Evaluation of HCV RNA testing from finger-stick capillary dried blood spot and venepuncture-collected samples: A cohort study

Beth Catlett[1,2], Sahar Bajis[1], Mitchell Starr[2], Behzad Hajarizadeh[1], Gregory J Dore[2], Jason Grebely[1*] and Tanya L Applegate[1*]
[1]The Kirby Institute, UNSW Sydney, Sydney, NSW, Australia, [2]St Vincent's Hospital, Sydney, NSW, Australia
*Joint senior authors, equally contributed to the work
bcatlett@kirby.unsw.edu.au

Introduction

- Simplified HCV diagnostic strategies to increase testing and linkage to care are needed.
- Novel collection methods such as finger-stick capillary dried blood spots (DBS) have advantages over standard phlebotomy, especially among people who inject drugs where venous access may be a barrier to testing.

Aims

To evaluate the diagnostic performance of the Hologic Aptima HCV Quantitative assay for the detection and quantification of HCV RNA with finger-stick capillary DBS and venepuncture samples.

Methods

Study design and participants

- LiveRLife is an observational cohort study evaluating the effectiveness of an intervention integrating non-invasive liver disease screening on HCV assessment and treatment uptake.
- To be included in the study, participants were 18 years or older, had written informed consent and a history of injecting drug use. Current pregnancy was the only exclusion criterion.
- Plasma and finger-stick capillary DBS samples were collected from participants enrolled at six sites in Australia (four drug treatment clinics, one homelessness service and one needle and syringe programme).
- Paired plasma and DBS samples were analysed for HCV RNA on the Hologic Aptima HCV Quant assay (DBS were punched out manually using 1cm punch size and eluted in 1mL Aptima transfer media for 30 minutes on a blood wheel).

Statistical analyses

- Baseline characteristics of participants enrolled in the study (Table 1).
- Assessment of sensitivity and specificity in DBS compared with the plasma at different thresholds of HCV RNA levels.
- Distribution of HCV RNA viral load including Deming Regression analysis.
- Bland-Altman analysis to show bias and agreement in detectable HCV RNA.

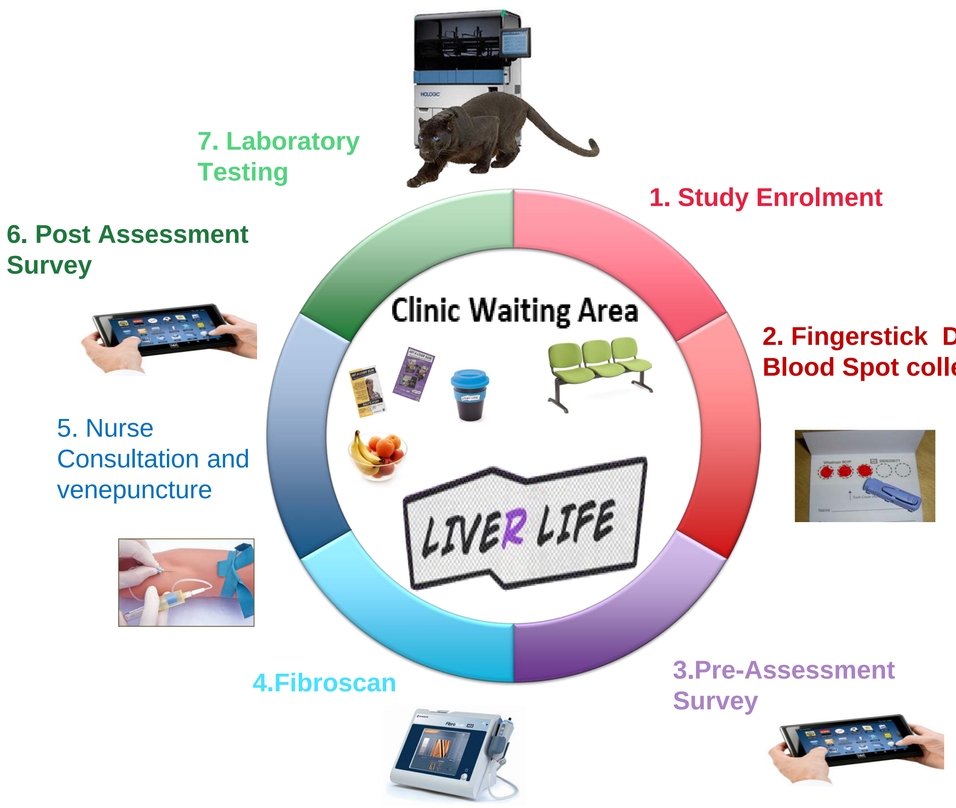


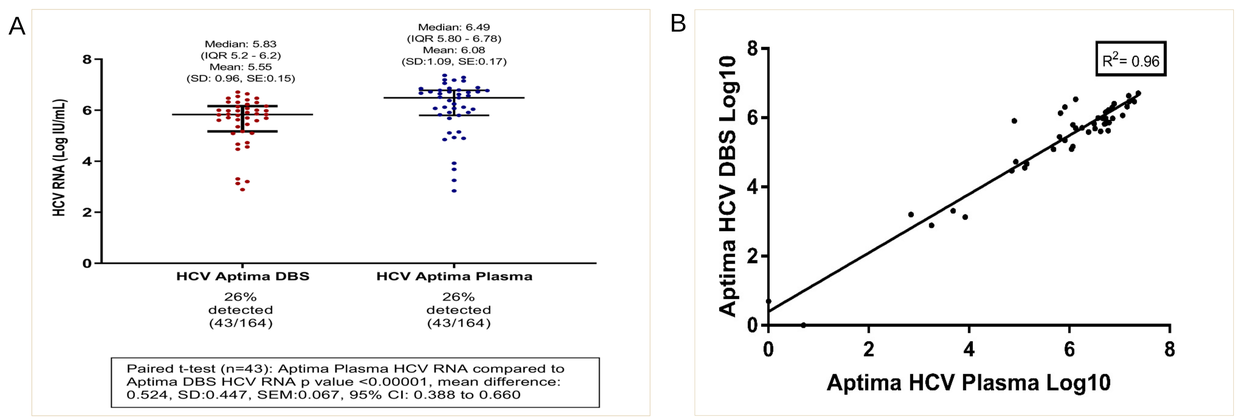
Figure 1. LiveRLife study assessments

Results

Table 1. Baseline characteristics of participants enrolled in LiveRLife study (N=164[1])

Characteristics	N (%)
Age, median (25%, 75%)	46 (36, 52)
Gender	
Male	124 (76)
Female	25 (15)
Transgender	1 (1)
Unknown[2]	14 (8)
History of ever injecting drugs	
No	52 (32)
Yes	98 (60)
Unknown[2]	14 (8)
Injecting drug use in the last month[3]	
No	34 (35)
Yes	63 (64)
Unknown[2]	1 (1)
Opioid substitution therapy[3]	
No	29 (30)
Yes, previously	18 (18)
Yes, currently	50 (51)
Unknown[2]	1 (1)
FibroScan® liver disease stage	
F0-1	106 (65)
F2	22 (13)
F3	5 (3)
F4	11 (7)
Invalid score	20 (1)

F0-1 absent of mild fibrosis, F2 significant fibrosis, F3 severe fibrosis, F4 Cirrhosis [1]11 individuals receiving HCV treatment at enrolment were excluded from the analysis[2] Missing due to loss of data during data transfer from tablet computer and one stolen tablet. [3]Among people with a history of injecting drug use



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Threshold	Sensitivity	Specificity
Detectable HCV RNA	95.6%	94.1%
95% CI	85-99.5%	88-97.6%
≥10 IU/mL	100%	100%
95% CI	91.8-100%	97-100%
≥1,000 IU/mL	100%	100%
95% CI	91.8-100%	97-100%

Figure 2 A: Distribution of paired venous and capillary DBS paired samples (among detectable HCV RNA). Figure B: Deming correlation analysis showing agreement between paired samples. Figure C: Bland-Altman analysis to show bias and agreement in detectable HCV RNA in both DBS and Plasma specimens. Figure D: Sensitivity and specificity of HCV RNA detection among DBS samples compared to venous plasma (gold standard).

- HCV RNA was detected in 45 plasma samples (27% [95% CI 21-35]).
- A small bias of 0.37 Log10 in plasma over DBS (95% CI -0.8-1.6) was observed with good agreement (R^2=0.96).
- Sensitivity of the Aptima HCV assay for HCV RNA detection in DBS was 95.6% (95% CI 84.9-99.5%) and specificity was 94.1% (95% CI 88.3-97.6%).
- Sensitivity for HCV RNA quantification in DBS (≥10 IU/mL in plasma) was 100% (95% CI 91.8%-100%) and specificity was 100% (95% CI 97%-100%).

Conclusion

The Aptima HCV Quant assay can detect active infection from DBS with acceptable diagnostic performance and is clinically comparable to plasma. This data will strengthen the case for the registration (with stringent regulatory authority approval) of a DBS kit insert claim, enabling future clinical utility.

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