



# Point-of-care testing (POCT) in molecular diagnostics: Performance evaluation of GeneXpert HCV RNA test in diagnosing and monitoring of HCV infection

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## ARTICLE INFO

### Article history:

Received 9 September 2016

Received in revised form 16 January 2017

Accepted 23 January 2017

### Keywords:

Hepatitis C infection

Point-of-care of molecular diagnostics

## ABSTRACT

**Background:** Molecular testing at the point-of-care may turn out to be game changer for HCV diagnosis and treatment monitoring, through increased sensitivity, reduced turnaround time, and ease of performance. One such assay GeneXpert® has recently been released.

**Objectives:** Comparative analysis between performances of GeneXpert® and Abbott HCV-RNA was done. **Study design:** 174 HCV infected patients were recruited and, one time plasma samples from 154 patients and repeated samples from 20 patients, obtained at specific treatment time-points (0, 4, 12 and 24) weeks were serially re-tested on Xpert®.

**Results:** Genotype 3 was the commonest, seen in 80 (66%) of the cases, genotype 1 in 34 (28.3%), genotype 4 in 4 (3.3%) and genotypes 2 and 5 in 1 (0.8%) each. Median HCV RNA load was 4.69 log<sub>10</sub> (range: 0–6.98 log<sub>10</sub>) IU/ml. Overall a very good correlation was seen between the two assays ( $R^2 = 0.985$ ), concordance of the results between the assays was seen in 138 samples (89.6%). High and low positive standards were tested ten times on Xpert® to evaluate the precision and the coefficient of variation was 0.01 for HPC and 0.07 for the LPC. Monitoring of patients on two different regimes of treatment, pegylated interferon plus ribavirin and sofosbuvir plus ribavirin was done by both the systems at baseline, 4, 12 and 24 weeks. Perfect correlation between the assays in the course of therapy at different treatment time-point in genotypes 3 and 1 was seen.

**Conclusion:** The study demonstrates excellent performance of the Xpert® HCV assay in viral load assessment and in treatment course monitoring consistency.

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## 1. Background

HCV infection is a major public health problem and is associated with significant liver-related morbidity and mortality globally [1,2]. People with untreated HCV infection are at increased risk of liver cirrhosis, hepatocellular carcinoma, and liver failure [3]. Owing to the attributes of HCV which make it a likely candidate for elimination [4], world health organisation (WHO) in 2012, has launched its global action framework for elimination of hepatitis C in near future by increasing screening, diagnosis and referral to care of HCV cases, and move towards public health approach in screening and diagnosis [5]. HCV RNA testing, which is done after a positive anti-HCV antibody screening test in a clinical setting, allows the detection of current HCV infection, thus indicating individuals for treatment

[6]. The treatment landscape for hepatitis C is currently undergoing a dramatic transformation with the advent of direct acting antivirals (DAAs) [7–9]. Viral load monitoring while on therapy serves to predict treatment response and acts as a measure of treatment adherence; and recurrence after end of treatment, especially in high risk groups [10]. In the contemporary era, viral load monitoring may also help to prevent resistance development by allowing for switching or stopping therapy if HCV RNA does not decrease or return to baseline levels while on treatment. There are currently several commercially available HCV viral load assays; real-time PCR assays are generally preferred because of their wide dynamic ranges and good sensitivities [11,12]. The need of the hour is the development of platforms which are fully automated, are less prone to contamination, have a high throughput and low turnaround time, and can serve to decentralize molecular testing facilities especially in low income countries. Point-of-care diagnostics has revolutionised early diagnosis and rapid referral to treatment and care in many infectious diseases especially tuberculosis [13,14].

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Xpert<sup>®</sup> HCV Viral Load assay (Cepheid, CA, USA) is launched in 2015 as point-of-care molecular test for HCV RNA estimation, which is a fully automated real time reverse transcriptase polymerase chain reaction (qRT-PCR) assay. This assay combines the steps of sample preparation, nucleic acid extraction, amplification and detection of target sequences in one cartridge [15].

## 2. Objectives

The aim of this study was to evaluate the performance of the Xpert<sup>®</sup> HCV for viral load estimation and monitoring of patients on antivirals and to compare it with the FDA approved Abbott Real Time HCV quantitative assay (Abbott, Wiesbaden, Germany).

## 3. Study design

### 3.1. Subjects and samples

This was a prospective study conducted in the Department of Clinical Virology, Institute of Liver and Biliary Sciences (ILBS), a tertiary care liver centre. Overall, 174 consecutive anti-HCV antibody positive patients between July–December 2015 either coming to the outpatient department or admitted in the hospital were recruited in this study. Informed consent was taken from the participants. Patients with co-infection with HBV or HIV, patients with low serum/cut-off (S/Co) ratio ( $\geq 1$  to  $<4$ ) on serological HCV assay and who failed to give consent were excluded from the study. Patients with low S/Co were not included in the study as the probability of detecting HCV viremia in them would be low [16]. Plasma samples from these patients were received in the department for HCV viral load testing as a part of their routine clinical management. Patients with detectable viral load were put on treatment as per the Institutes protocol taking into consideration the genotype of the virus and patients clinical condition, consisting of interferon plus ribavirin or direct acting antiviral (DAA) i.e. sofosbuvir plus ribavirin. Samples were aliquoted in two parallel aliquots and stored at  $-80^{\circ}\text{C}$  till further testing. Abbott Real Time HCV quantitative assay (hereafter, Abbott) was used as the routine assay for HCV viral load estimation. Clinical decisions to initiate HCV treatment were based on HCV RNA viral load results obtained with this assay. All the samples were re-tested in parallel on the Xpert<sup>®</sup> HCV Viral Load assay (hereafter, Xpert). In 20 patients who were on anti-HCV treatment, follow up plasma samples obtained at different treatment time points (baseline, 4 weeks, 12 weeks and 24 weeks) were also serially re-tested on Xpert. The HCV genotype was determined by direct PCR sequencing using in-house designed pan genotypic primers. The study was approved by the Institutes Review/Ethics committee.

### 3.2. Standards

5th WHO international standard for HCV (NIBSC code: 14/150) comprising of genotype 1a was used to evaluate the precision and linearity of Xpert assay. The lyophilized standard was reconstituted with 1.1 ml of deionized, nuclease-free molecular grade water. This material was assigned a unit of  $5 \log_{10}$  IU/ml in the final concentration as per the literature. Serial 10-fold dilutions of this were made in human plasma tested negative for all markers of HBV, HCV and HIV. Standard with  $5 \log_{10}$  value was considered as High positive control (HPC) and with  $2 \log_{10}$  value as low positive control (LPC).

### 3.3. Abbott real time HCV assay

750  $\mu\text{l}$  of plasma samples were tested by the Abbott Real Time HCV assay on the automated m2000sp/m2000rt platform, as per

the manufacturer's instructions. Results from the routine testing were recorded and used for the comparative analysis in the present study. The lower limit of detection (LOD) and lower limit of quantification (LLOQ) of this assay is similar i.e. 12 IU/ml and linear range of the assay is from 12 to  $10^8$  IU/ml.

### 3.4. Xpert<sup>®</sup> HCV viral load assay

Parallel plasma samples were used for determination of HCV RNA on Xpert system. 1000  $\mu\text{l}$  of the plasma was transferred into the cartridge provided by the manufacturer which was then scanned and loaded into the GeneXpert Dx instrument as per manufacturer's instructions, and results were obtained in 105 min. The LOD of this assay is 4 IU/ml and LLOQ is 10 IU/ml and linear range of the assay is from 10 to  $10^8$  IU/ml.

### 3.5. HCV genotyping

Samples with viral load  $>3 \log_{10}$  IU/ml were further processed for genotyping. 5'UTR region was used for genotyping and in genotype 1 cases further confirmation was done by using the NS5B region of the virus [17]. HCV RNA isolation was done using High pure viral nucleic acid kit (Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's instructions. Viral RNA was reverse-transcribed to cDNA using quantitect reverse transcription kit (Qiagen, GmbH, Germany). 5  $\mu\text{l}$  of cDNA was subjected to PCR using in-house designed primers for 5'UTR (forward primer 5'ATGGATCACTCCCTGTGAGGAAGT3', reverse primer 5'GTCTACGAGACCTCCGGGGCACT3') and NS5B regions also using in-house designed primers (forward primer 5'ACYACCATYATGGCNAARARYGAGGT3') and reverse primer 5'TAYCTRGTCATRGCTCCGTGAAGRC3'. PCR was done using Phusion high fidelity DNA polymerase (Thermoscientific Inc., Waltham, MA). Amplified products were purified by gel-excision using QIAquick gel extraction kit (QIAGEN, GmbH, Mannheim, Germany) to remove unincorporated dNTPs and primers. This was followed by bidirectional sequencing using ABI Big Dye chemistry on the ABI 3500Dx series genetic analyzer (Life Technologies, Waltham, MA). Forward and reverse sequence reads were aligned and assembled using DNA Baser v3.5.1 (Heracle BioSoft SRL, Romania). Genotype assignment was done by comparing the obtained sequences with the reported sequences on the Basic Local Alignment Search Tool (BLAST) database of NCBI.

### 3.6. Statistical analysis

The HCV RNA values were  $\log_{10}$  transformed for analysis. Linear regression analysis was done and correlation coefficients were calculated using SPSS version 22. Agreement between the two assays was determined using Bland-Altman plots. For comparison of the two assays in treatment monitoring repeated measure analysis was done followed by post hoc comparison by Bonferroni method.

## 4. Results

Of the 174 subjects recruited in the study, male preponderance was seen with M:F ratio of 1.3:1. Genotyping could be done in 120 samples with detectable viral load more than  $3 \log$  IU/ml. Genotype 3 was the commonest, seen in 80 (66%) of the cases, followed by genotype 1 in 34 (28.3%), genotype 4 in 4 (3.3%) and genotypes 2 and 5 in 1 (0.8%) each (Table 1). HCV RNA load in the samples ranged from 0 to  $6.98 \log_{10}$  IU/ml, with a median value of  $4.69 \log_{10}$  IU/ml.

**Table 1**  
Baseline characteristics of the study population.

Variable	Number
Age (mean $\pm$ SD) years	48.3 $\pm$ 13.5
Male:Female	1.3:1
Genotype (subtype) <sup>a</sup>	n
1(1a/1b/1c)	34(14/10/10)
2(2a)	1
3(3a/3b/3i)	80(60/16/4)
4a	4
5a	1
Median RNA levels (log <sub>10</sub> IU/ml) (Range) <sup>b</sup>	4.69 (0–6.98)
ALT (mean $\pm$ SD) IU/ml	56.85 $\pm$ 46.67
AST (mean $\pm$ SD) IU/ml	65.67 $\pm$ 53.69

RNA: ribonucleic acid; IU: international unit; ALT: Alanine transaminase; AST: aspartate aminotransferase; SD: standard deviation; ml: millilitre.

<sup>a</sup> Done in 120 samples with detectable viral load more than 3 log IU/ml.

<sup>b</sup> As determined by the routine assay, Abbott Real Time HCV assay.

**Table 2**  
Comparison of HCV RNA levels between Abbott and Xpert assay.

		Abbott			Total
		Negative	<12 IU/ml	>12 IU/ml	
Xpert	Negative	13	7	3	23
	<10 IU/ml	2	7	4	13
	>10 IU/ml	0	0	118	118
	Total	15	14	125	154

IU: international unit; ml: millilitre.

#### 4.1. Concordance between the assays

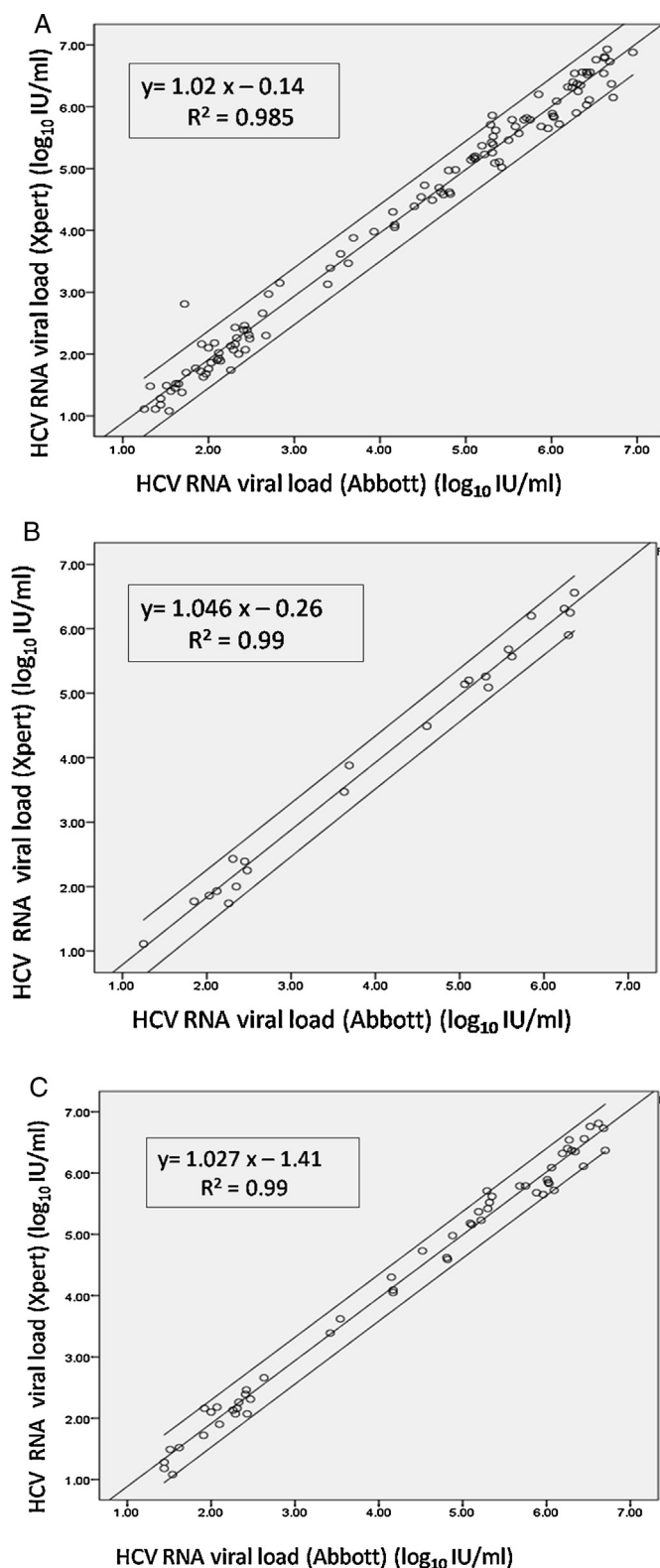
A total of 154 serum samples were available for comparative analysis. During routine monitoring by Abbott, 15 (9.7%) samples had undetectable viral load (negative) and 139 (90.2%) had detectable HCV RNA [quantifiable HCV RNA load in 125(81.1%) and in 14(9%) samples it was <LLOQ] (Table 2). Comparing the results of the Xpert with Abbott, overall concordance was seen in 138 samples (89.6%), of which 118 were quantifiable, 7 were less than LLOQ, (<12 IU/ml for Abbott and <10 IU/ml for Xpert) and 13 were negative by both the assays. Amongst the 16 discrepant results, most of the differences were in viral loads that were below the LLOQ of one assay and were read as undetectable by another assay, 7 of Abbott were detected as negative by Xpert while out of 23 negatives detected by Xpert in 20 viral load was either negative or below LLOQ by Abbott, but in 3 samples HCV RNA was quantifiable above the LLOQ by Abbott. These three samples were repeatedly re-tested and viral load was again found to be detectable by Abbott but negative by Xpert. Similar false negative results were not seen by Abbott.

#### 4.2. Correlation between the two assays

On linear regression analysis of the quantifiable viral loads as obtained by both the assays (Fig. 1), a very good correlation was seen between the two assays ( $R^2=0.985$ ) (Fig. 1A). The correlation was strong in both the HCV genotypes 3 and 1 independently ( $R^2=0.99$  and  $0.99$  respectively) (Fig. 1B and C).

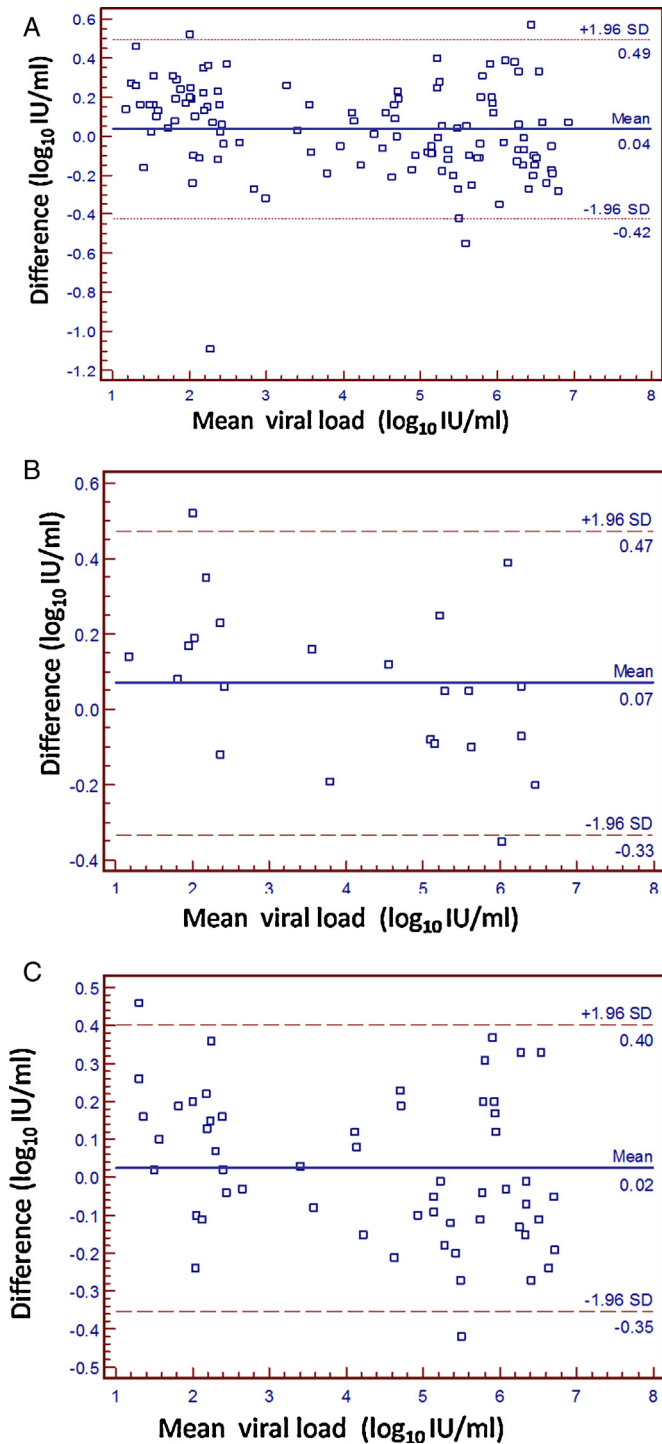
#### 4.3. Agreement between the two assays

To determine the level of agreement between the assays a Bland-Altman plot was used, wherein the difference of HCV viral loads by the two assays were plotted against the mean of the techniques (Fig. 2). The mean difference between the Abbott and Xpert systems was 0.04 log<sub>10</sub> IU/ml, with limits of agreement ranging from −0.42 to 0.49 log<sub>10</sub> IU/ml. The two systems showed a good agree-



**Fig. 1.** Linear regression analysis for correlation between the assays, for all genotypes (A), genotype 1 patients (B), genotype 3 patients (C).

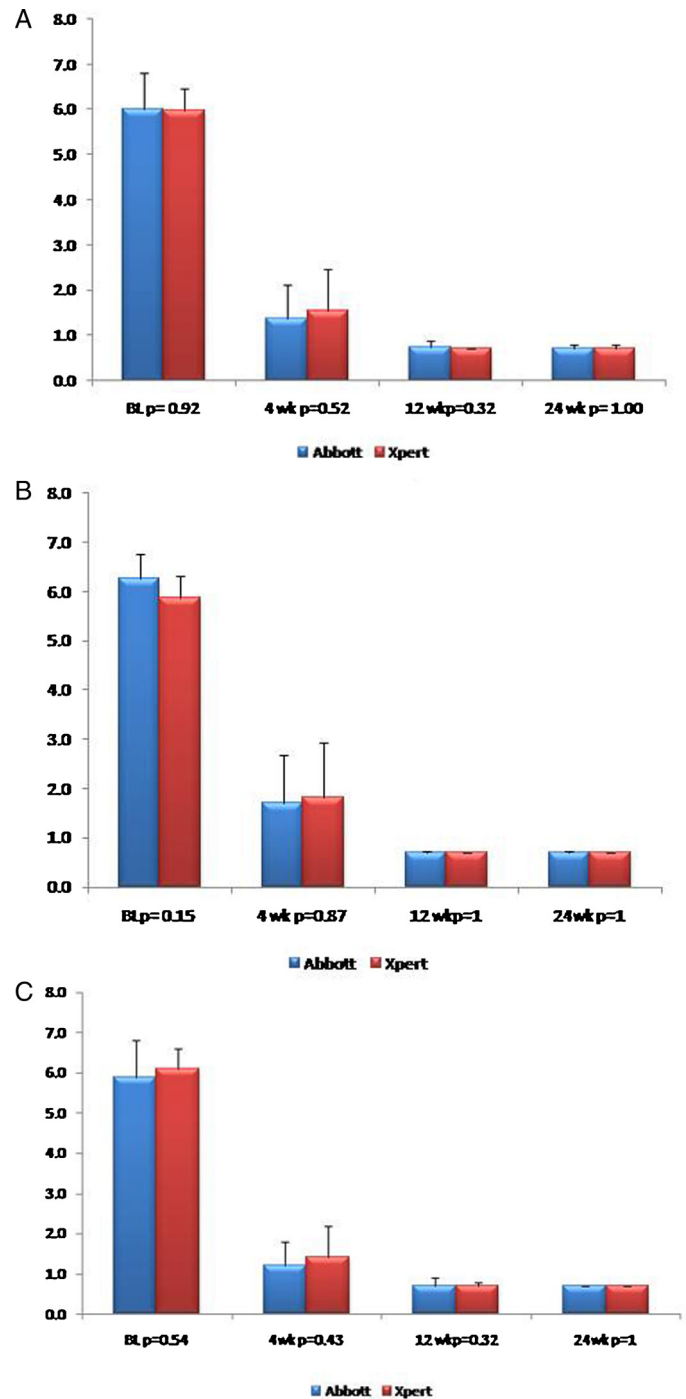
ment (Fig. 2A), with an Intraclass correlation coefficient (ICC) of 0.996 (95% CI: 0.994–0.007,  $p < 0.001$ ). There was good agreement for genotype 3 (ICC = 0.997, 95% CI: 0.995–0.998,  $p < 0.001$ ) as well as genotype 1 (ICC = 0.997, 95% CI: 0.992–0.999,  $p < 0.001$ ) (Fig. 2B and C).



**Fig. 2.** Level of agreement between the assays by Bland-Altman plot for all genotypes (A), genotype 1 patients (B), genotype 3 patients (C).

#### 4.4. Comparison of the two assays at different treatment time points

A set of 20 samples collected at the different treatment time points [baseline (BL), 4 weeks, 12 weeks and 24 weeks] was available for comparative analysis (Fig. 3). Two types of therapy were considered 15 were on direct acting antiviral: sofosbuvir plus ribavirin and 5 patients were on Interferon plus ribavirin. Overall good agreement between the assays was seen in treatment monitoring (ICC=0.73, 95% CI: 0.57–0.82,  $p < 0.05$ ) (Fig. 3A). There were



**Fig. 3.** Comparison between Abbott and Xpert assays at different treatment time points: baseline (BL), 4 week, 12 week and 24 weeks post initiation of the treatment, for all genotypes (A), genotype 1 patients (B), genotype 3 patients (C).

no significant differences at any of the four times between the assays and in any of the therapy. Cases of genotypes 1 and 3 were analysed separately as well (Fig. 3B and C), but no inter assay differences in the mean viral loads were seen at any measurement. No difference in different treatment regimens was seen between the assays. On posthoc analysis, there was a significant decrease in the viral load estimated by both the instruments over a period of time ( $p < 0.001$ ). The fall in viral load was significant from BL to 4th week as seen by both the assays, however no significant difference was seen in viral load decrease from 4th week to 12th and 24th week



**Table 3**  
Evaluation of Xpert using reference standards.

Reference standard	Mean (log IU/ml)	Standard deviation	Coefficient of variation
HPC	5.65	0.09	0.01
LPC	2.32	0.17	0.07

IU: international unit; ml: millilitre; HPC: high positive control; LPC: low positive control.

( $p > 0.05$ ). Both the instruments were comparable at 12th and 24th week in calculating the viral load ( $p = 1$ ) (Fig. 3).

#### 4.5. Evaluation of xpert using reference standards

High and low positive standards were tested ten times on Xpert to evaluate the precision of the assay (Table 3). The coefficient of variation for the ten viral load measurements was 0.01 for the HPC and 0.07 for the LPC, thus demonstrating good precision of the assay. For evaluation of linearity of Xpert, five serial 10-fold dilutions of the HPC were tested. On linear regression analysis, good correlation was seen between the measured HCV RNA concentrations and the expected concentrations ( $R = 0.99$ ).

## 5. Discussion

In this study, we evaluated the performance characteristics of the recently launched Xpert assay which is a point-of-care molecular test for HCV RNA viral load measurement. A good correlation between the performances of Xpert with Abbott assay was seen in the present study. Both the assays are based on Real Time PCR based method for quantification of viral load. Though Abbott 2000 SP platform used in the study is a fully automated Real time PCR system with automation for RNA extraction but it still requires biosafety set-up to prevent contamination. On the other hand, Xpert system is a small bench-top system that is free from biosafety handling. Both the assays when analysed for different ranges of viral loads demonstrated good concordance, irrespective of the viral genotype. Few discrepant results between the assays were mostly in samples where the viral load was less than the LLOQ of either assay. Both the assays had comparable LLOQ which is 12 IU/ml for Abbott and 10 IU/ml for Xpert, this makes both of them sensitive enough to be used as a confirmatory assay to serologically reactive HCV cases. If we consider undetectable and samples with loads <LLOQ as negative, the sensitivity of the Xpert assay for HCV RNA measurement was found to be 94.4% with a specificity of 100%. However, in our study false negativity was seen in 3 samples with Xpert assay. This discordance remains to be explained. Differences in technical features or concentration of RNA standard might be involved. The turnaround time for the Abbott assay was roughly 5 h as compared to 105 min for the Xpert assay. Such molecular tests with very less turn-around time and requiring minimal infrastructure and technical expertise would be ideal for de-centralization of molecular testing especially in low income countries, where viral load testing facilities are currently available only at a handful of apex centres. Utility of Xpert system in large laboratory set-up may not be justified because of the ability of the assay to handle fewer samples at one time as compared to Abbott system which can handle 96 samples in a single run. Nevertheless, Xpert assay has proven to be the game changer for tuberculosis by revolutionising the diagnosis and drug resistance detection in low resource settings, thus significantly decreasing morbidity associated with diagnostic delay and mistreatment [18,19]. Optimistically, it could be expected Xpert HCV may turn out to be an important step towards public health approach in rapid HCV diagnosis and referral to care and finally achieving elimination of HCV.

Given the importance of measuring viremia in the monitoring of HCV therapy, one concern is to select the best performing assay. Differences in the sensitivity of various viral load assays, especially for low amounts of HCV RNA could affect the decisions regarding treatment and further patient management [20]. Therefore comparison between the assays for treatment monitoring was also done in the present study. Both the assays demonstrated equal reliability in monitoring the patients on antivirals, whether Interferon based or on direct acting antivirals. However, small sample size of the study and lack of follow up of patients in terms of achieving SVR was a major limitation. Larger studies should be undertaken to confirm these observations and the usefulness of this assay for assessment of responses to treatment.

## 6. Conclusions

Molecular testing at the point-of-care, a step towards the decentralization of services, is beginning to enter clinical practice in low income countries. Xpert HCV, a point-of-care molecular test, demonstrated good performance in viral load measurement and in treatment course monitoring consistency as compared to Abbott Real Time PCR test.

## Competing interest

None declared.

## Ethical approval

The ethical approval for the study was taken from the Institutes Ethical Committee.

## Funding

None.

## Acknowledgement

We acknowledge Mr Gaurav Singh for the technical support.

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