Improving linkage to care of hepatitis C: Evaluation of GeneXpert® HCV Viral Load Point-of-Care Assay in Indonesia

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Abstract

Background: Lack of simple, rapid, and reliable point-of-care (POC) test hampers large-scale diagnosis and treatment of hepatitis C virus (HCV) infection and pose a challenge for its elimination as a public health threat. This study aimed to evaluate Cepheid Xpert® HCV Viral Load performance in comparison to Roche Cobas® TaqMan® HCV Test using HCV-samples with various genotypes in Indonesia.

Methods: Viral load (VL) quantification was performed on 243 anti-HCV positive patients' samples using both Xpert and Roche HCV tests, followed by HCV genotyping by reverse hybridization. Strength of relationship between the assays was measured by Pearson correlation coefficient, while level of agreement was analyzed by Deming regression and Bland-Altman plot analysis using $\log_{10}$-transformed VL values.

Results: Quantifiable VL was detected in 180/243 (74.1%), with Xpert sensitivity of 100% (95% CI 0.98, 1.00) and specificity of 98.41% (95% CI 0.91, 0.99), while HCV genotypes were determined in 172/180 (95.6%) samples. There was a very good correlation between both assays (r = 0.97, P < 0.001), overall and per genotype, with good concordance by Deming regression and mean difference of −0.25 $\log_{10}$ IU/mL (95% CI −0.33, −0.18) by Bland-Altman plot analysis.

Conclusion: Good performance of Xpert HCV Viral Load test was demonstrated as a POC platform for HCV diagnosis and treatment decision, which would be beneficial for decentralized service in resource-limited areas.

Background

Globally, an estimated 170 million people have serological evidence of current or past hepatitis C virus (HCV) infection and 71 million people have chronic viremic infection. Approximately 399,000 people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. This disease also imposes a great multifaceted economic burden worldwide that includes direct medical expenses and indirect costs due to impaired quality of life and loss of work productivity [3]. In response to this concern, the World Health Organization (WHO) developed the Global Health Sector Strategy (GHSS) on Viral Hepatitis 2016-2021, which was endorsed by the World Health Assembly in May 2016. This strategy outlines a set of service coverage targets—diagnosing 90% of chronic infections and treating 80% of eligible people, in order to achieve a global impact including a 90% reduction in new chronic infections and a 65% reduction in mortality by 2030, toward the elimination of viral hepatitis as a major public health threat [4].

The advent of direct-acting antiviral (DAA) drugs for HCV heralded a significant breakthrough for hepatitis C treatment, providing an opportunity to achieve the targeted global HCV elimination [5]. The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) had approved 13 DAAs and several fixed-dose combinations for the treatment of HCV infection. These regimens have shown high efficacy across all six major HCV genotypes and the newly discovered genotypes 7 and 8 [6, 7].

Despite the increased options, expansion of access, and steep price reduction of DAAs, only 20% of infected persons have been diagnosed and 7% have received treatment worldwide, with the majority in higher income settings [8, 9]. In many low- and middle-income countries (LMICs), less than 1% of infected people have been diagnosed and treated [10]. While the world attention is focused on the final steps within the cascade of care for HCV infection, uptake of DAA treatment is progressing slowly and unevenly [9, 11]. Complex clinical management and the cost of HCV testing appear as major impediments to the screening and diagnostic coverage [7, 12-14]. In some countries, the cost of HCV diagnostics may exceed the cost of treatment [11]. A market research in 29 LMICs on the HCV diagnostic landscape highlighted the wide diagnosis-treatment gap that needs to be addressed [15]. In the light of these facts, efforts are needed to scale up access to simple, rapid, and affordable diagnostics to ensure reaching people with or at risk of HCV infection [7, 16, 17].

HCV diagnosis requires the detection of antibodies (anti-HCV) against HCV to identify persons with current or past infection, followed by confirmation of viremic infection by qualitative or quantitative HCV RNA nucleic acid testing (NAT) [18]. Quantitative NAT is used for measuring viral load (VL), identifying patients who need treatment and assessing treatment response. Abbott RealTime HCV® and Roche Cobas® TaqMan® are two market leaders for NAT-based VL tests used in the private sector and tertiary hospital-level laboratories in the public sector [11]. Although NAT technologies are very sensitive and specific for the detection of viremia, they require skilled technicians and centralized laboratories with an established infrastructure, including laboratory network and cold-chain transportation [19]. Samples are tested in batches with a turn-around time up to several days to get the result, leaving the potential for loss to follow-up. In addition, multiple clinical visits are required for treatment decision (e.g. serological diagnosis, followed by NAT). These conditions impede the large-scale screening and diagnosis of HCV infection [9]. To support the scale-up of HCV elimination program, there is an urgent need for alternative simple, rapid, and reliable point-of-care (POC) HCV VL tests that can be suited for decentralized services linking diagnosis to care [7, 16, 20].

Several POC assays including venipuncture-based testing, finger-stick capillary whole-blood testing, and oral fluid diagnostic testing that facilitate HCV RNA confirmation in a single visit are currently available or in the last-stage development [19, 21, 22]. Of these assays, Xpert® HCV Viral Load (Cepheid, Sunnyvale, CA) is the only CE marked and WHO prequalified platform suitable for use in resource-limited settings [15, 19, 23]. Nucleic acid extraction, amplification, and detection of target sequences are carried out in a cartridge and processed in a GeneXpert® Instrument with a quantitative HCV RNA result in 105 minutes. This Cepheid system is semiportable, implementable with minimal laboratory set-up, and does not require batch testing. It is also a modular platform that enables testing of other infections [19, 24, 25].

Spread across more than 17,000 islands, Indonesia represents an ‘island country’ (in contrast to ‘continental country’) where specific strategies for decentralization of healthcare should be implemented. This country has the highest number of HCV prevalence in Southeast Asia, with an estimated 1,289,000 people have viremic infection in 2015 [1]. Beginning in 2017, government assistance programs provided testing and treatment with DAA therapies for 6,000 patients with active HCV infection in seven provinces

in Indonesia, and were gradually expanded to other provinces [26]. Newly installed GeneXpert devices, together with those that have been placed by Tuberculosis program, are integrated to support HCV scale-up program in Indonesia [27]. Given the presence of various HCV genotypes/subgenotypes (genotypes 1, 2, 3, 4, 6, and mix genotypes) in Indonesia which may implicate the reliability of HCV RNA quantification [28, 29], assessment of this system is needed prior to using for confirmation of viremic infection and monitoring of HCV treatment [7, 16, 30]. This study aimed to evaluate the performance of the Xpert® HCV Viral Load and compared it with the FDA-approved Roche Cobas® TaqMan® HCV Test, v2.0 [31] using Indonesian HCV-samples with various genotypes.

Methods

Study participants and clinical samples

This was a prospective study conducted at the Hepatitis Research Unit, Eijkman Institute for Molecular Biology, Jakarta, Indonesia. Between 1 June 2018 and 31 January 2019, 243 anti-HCV positive patients who were either referred to the Eijkman Institute or coming to outpatient department of Dharmais Cancer Hospital in Jakarta —a national referral center for cancer diagnosis and treatment, or Abdoel Wahab Sjahranie Regional General Hospital in Samarinda, Kalimantan Island, were consecutively enrolled. Data were collected on patient age, sex, previous HCV treatment history, as well as results of transient elastography (TE) using FibroScan®, which defined cirrhosis as having a value of 14.1 kPa or higher (fibrosis stage 4) [32].

After written consent was obtained, venous whole blood was collected from each eligible patient in a 9-mL ethylene-diamine-tetraacetic acid (EDTA) tube. After centrifugation, plasma was extracted from each tube and divided into four 1.2-mL aliquots¾two were kept for VL testing, one for genotype determination, and one for backup. The study was in accordance with and approved by the Eijkman Institute Research Ethics Commission (EIREC No 115/2017).

Xpert® HCV viral load testing

The Xpert® VL test (hereafter referred to as Xpert) was performed by laboratory technicians who were recruited from district laboratories and directly supervised by trained research scientists at the Eijkman Institute, as part of capacity building for decentralization of HCV care in the country. Briefly, a total of 1000 mL plasma was placed into the Xpert cartridge, which was scanned and loaded into the GeneXpert® IV instrument according to the manufacturer's instructions. Results were recorded as undetected when VL was below 4 IU/mL (0.6 $\log_{10}$ IU/mL), detected below 10 IU/mL (1.0 $\log_{10}$ IU/mL) or above 10^8 IU/mL (8.0 $\log_{10}$ IU/mL)¾which is the lower and upper limits of quantification (LOQ), or within the LOQ (between 1.0 $\log_{10}$ IU/mL and 8.0 $\log_{10}$ IU/mL), defined as quantifiable [30].

Cobas® TaqMan® (Roche) HCV RNA viral load testing and HCV genotype determination

850 ml of plasma samples was tested for VL using the COBAS® AmpliPrep/Cobas® TaqMan® HCV Quantitative Test v2.0 (hereafter referred to as Roche) according to the manufacturer's instruction on Cobas®Taqman® 48 instrument. Results were recorded as undetected, detected under 15 IU/mL (1.2 $\log_{10}$ IU/mL) or above 10^8 IU/mL (8.0 $\log_{10}$ IU/mL) the LOQ, or detected within the LOQ (between 1.2 $\log_{10}$ IU/mL and 8.0 $\log_{10}$ IU/mL), defined as quantifiable [30]. One aliquot of all samples with detectable HCV RNA by the Roche platform was tested for HCV genotype (GT) by a second-generation LiPA-HCV genotype assay (Versant HCV Genotype 2.0; Siemens Healthcare Diagnostics) [33].

Statistical analysis

Baseline characteristics of the patients were summarized descriptively. The sensitivity and specificity of the Xpert HCV VL was assessed qualitatively using both detectable and quantifiable thresholds (LOQ > 1.0 $\log_{10}$ IU/mL) compared with the Roche HCV RNA assay as the reference (LOQ > 1.2 $\log_{10}$ IU/mL). Undetectable or detectable VL under the lower LOQ of respective platforms was considered as a negative result, whereas VL within the LOQ boundaries was considered as positive result. The strength of relationship between the two assays was measured by the Pearson correlation coefficients, while the level of agreement was analyzed by Deming regression and Bland-Altman plot analysis using the $\log_{10}$-transformed VL values in IU/mL. Deming regression takes account of measurement errors for both methods [34], while Bland-Altman plot measures the mean difference (bias) and concordance, including limits of agreement (LOA) and their 95% confidence intervals (CI) between the quantification results of both assays [35].

Samples with unquantifiable VL on either platform were excluded from the quantitative analysis. The performance of the Xpert HCV VL test for different genotypes was also analyzed. Tests were two sided and *P* values < 0.05 were considered statistically significant. Data were analyzed using the Statistical Program for Social Sciences (IBM SPSS version 22.0 for Windows; SPSS, IL, USA) and NCSS Statistical Software version 12 (NCSS, LLC, Kaysville, Utah, USA).

Results

Characteristics of study population

Among all enrolled participants (n = 243), the median age was 49 years, 64.6% (n = 157) were male, and 48 (19.8%) had a previous history of HCV treatment. Fibrosis assessment by transient elastography (TE) was performed on 66 patients, of whom, 20 (30.3%) had cirrhosis. Among 180 patients with quantifiable VL on Roche, HCV genotypes were successfully determined on 172 samples, with GT1 (108/62.8%) and GT3 (26/15.1%) being the most commonly found, followed by GT2 (20/11.6%), GT4 (17/9.9%) and GT6 in one (0.6%) sample (Table 1).

Table 1 Characteristics of study population (N = 243)

Characteristics	n (max, min)	%
Age (years)[a]	49 (10, 87)	
Male	157	64.6
Fibrosis stage[b] (n=66)		
F0 (<5.1 kPa)	3	4.5
F1 (≥5.1 and <7.2 kPa)	4	6.1
F2 (≥7.2 and <9.6 kPa)	19	28.8
F3 (≥9.6 and <14.1 kPa)	20	30.3
F4 (≥14.1 kPa)	20	30.3
History of HCV treatment (n=48)[c]		
IFN or PegIFN	44	91.7
DAA[d]	7	14.6
Both IFN/PegIFN and DAA	3	6.3
HCV genotypes (n=172)[e]		
1	108	62.8
1a	57	33.1
1b	40	23.3
Subtype unknown	11	6.4
2	20	11.6
2a/c	14	8.1
Subtype unknown	6	3.5
3	26	15.1
3a	15	8.7
3k	11	6.4
4	17	9.9
4h	6	3.5
Subtype unknown	11	6.4
6	1	0.6

6c	1	0.6
Indeterminate	8	4.7

[a]Median (minimum, maximum)

[b]By transient elastography (TE) using FibroScan®, cirrhosis was defined as having a TE value of 14.1 kPa or above

(fibrosis stage 4) [31]

[c]With previous HCV treatment

[d]Either daclatasvir/sofosbuvir or sofosbuvir/ledipasvir with or without ribavirin

[e]Genotypes are among 180 patients with quantifiable viral load by Roche

Sensitivity and specificity of Xpert

Of the 243 patients, 180 (74.1%) had detectable and quantifiable VL above the lower LOQ on both platforms, and 55 (22.6%) had undetectable VL on both platforms (Table 2). One sample was unquantifiable and detectable by Roche but quantifiable by Xpert with a VL of 1.04 $\log_{10}$ IU/mL. This was one of the eight samples with indeterminate HCV genotype. There were no samples quantifiable by Roche but unquantifiable by Xpert. Considering quantifiable VL as positive and unquantifiable VL as negative results, the sensitivity of Xpert was 100% (95% CI 0.98, 1.00) and the specificity was 98.41% (95% CI 0.91, 0.99). VL values and bias between the two tests at percentiles are shown in Table 3.

Table 2 Comparison of Viral Load test results between Xpert and Roche (n = 243)

		Roche Cobas® TaqMan® HCV Test			
		Detectable & Quantifiable	**Detectable & Unquantifiable**	**Undetectable**	**Total**
Xpert® HCV Test	**Detectable & Quantifiable**	180	1	0	181
	Detectable & Unquantifiable	0	7	0	7
	Undetectable	0	0	55	55
	Total	180	8	55	243

Table 3 Viral load values and bias between Xpert and Roche at percentiles

Variable	Xpert HCV viral load (95% CI) (log_{10} IU/mL)	Roche HCV viral load (95% CI) (log_{10} IU/mL)	Bias (95% CI) (log_{10} IU/mL)
10^{th} percentile	4.56 (3.69, 4.83)	4.43 (4.01, 4.88)	-0.90 (-1.03, -0.79)
25^{th} percentile	5.35 (5.14, 5.48)	5.42 (5.13, 5.76)	-0.55 (-0.68, -0.49)
50^{th} percentile	5.95 (5.75, 6.09)	6.19 (5.98, 6.37)	-0.26 (-0.36, -0.20)
75^{th} percentile	6.42 (6.34, 6.52)	6.76 (6.58, 6.86)	0.04 (-0.06, 0.18)
90^{th} percentile	6.70 (6.62, 6.85)	7.24 (7.06, 7.44)	0.39 (0.25, 0.54)

Concordance between Xpert and Roche

A very good correlation was seen between Xpert and Roche (Pearson's correlation coefficient (r) = 0.97, P < 0.001). When analyzed separately by HCV genotype, the correlation was significant in GT1 (r = 0.87, P < 0.001), GT2 (r = 0.93, P < 0.001), GT3 (r = 0.87; P < 0.001), GT4 (r = 0.88, P < 0.001), and indeterminate genotype (r = 0.99, P < 0.001). Further analysis by Deming regression for the 180 samples showed the overall Deming regression equation $Y = 0.87X + 0.53$ (Figure 1A). When analyzed by genotype, the Deming regression equations were $Y = 0.88X + 0.50$ for GT1, $Y = 0.69X + 1.37$ for GT2, $Y = 0.71X + 1.60$ for GT3, $Y = 0.97X + 0.13$ for GT4, and $Y = 0.97X - 0.07$ for indeterminate genotype (Figure 2A, 2C, 2E, 2G, 2I). Detailed correlation and Deming regression results are shown in Table 4.

Table 4 Pearson's correlation and Deming Regression analysis of Xpert against Roche

Genotype	N	Pearson's Correlation		Deming Regression			
		r	*P* value	Intercept (95% CI)	Slope (95% CI)	T-value	Df
All	180	0.97	<0.001	0.70 (0.32, 1.08)	0.84 (0.79, 0.90)	1.97	178
GT1	108	0.87	<0.001	0.71 (0.11, 1.31)	0.84 (0.74, 0.94)	1.98	106
GT2	20	0.93	<0.001	1.54 (0.84, 2.25)	0.66 (0.53, 0.80)	2.10	18
GT3	26	0.87	<0.001	1.96 (0.59, 3.35)	0.65 (0.44, 0.86)	2.06	24
GT4	17	0.88	<0.001	0.16 (-0.79, 1.11)	0.97 (0.79, 1.45)	2.13	15
GT6	1	n.a.	n.a.	n.a.	n.a.		
Indeterminate	8	0.99	<0.001	-0.06 (-1.06, 0.93)	0.97 (0.77, 1.17)	2.45	6

CI = confidence interval, Df = N-2 degrees of freedom; n.a = not applicable

By Bland-Altman plot analysis, the mean difference between the two platforms was -0.25 $\log_{10}$ IU/mL (95% CI -0.33, -0.18) with differences between the platforms ranging from −1.62 to 1.56 $\log_{10}$ IU/mL. The lower and upper LOAs were −1.24 $\log_{10}$ IU/mL (95% CI −1.37, −1.12) and 0.74 $\log_{10}$ IU/mL (95% CI 0.61, 0.87), respectively. One hundred sixty-nine (93.9%) samples were within the LOA, while eleven (6.1%) samples (five GT1, one GT2, two GT3, and three GT4) were outside the LOA (Figure 1B). By genotype, the mean differences across the two platforms were -0.26 ± 0.49 (95% CI -0.36, -0.17) $\log_{10}$ IU/mL for GT1, -0.45 ± 0.49 (-0.68, -0.22) $\log_{10}$ IU/mL for GT2, -0.21 ± 0.52 (-0.42, 0.00) $\log_{10}$ IU/mL for GT3, -0.01 ± 0.66 (-0.35, 0.33) $\log_{10}$ IU/mL for GT4, and -0.20 ± 0.27 (-0.43, 0.02) $\log_{10}$ IU/mL for indeterminate genotype. Detailed Bland-Altman analysis results are shown in Figure 2B, 2D, 2F, 2H, 2J, and Table 5.

Table 5 Bland-Altman Plot analysis of Xpert against Roche

Genotype	N	Mean difference ± SD (95% CI)	Lower LOA (95% CI)	Upper LOA (95% CI)	Inside LOA	Outside LOA
		(log_{10} IU/mL)	(log_{10} IU/mL)	(log_{10} IU/mL)	n (%)	n (%)
All	180	-0.25 ± 0.51 (-0.33, -0.18)	-1.24 (-1.37, -1.12)	0.74 (0.61, 0.87)	169 (93.9)	11 (6.1)
GT1	108	-0.26 ± 0.49 (-0.36, -0.17)	-1.22 (-1.37, -1.06)	0.69 (0.53, 0.84)	100 (92.6)	8 (7.4)
GT2	20	-0.45 ± 0.49 (-0.68, -0.22)	-1.40 (-1.81, 1.01)	0.51 (0.11, 0.91)	19 (95.0)	1 (5.0)
GT3	26	-0.21 ± 0.52 (-0.42, 0.00)	0.18 (-1.61, 0.88)	0.83 (0.45, 1.20)	24 (92.3)	2 (7.7)
GT4	17	-0.01 ± 0.66 (-0.35, 0.33)	-1.30 (-1.89, -0.71)	1.28 (0.67, 1.87)	16 (94.1)	1 (5.9)
GT6	1	n.a.	n.a.	n.a.	n.a.	n.a.
Indeterminate	8	-0.20 ± 0.27 (-0.43, 0.02)	-0.744 (-1.14, -0.33)	0.32 (-0.08, 0.74)	8 (100.0)	0 (0.0)

n.a. = not applicable

Discussion

This study showed Xpert HCV Viral Load assay accurately quantifies HCV viral load compared to the Roche HCV RNA assay, which is a leading assay used worldwide [36]. The sensitivity of the Xpert for HCV VL measurement was found to be 100% (95% CI 97.9, 100.0). This finding confirms the studies of Iwamoto *et al*. among mostly GT1 and GT6 patients with a sensitivity of 100% (95% CI, 99.2, 100.0) in comparison to the Roche COBAS® Ampliprep/Cobas® TaqMan® HCV Quantitative Test v2.0 [30], and those of Gupta *et al*. at 94.4% (95% CI 48.8, 99.8) and McHugh *et al*. at 98.0% (95% CI 96.1, 99,1) among mostly GT1 and GT3 patients against the Abbott RealTime HCV assay [25, 37]. No false negativity by Xpert was seen in this study. We found a specificity of 98.41% (95% CI 0.91, 0.99) compared to 98.5% (95% CI 94.8, 99.8), 100% (95% CI 88.1, 100.0), and 98.1% (95% CI 95.2, 99.5) in the Iwamoto, Gupta, and McHugh's studies, respectively. This assay provides a rapid, simple, and accurate POC molecular test for HCV viremia, fulfilling the requirements published by Foundation for Innovative New Diagnostics FIND/WHO (diagnostic sensitivity > 95% and specificity > 98%) [38]. With minimal requirement of infrastructure and less turn-around time (105 minutes) than that of Roche (around 4 hours), this test would be ideal for decentralization of molecular testing in a resource-limited setting.

Quantitative analysis revealed a significant correlation (r = 0.97, P <0.001) between the Xpert and Roche, which was also comparable when calculated in individual genotypes. The Xpert VL bias against the

Roche VL values varies from −0.90 $\log_{10}$ IU/mL at the 10^{th} percentile to 0.39 $\log_{10}$ IU/mL at the 90^{th} percentile. The negative bias at the lower end is likely due to sparse data in the range of 10^{th} to 25^{th} percentiles, while data for higher VL across the range 50^{th} to 75^{th} percentiles is more equally distributed and therefore the regression curve is less driven by the high-end samples.

Bland Altman analysis showed a mean difference of -0.25 ± 0.51 $\log_{10}$ IU/mL (95% CI -0.33, -0.18) between the two assays; the LOA was between -1.24 $\log_{10}$ IU/mL to 0.74 $\log_{10}$ IU/mL, with 11 (6.1%) of samples fell outside the LOA. Our overall LOA was wider compared to previous studies by Iwamoto (mean difference -0.01 $\log_{10}$ IU/mL; LOA −0.76 and 0.73), Gupta (mean difference 0.04 $\log_{10}$ IU/mL; LOA −0.42 and 0.49), McHugh (mean difference 0.03 $\log_{10}$ IU/mL; LOA −0.41 and 0.47), and Grebely (mean difference −0.036 $\log_{10}$ IU/mL; LOA −0.28 to 0.35) [25, 30, 37, 39]. This could be attributable to smaller number of samples, particularly in the range of lower VL values [40, 41].

Assays can perform differently by genotype [36, 42, 43], while detection of HCV RNA and measurement of VL for the different genotypes is crucial to clinical management of HCV-infected patients [12-14]. This study showed a high efficiency and accuracy of Xpert HCV VL assay for quantitation HCV RNA of GT1, GT2, GT2, GT4, and even indeterminate genotype. This finding, together with other studies [30, 37], proved that Xpert could identify all major HCV genotypes in different part of the world. As highly potent pangenotypic regimens for HCV treatment are yet available in most countries, this assay may provide an important contribution for simplified diagnosis strategies to skip the determination of the viral genotype in the cascade of care of HCV infection.

A major strength of this study was that it was performed using samples from various places of Indonesia, covering major HCV genotypes that prevailed in Indonesia and its surroundings. In parallel, the study was utilized as training forum of district laboratory technicians, who were directly supervised by research scientists at the Eijkman Institute in Jakarta. Thus, it exemplified a capacity-building project for decentralized service for HCV VL determination.

Several other POC tests for HCV have been developed, including Xpert® HCV Viral Load Finger-Stick (Xpert HCV VL FS) that can detect and quantify HCV RNA from 100 µL of capillary whole blood within 60 minutes [22, 39]. However, this assay has not been commercially available, and according to a preliminary study, further improvement and evaluation studies are still needed before it is ready for routine use [44, 45]. Another device is the Genedrive® HCV (Genedrive Diagnostics, Manchester, UK), which can detect and semiquantify HCV RNA from 30 µL of plasma in less than 90 minutes [46]. Nevertheless, this test still requires a venous puncture for the collection of plasma samples and needs centrifugation, which is not easily accessible in remote areas [47]. Therefore, in this current situation, Xpert VL test could be an option to support the ‘one-step diagnosis strategy’ that testing HCV viremia can be performed at any level of patient care to achieve elimination of HCV by non-laboratory trained individuals such as physicians, nurses, and nursing assistants [38, 48].

We acknowledge several limitations in this study. The sample size could be a potential limitation. However, the sensitivity and specificity of Xpert against the comparator assay was good and the correlation was strong, both among overall samples and by genotype. Further, a selection bias in participants recruited (i.e. patients with anti-HCV referred for treatment decision), who were more likely to be HCV RNA positive, could be one limitation. This also occurred in other studies, that most subjects were patients engaged in health services that could lead to a greater sensitivity and specificity of the test [22, 25, 30, 37]. Further studies on specimens from general population would be needed to augment confidence in the sensitivity and specificity of the test. Another limitation is that we did not include samples during or at the end of treatment that could represent those with low levels of quantifiable HCV RNA. Although our finding showed that both platforms have comparable sensitivity, there were only a limited number of samples in the low-range VL values. Additional study on samples during or post treatment would be useful to ensure the performance of Xpert against other assays among low VL samples [30, 49].

Conclusions

This study demonstrates a good performance of the Xpert HCV VL test as a POC platform comparable to that of a market leading assay for treatment decision and determining the outcome of HCV antiviral treatment. The robustness of data resulted in this study suggests that this assay can be used for decentralised HCV VL testing, which would streamline the cascade of care the patients in areas with resource-limited setting.

Abbreviations

CI = confidence interval; DAA: direct-acting antiviral agent; GT: genotype; HCV: hepatitis C virus; IFN: interferon; LMIC: low- and middle- income countries; LOA: limits of agreement; LOQ: limits of quantification; Peg-IFN: pegylated interferon; POC: point of care; TE: transient elastography; VL: viral load.

Declarations

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Author contributions

MDT and DHM conceptualized the study and developed the proposal; MDT and DPW performed data management, analysis, and manuscript writing; DPW and T supervised and performed part of the laboratory work, performed data collection and analysis; KEK performed data analysis, result interpretation, and manuscript writing; SII participated in data analysis and manuscript writing; LS and

ISM provided clinical samples and performed data collection; DHM provided critical review of the manuscript. All authors have read and approved of the final manuscript.

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Availability of data and materials

The data generated and analyzed in this study are included in this published article.

Ethics approval and consent to participate

The study was in accordance with and approved by the Eijkman Institute Research Ethic Commission (EIREC No. 115/2017). Written informed consent was obtained from each patient.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

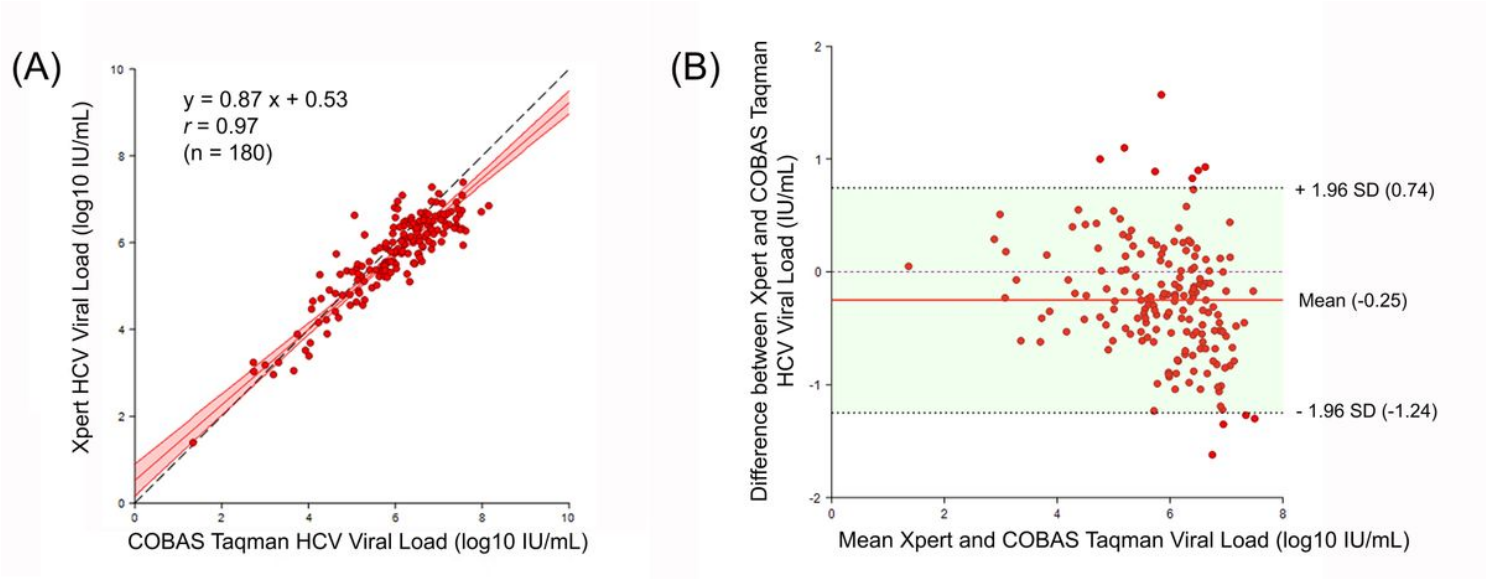

Figure 1

Deming regression and Bland-Altman plot analyses for samples' HCV quantification by Xpert and Roche assays. A. The Deming regression plot shows the Deming fitted regression line (red), associated confidence interval bounds (red shadow), and Pearson's correlation. The black dashed line represents the 45° Y = X line (identity line). B. The Bland-Altman plot shows the difference between the HCV RNA levels obtained by the two assays; the mean difference is depicted by the red line; and dotted lines indicate the upper and lower limit of agreements (LOAs) corresponding to ± 1.96 standard deviation.

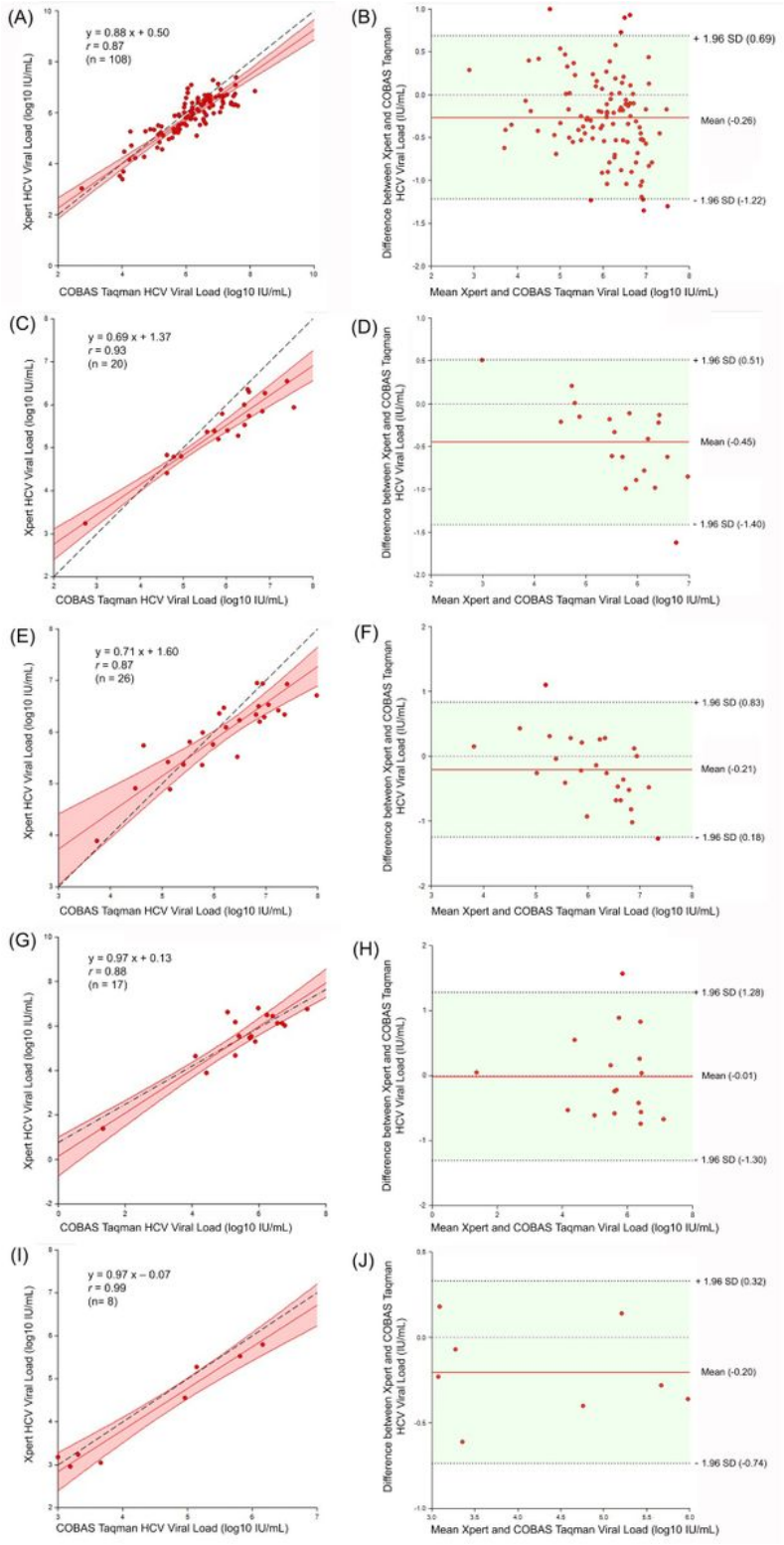

Figure 2

Deming regression and Bland-Altman plot analyses by HCV genotype. Deming regression plot and correlation of genotype 1 samples (A), genotype 2 samples (C), genotype 3 samples (E), genotype 4 samples (G), and indeterminate genotype samples (I). Bland-Altman plot of genotype 1 samples (B), genotype 2 samples (D), genotype 3 samples (F), genotype 4 samples (H), and indeterminate genotype samples (J). The Deming regression plots show the Deming fitted regression lines (red), associated

confidence interval bounds (red shadow), Pearson's correlation, and the identity line (45° Y = X) (black dashed). The Bland-Altman plots show 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 (red) indicates the mean difference and the dotted lines indicate the upper and lower limits of agreement (LOAs) corresponding to ± 1.96 standard deviation.