HCV-RNA Detection In Small Volume Capillary Blood For Point-Of-Care Applications

P 549

UNIVERSITY OF LIVERPOOL
INSTITUTE OF INFECTION AND GLOBAL HEALTH

Mark Hopkins[1], Rachael Dagnall[2], Athanasios Papadimitropoulos[2], Paula Davies[1], Jillian Williams[1], Eimear Railton[1], Sarah Hau[1], Apostolos Beloukas[2], Anna Maria Geretti[2]

[1]Royal Liverpool University Hospital and [2]Institute of Infection & Global Health, University of Liverpool, Liverpool, UK

Background

- The rapid diagnosis of HCV infection is hampered by slow antibody responses and lack of reliable point-of-care tests (POCT)
- Patients often report preferring blood collection by fingerprick rather than by venous puncture
- The Cepheid GeneXpert platform provides a closed system for nucleic acid amplification and detection that makes it suitable for use outside of specialized laboratory settings including POCT; however, there are no published data on the use of small volume capillary blood for HCV RNA detection

Aim

To assess the performance of the GeneXpert quantitative HCV RNA assay in paired blood samples collected by venous puncture and by fingerprick in subjects with known HCV, HBV and HIV status

Methods

- **Study population**: With ethics approval, patients whose HCV RNA status had been determined in routine practice within the previous three months underwent paired blood collection by fingerprick (FP) and venous puncture in Mar 2016-Jan 2017 (Table 1)
- **Capillary blood** was collected by nursing staff using the Roche Accu-Chek Safety Safe-T-Pro Plus Lancet and the EDTA-containing Sarstedt Microvette® 100 K3, following established FP procedure. **Venous blood** was collected in parallel in EDTA tubes. Samples were transported at room temperature to the laboratory to be tested immediately by laboratory personnel using the GeneXpert XVI analyzer. FP blood (100µl) was added first to a cartridge, followed by 1ml Xpert diluent. In parallel, plasma (1.1 ml) separated from venous blood was added to another cartridge without sample diluent. Personnel was blind to the sample source
- **Assay sensitivity** with small (100µl) sample volume was tested using HCV RNA positive plasma from either clinical samples or external quality assurance (QA) panels, representing HCV genotypes 1a, 1b, 2b, 3a, and 4d (Fig 1)
- To verify **HCV RNA stability** in small sample volumes, 100µl venous blood was spiked with 100 IU/ml of the 4th International Standard for HCV RNA (NIBSC code 06/102), transferred into 2ml EDTA tubes, and left at room temperature for up to 10 days prior to testing (Table 3)

Table 1. Study population by known HCV RNA status

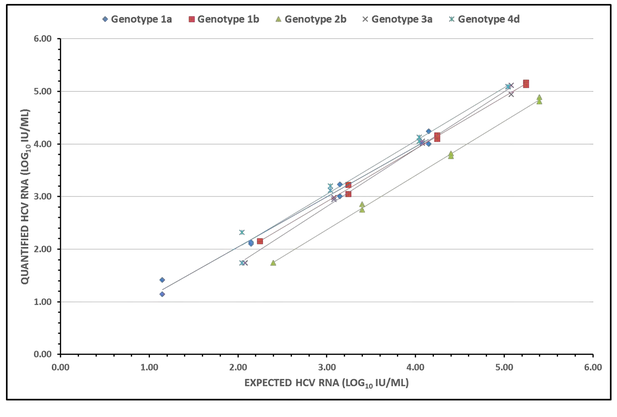
	HCV RNA positive N	HCV RNA negative N
Total	41	12
HCV Ab positive	41	3
Previous SVR	6	3
HIV Ab positive	9	7
HBsAg positive	1	3

Ab= Antibody; SVR= Sustained virological response, following pegylated IFN-alpha+ribavirin or ledipasvir/sofosbuvir; HBsAg= Hepatitis B surface antigen

Results: Assay performance

- Plasma samples in serial dilution representing five HCV genotypes were tested in duplicate using the small (100µl) volume GeneXpert® protocol. HCV RNA had been previously quantified using the standard protocol of the Roche COBAS® AmpliPrep/COBAS® TaqMan® assay, which provided the expected value (Fig 1)
- Relative to the expected quantification, the small volume protocol measured HCV RNA with a 100% detection rate down to a concentration of 140, 175, 250, 120 and 110 IU/ml for genotypes 1a, 1b, 2b, 3a and 4d, respectively
- There was good linearity (R^2 ≥0.99) across the five HCV genotypes. For each dilution series, median SDs (IQR) of duplicate HCV RNA measurements were were 0.17 (0.13-0.18), 0.04 (0.02-0.06), 0.05 (0.03-0.06), 0.03 (0.02-0.05) and 0.06 (0.04-0.15) for genotypes 1a, 1b, 2b, 3a and 4d, respectively
- Using a Qnostic QA HCV RNA panel representing genotypes 1b and 3a and spanning 200-20,000 IU/ml for genotype 1b and 63-6,300 IU/ml for genotype 3a, the small volume protocol similarly showed 100% HCV RNA detection rate at all concentrations (not shown)

Fig 1. Assay performance with small sample volume



Contact details: geretti@liverpool.ac.uk; beloukas@liverpool.ac.uk

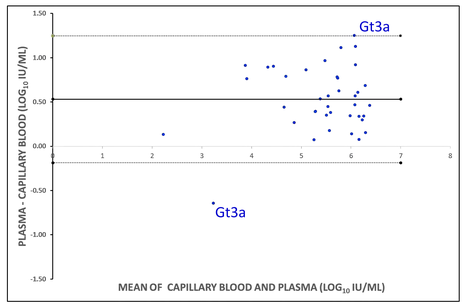
Results: HCV RNA detection in capillary blood vs. plasma

Table 2. HCV RNA detection and quantification in paired capillary blood and plasma

	Small volume *Capillary blood*	Standard volume *Plasma*
HCV RNA positive	39	39
HCV RNA, median log_{10} IU/ml (IQR)	5.3 (4.9-5.3)	6.0 (5.4-6.4)
HCV RNA negative	11	11
Invalid	1	1
Insufficient sample	1	

- Overall 50/53 patients yielded a valid result
- Among 50 patients with a valid result, HCV RNA detection showed 100% concordance between capillary blood and plasma, and with the patients' known HCV RNA status (Table 2)
- Median HCV RNA load was higher in plasma than in capillary blood. This was attributed to the fact that capillary blood collection tubes were frequently under-filled, reflecting the variability of FP blood collection in clinical practice

Fig 2. HCV RNA quantification in capillary blood vs. plasma



- The Bland–Altman analysis includes 39 paired samples that showed positive HCV RNA detection (Fig 2)
- The solid horizontal lines indicate the mean differences between the values; the horizontal dotted lines indicate the mean differences ±1.96 SD (representing the 95% confidence limits of the agreement)
- Two samples (both genotype 3a) fell outside the 95% level of agreement; one was underquantified in capillary blood and one in plasma

Table 3. HCV RNA stability at room temperature in small volume

Length of storage (hrs)	HCV RNA detection Run 1	HCV RNA detection Run 2
0	+	+
16	+	+
70	+	+
168	+	+
240	+	+

- In the stability experiments, there was no loss of HCV RNA detection in small volume (100µl) plasma spiked with HCV RNA (100 IU/ml) for up to 240 hours prior to testing in the small volume protocol (Table 3)

LIFE CHANGING | World Shaping

Conclusions

- **Small volume capillary blood collected by fingerprick offers a reliable method for HCV RNA detection by GeneXpert®**
- **In its described application, the approach is suitable for screening undiagnosed populations even when accounting for the variability of sampling by fingerpick in routine practice**
- **Viral load measurements were overall consistent between small and large volume testing. The agreement can be improved by ensuring that capillary collection tubes are not underfilled, allowing a full 100µl sample to be tested**
- **Results provide the evidence base for evaluating use as POCT in non-specialist laboratory settings**