa)	118 (20.0%)
F4 (≥14.1 kPa)	150 (25.4%)
Missing FibroScan values (fibrosis stage)	7 (1.2%)
History of HCV treatment	
Yes	19 (3.2%)
IFN or PegIFN	8 (1.4%)
Ribavirin	3 (0.5%)
Direct-acting antivirals <sup>b</sup>	4 (0.7%)
Unknown regimen	4 (0.7%)
HCV genotype <sup>c</sup>	
1a	15 (3.3%)
1b	185 (40.8%)
2a	35 (7.7%)
2b	2 (0.4%)
6a	10 (2.2%)
6e	94 (20.7%)
6f	52 (11.5%)
6p	7 (1.5%)
6q	21 (4.6%)
6s	9 (2.0%)
6 (other subtypes)	2 (0.4%)
Indeterminate	22 (4.9%)

<sup>a</sup>Parentheses for age indicate IQR; percentages in parentheses are among 590 seropositive patients in the study unless otherwise indicated.

<sup>b</sup>Either daclatasvir/sofosbuvir or sofosbuvir/ledipasvir with or without ribavirin.

<sup>c</sup>Genotypes are among the 454 patients with quantifiable viral load on Roche.

by GT, the proportion of samples falling outside this  $\pm 0.5 \log_{10}$  IU/mL range was 15.5% ( $n = 31/200$ ) for GT1 and 10.8% ( $n = 21/195$ ) for GT6. For these 63 samples falling outside the predefined clinical agreement limit, the VL values for Xpert were larger than Roche for 82.5% ( $n = 52/63$ ) of the samples. Variability in the difference between the two platforms was greater for mean VL values around 4-6  $\log_{10}$  IU/mL, but smaller for higher VL values of 6.5-7.5  $\log_{10}$  IU/mL (Figure 2B).

In the analyses by genotype (Figure 3), for GT6 ( $n = 195$ ), the difference in means across the two platforms was  $-0.07 \log_{10}$  IU/mL (95% CI  $-0.12, -0.02$ ) with the LOA between  $-0.76$  (95% CI  $-0.85, -0.68$ ) and  $0.62$  (95% CI  $0.53, 0.71$ )  $\log_{10}$  IU/mL. For GT1 ( $n = 200$ ),

the mean difference was 0.05 (95% CI  $-0.003, 0.10$ ) with a LOA of  $-0.69$  (95% CI  $-0.78, -0.60$ ) and  $0.79$  (95% CI  $0.70, 0.88$ )  $\log_{10}$  IU/mL.

The median number of Xpert tests performed for the study per day was 22 patient samples (IQR 18-25, minimum 12 to maximum 29) using two-four-module Xpert machines. The majority of the samples (96.8%,  $n = 571/590$ ) were tested on Xpert on the same day as the blood draw, and 97.8% ( $n = 577/590$ ) were tested on fresh plasma. The frequencies of no results and errors for Xpert were 0.7% ( $n = 4/590$ ) and 0.5% ( $n = 3/590$ ), respectively. There were no invalid results.

The median TAT for the Xpert VL was 0 days (IQR 0-0, minimum 0 to maximum 7) compared to 4 days (IQR 3-5, minimum 1 day to maximum 29) using Roche at the reference laboratory. Only two samples tested on Xpert had a TAT of 7 days. This was due to the patient load exceeding the laboratory capacity. These samples were stored at  $-20^{\circ}\text{C}$  and processed when the patient returned a week later. For the Roche VL results, the TAT was greater than 1 week for six samples ( $n = 6/590$ ). This occurred due to the rare occasion of printed results not being delivered to the clinic despite having been tested at IPC; the inquiry and response for missing results required days to process. The median TAT for genotyping was 21 days (IQR 20-30, minimum 16 days to maximum 79). For samples that were difficult to subtype, IPC consulted virologists for a second opinion to assure the quality of the results, which resulted in a long TAT for some samples ( $n = 25/454$  with a TAT >42 days).

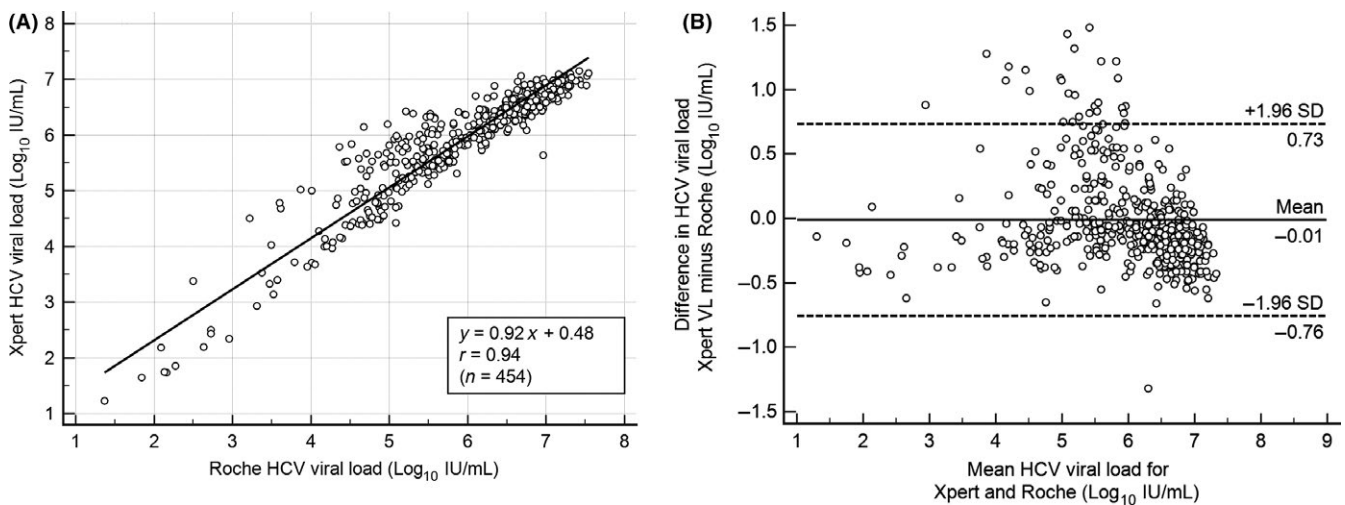
## 4 | DISCUSSION

We showed that in a resource-limited clinical setting, among a patient population with a high prevalence of GT6 HCV infection, the Xpert assay performed well under field conditions compared to the Roche VL conducted in a reference laboratory. In our study, the sensitivity of Xpert compared to Roche was 100% (95% CI 99.2, 100.0). This finding is similar to two previous studies that compared the performance of Xpert against Abbott RealTime among mostly GT1 and GT3 patients: Gupta et al found a sensitivity of 94.4% (95% CI 88.8, 97.7) and McHugh et al 98.0% (95% CI 96.1, 99.1).<sup>5,12</sup> While both Gupta and McHugh reported false negatives, none occurred in our study. We found a specificity of 98.5% (95% CI 94.8, 99.8), compared to 100% (95% CI 88.1, 100.0) and 98.1% (95% CI 95.2, 99.5) in the Gupta and McHugh's studies, respectively. Their studies used Abbott RealTime as the comparison test, which has a lower LOQ (12 or 1.1  $\log_{10}$  IU/mL) compared to Roche (15 or 1.2  $\log_{10}$  IU/mL), and they used mostly frozen samples. The delays between the blood sampling and VL testing using Roche performed at the reference laboratory, which were greater than the delay for the Xpert VL test performed at the clinic using fresh samples, may have affected the sensitivity of the Roche platform for samples close to the LOD. These differences may have led to small variations in findings. If we consider quantifiable VL to be a positive VL result, two among 456 patients (0.4%) with very low, but above the LOQ, VL would not have

**TABLE 2** Comparison of results of Xpert and Roche (n = 590)

	Cobas TaqMan (Roche)			Total
	Detectable and quantifiable $\geq 1.2$ $\log_{10}$ (15) IU/mL	Detectable and unquantifiable $< 1.2$ $\log_{10}$ (15) IU/mL	Undetectable	
Xpert (Cepheid)				
Detectable and quantifiable $\geq 1.0$ $\log_{10}$ (10) IU/mL	454	2	0	456
Detectable and unquantifiable $< 1.0$ $\log_{10}$ (10) IU/mL	0	3	6	9
Undetectable	0	0	125	125
Total	454	5	131	590

IU: international units, mL: millilitre.



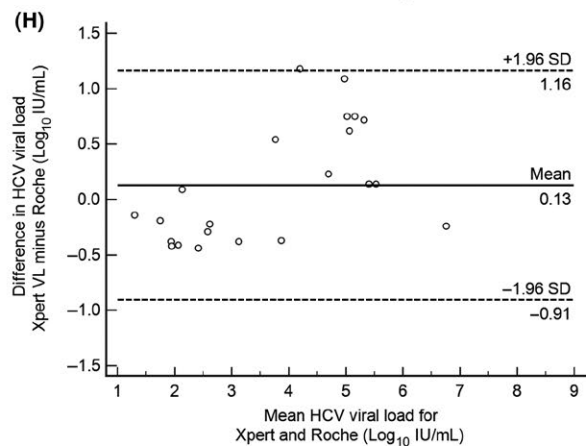
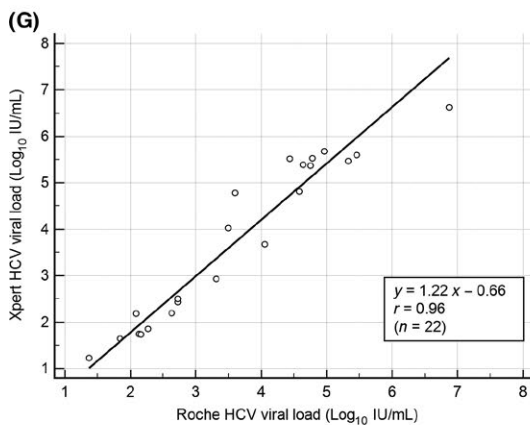
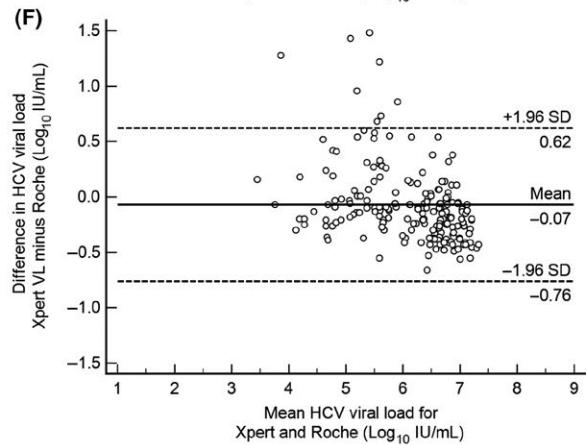
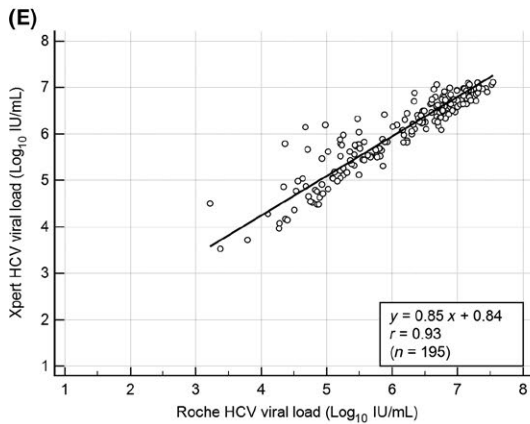
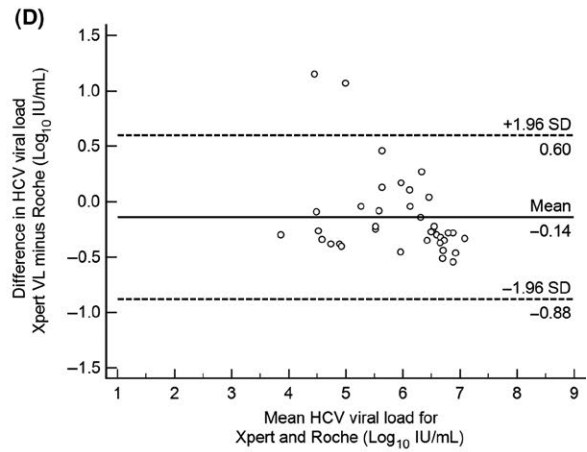
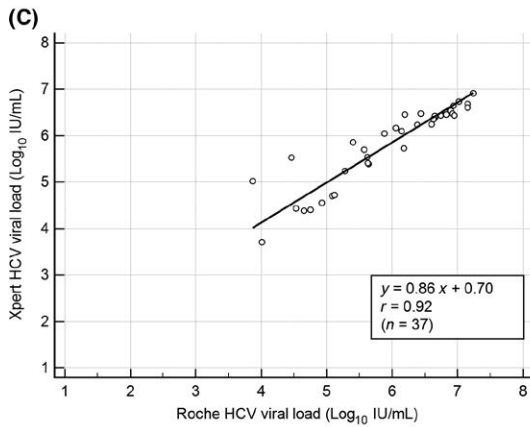
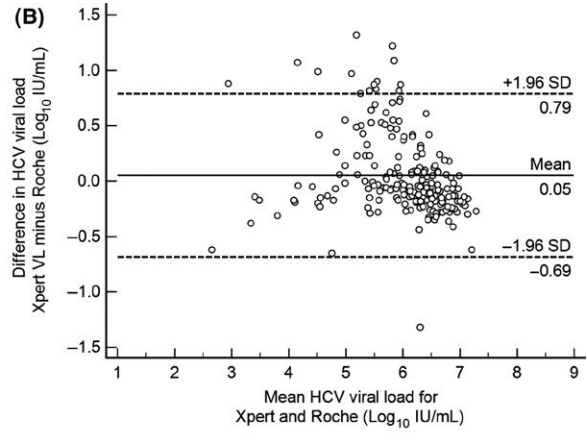
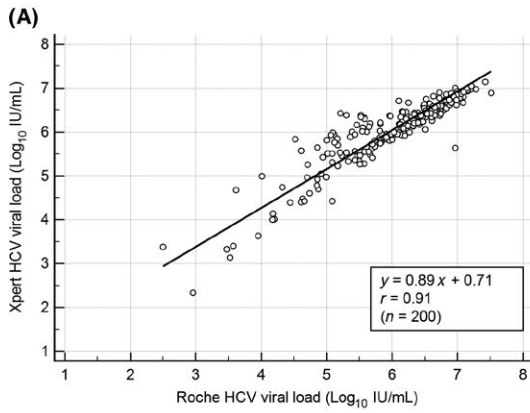
**FIGURE 2** Deming regression and Bland-Altman plot for GeneXpert<sup>®</sup> HCV (Xpert) and Roche Cobas Ampliprep-Cobas TaqMan<sup>®</sup> HCV v2.0 (Roche) viral load values for 454 samples quantifiable on Roche for all genotypes. A, Deming regression plot and correlation. B, Bland-Altman plot

been diagnosed with chronic HCV infection had they used the Roche platform. In the diagnosis of chronic HCV, more research is needed to understand the clinical significance and proper interpretation of VL values close to the lower LOQ.

For the quantitative analysis, we found a small mean difference between the Xpert and Roche assays:  $-0.01 \log_{10}$  IU/mL (95% CI  $-0.05, 0.02$ ) for all GTs indicating the absence of systematic bias. The mean difference for GT6 was  $-0.07 \log_{10}$  IU/mL (95% CI  $-0.12, -0.02$ ) and was comparable to the results from GT1 patients. In addition, the LOA was  $-0.76$  to  $0.73$  for all GTs, with similar results for GT6, for

which the LOA was  $-0.76$  to  $0.62$ . Our LOA was greater than the pre-defined clinical agreement limit for the maximum allowed difference of  $0.5 \log_{10}$  IU/mL, and 13.9% ( $=63/454$ ) of samples fell outside this limit. Our LOA was also wider compared to previous studies by: Gupta (mean difference of  $0.04 \log_{10}$  IU/mL and LOA of  $-0.42$  and  $0.49$ ), McHugh (mean difference of  $0.03 \log_{10}$  IU/mL and LOA of  $-0.41$  to  $0.47$ ), and Grebely (mean difference of  $-0.036 \log_{10}$  IU/mL and LOA of  $-0.28$  to  $0.35$ ).<sup>5,12,20</sup> This may be attributable to differences in sample preparation procedures and the differences in the comparator platforms. Contrary to the three previous studies, we also did not create a

**FIGURE 3** Deming regression and Bland-Altman plot for GeneXpert<sup>®</sup> HCV (Xpert) and Roche Cobas Ampliprep-Cobas TaqMan<sup>®</sup> HCV v2.0 (Roche) viral load values for 454 samples quantifiable on the Roche platform, by HCV genotype. A, Deming regression plot and correlation of genotype 1 samples (n = 200). B, Bland-Altman plot of genotype 1 samples (n = 200). C, Deming regression plot and correlation of genotype 2 samples (n = 37). D, Bland-Altman plot of genotype 2 samples (n = 37). E, Deming regression plot and correlation of genotype 6 samples (n = 195). F, Bland-Altman plot of genotype 6 samples (n = 195). G, Deming regression plot and correlation of indeterminate genotype samples (n = 22). H, Bland-Altman plot of indeterminate genotype samples (n = 22). The Deming regression plots demonstrate the Deming fitted regression lines. The Bland-Altman plots depict the mean HCV viral load of the two platforms (Xpert and Roche) against the difference in viral load values (Xpert minus Roche). The central horizontal line indicates the mean difference and the wider dotted lines (1.96 SD) demonstrate the lower and upper limits of agreement



homogenous plasma sample before aliquoting the samples designated for testing on the two platforms, although all samples were obtained at the same time from the patients. Venous whole blood samples were centrifuged and prepared to be processed on the PCR assay in their respective laboratories. There was also a greater lag time between blood sampling and VL testing for samples performed at the reference laboratory that may explain why, for the majority (82.5%) of the 63 samples falling outside the predefined clinical agreement limit, the VL values were greater for Xpert compared to Roche. Although HCV RNA is known to be stable even at room temperature, variability in sample storage and handling and lag times to testing, as well as dissimilarities inherent to the platforms, may have contributed to the differences in the RNA quantification of the 63 samples.<sup>21,22</sup>

However, from both the qualitative and quantitative study results, we find no evidence to suggest that the Xpert platform performs differently for GT6 HCV compared to other genotypes. The study supports the use of this platform in clinical settings where GT6 HCV is present.

Our study has some limitations. First, we did not further investigate samples that were outside of the predefined clinical agreement limit by repeating VL testing using other platforms as we did not see this as impacting the course of patient care. In addition, a few samples had very dissimilar VL results (variations of up to 1.48 log<sub>10</sub> IU/mL) between Xpert and Roche; it remains unknown whether sample handling procedures or the lag time between the blood draw and testing may fully account for these large differences. Another limitation is that we did not include the quantification of VL during or at the end of treatment. These samples may have low levels of quantifiable HCV RNA.<sup>23</sup> Although our findings suggest that the Xpert platform may have a higher sensitivity than Roche for VL testing for samples with low levels of HCV RNA, there were only a limited number of samples in the low-range VL values. Therefore, additional exploration of the performance of Xpert among low VL samples, or VL quantification during or post treatment, may be useful if the clinical importance of having low levels of quantifiable HCV RNA values during and after treatment is demonstrated.

A major strength of this study is that the assay was implemented in a resource-limited clinical setting where GT6 HCV is predominant, with locally hired and trained laboratory technicians performing the procedures. It thus captures the real-world performance of the Xpert platform for HCV. Xpert allows VL results to be available within the same day compared to a TAT of four days at the reference laboratory. Communication of missing results and retesting can be done immediately if testing is conducted near POC. If the number of patients is small, referral into care may occur on the same day and patients no longer need to wait and return for another visit to a health facility to receive their test results. These represent significant advantages for patients, as loss to follow-up tends to increase with longer TATs.<sup>24</sup> Same day diagnosis and treatment will have a significant impact on patient retention, especially for patients living in decentralized and remote areas.

Despite its excellent performance and advantages over conventional platforms, the Xpert platform still has major operational

constraints. The cost of set-up is high as it requires the instalment of a minimum laboratory infrastructure. The Xpert platform itself costs \$17 000 per unit,<sup>4</sup> and the expense of the entire laboratory set-up, including the cost of the two Xpert platforms as described in the methods section, was \$56 036 (breakdown of costs in the Appendix S1). Furthermore, the Xpert HCV cartridges contain guanidinium thiocyanate to facilitate the extraction of RNAs and require combustion in high-temperature incinerators for proper disposal.<sup>6</sup> These are enormous barriers for the decentralization of testing in resource-limited contexts and are often not sufficiently considered by manufacturers developing the assay. Therefore, further research and development of affordable and environmentally friendly POC HCV NAT platforms or HCV core antigen (HCVc-Ag) tests are urgently needed to reach the majority of the 71 million patients who remain undiagnosed and without access to diagnosis today.

Future studies should also develop procedures for, and evaluate the performance of, the Xpert platform using dried-blood spots (DBS). The use of DBS eliminates the need for cold chain and the set-up of the assay in a laboratory, helping to further improve access to the most underserved patient populations. A new Xpert platform, recently developed for use on finger-stick capillary whole blood, demonstrated excellent performance, with a shorter processing time (one hour), compared to the standard Xpert HCV assay<sup>25</sup> and avoids the special equipment and laboratory skills required for the centrifugation of plasma. However, the platform requires that testing immediately follows sample collection, nevertheless necessitating a similar laboratory set-up to the conventional Xpert HCV assay. Further improvement of access to testing for patients in remote areas and other difficult-to-reach places requires DBS or finger-stick cartridges that may be processed even hours after the insertion of capillary blood; DBS or finger-prick cartridges must also be robust to the conditions often found in resource-limited contexts (extreme and varying levels of temperature and humidity). Lastly, during product development, manufacturers should consider the inclusion of non-GT1/GT3 HCV patients in their studies to ensure the assessment of all genotypes and demonstrate the acceptability of the platform for different countries. The provision of comprehensive, standardized, and publicly available evidence would help reinforce the WHO Prequalification Programme, facilitating in-country test validation to better respond to the needs of populations in resource-limited settings across various regions.

Our study findings demonstrate the excellent performance, in terms of sensitivity (100%) and specificity (98.5%), of the Xpert platform compared to the Roche platform and support the use of the Xpert HCV assay in regions where GT6 HCV is prevalent in the patient population.

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### CONFLICT OF INTEREST

All authors declare no competing interests.

### AUTHORS' CONTRIBUTIONS

AL and DM conceptualized the study. MI, AC, AL, FR, JN, AD, CL, JD, and DM contributed to the formulation of the study methodology. MI and AC developed the research protocol and the standard operating procedures for the study and supervised the study. AC oversaw all laboratory processes, and CS managed and coordinated the study team. AC and CS made certain that the confidentiality of patients and standard operating procedures for the study were respected by the study team. AC, SY, and SP performed the laboratory testing for Xpert. SK and CD served as clinical advisors and ensured patient safety. MI designed the database and data collection processes, oversaw the acquisition of data, performed the data cleaning, analysis, and interpretation, and wrote the manuscript. AD drafted the results of the manuscript. RC provided technical advice on data interpretation and edited the manuscript. All authors critically reviewed the manuscript and gave their comments and approval for the final version of the manuscript. All authors agree to be accountable for the accuracy and integrity of this study.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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