Hepatitis C virus core antigen and dried blood spots as simplified hepatitis C virus diagnostic tools

François MJ Lamoury[1], Behzad Hajarizadeh[1], Angelica Soker[1], Danica Martinez[1], Camelia Quek[1], Philip Cunningham[2], Beth Catlett[2], Gavin Cloherty[3], Pip Marks[1], Janaki Amin[1], Jason Grebely[1], Gregory J. Dore[1], Tanya L. Applegate[1]

1 The Kirby Institute, UNSW Australia, Sydney, Australia. 2 St Vincent's Applied Medical Research, Darlinghurst, Sydney, Australia. 3 Abbott Virology, Abbott Park, IL, USA.

Introduction

- Simple, affordable diagnostic and treatment monitoring tools are urgently required to scale up interferon-free HCV treatment
- HCV core antigen (HCVcAg) provides an alternative tool to detect HCV viraemia in dried blood spot (DBS)

Background to DBS

- Concept of blotting blood on paper introduced by Robert Guthrie in the 1960s.
- DBS widely used for diagnostic screening of metabolic disorder in newborn babies
- Provide easier blood collection: DBS kit (Fig1A), sample by finger prick (Fig1B, 1C), dried DBS cards (Fig 1D), shipped by regular mail to central laboratory for testing (Fig1E)
- DBS and HCV: Rise of testing and diagnosis associated with Scotland's action plan on Hepatitis C with introduction of DBS HCV Ab testing in drug services (Fig 1F. McLeod, BMJ, 2014)

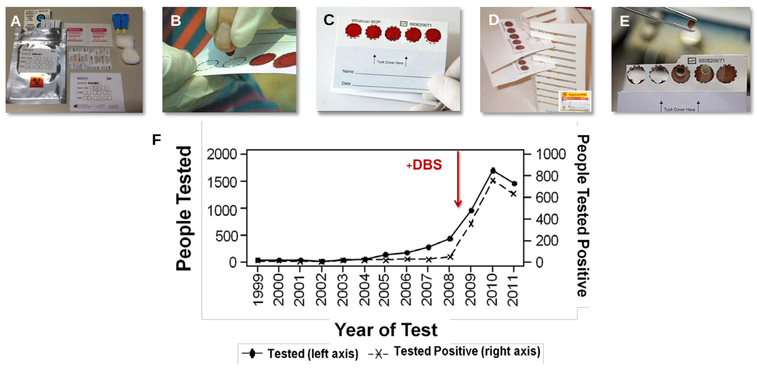

Figure 1: **DBS collection (A-E) and impact (F, McLeod, J Edidemiol Community health, 2014).**

Aim

To evaluate the diagnostic performance of HCV core antigen detection in plasma and DBS

Method

Study design and participants

- Paired plasma and venous DBS samples were prepared from remnant diagnostic samples
- DBS were spotted with 50μL of EDTA blood
- 2x10mm spots were eluted 1h at room temperature in 400mL of PBS-0.25% Triton X100

Study measurements and analysis

- HCV RNA in plasma - AmpliPrep/COBAS Taqman assay (Roche) (Gold standard).
- Core antigen - ARCHITECT HCV Ag (Abbott Diagnostics), in plasma and DBS.
- A conversion factor of 1fmol/L = 500IU/mL was used to to assess agreement between both test with Bland-Altman Bias plot (Chevaliez S et al. Antiviral therapy 2016).
- Sensitivity and specificity were assessed for the HCVcAg (>3fmol/L) at a threshold of HCV RNA > 1000IU/mL were calculated for both plasma and DBS.

Results

Table 1: **Characteristics of the paired plasma and venous DBS sample population**

	Total (n=120) n(%)
PLASMA HCV RNA detected	95 (79.2)
PLASMA HCV RNA non-detected	25 (20.8)
	Median concentration (n=120) (IQR)
Median LOG **HCV RNA** IU/mL in **PLASMA**	5.57 (2.52-6.16)
Median LOG **HCVcAg** fmol/L in **PLASMA**	2.29 (0.07-3.13)
Median LOG **HCVcAg** fmol/L in **DBS**	1.14 (0.00-1.91)

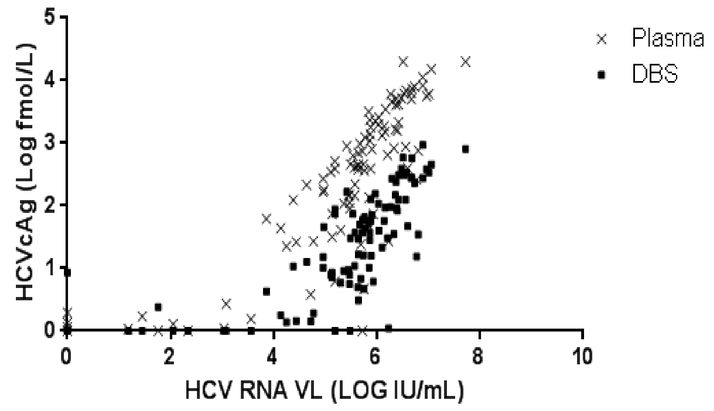

Figure 2: **Correlation between HCVcAg in plasma and DBS, with HCV RNA plasma.** HCVcAg is strongly related to HCV RNA for plasma samples (r=0.89, 95% CI: 0.85 to 0.92, p<0.0001) and DBS (r=0.81, 95% CI: 0.73 to 0.86, p<0.0001).

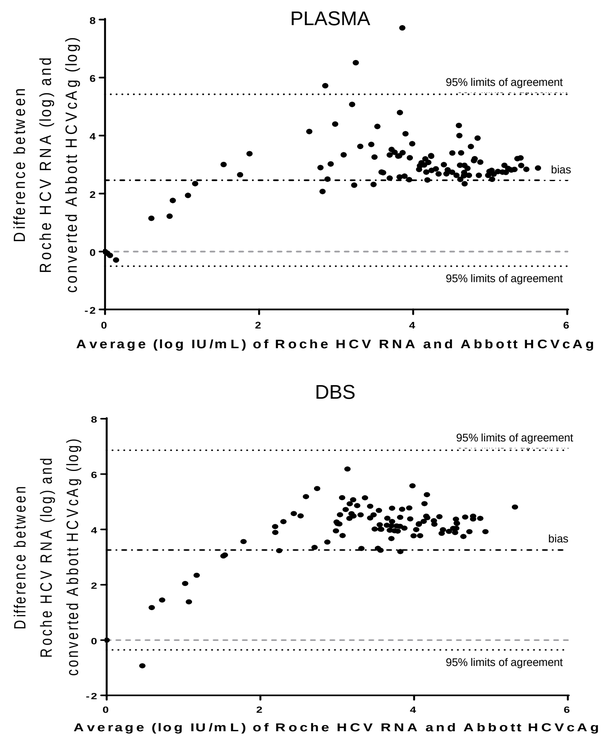

Figure 3: **Bland-Altman Bias plot: HCVcAg vs Roche HCV RNA for plasma (A) and DBS (B) paired samples.** These plots show the difference between the values of HCV RNA and HCVcAg as a function of the average of these two values. HCVcAg levels were converted to log IU/mL based on a conversion factor of 1fmol/L = 500IU/mL. A. The Bland-Altman Bias (95% limits of agreement) for plasma was 2.46 log IU/mL (-0.50, 5.42) with mean difference (95%CI) of 2.46 log IU/mL (2.19-1.51). B. The Bland-Altman Bias (95% limits of agreement) for DBS was 3.26 log IU/mL (-0.35, 6.86) with mean difference (95%CI) of 3.25 log IU/mL (2.92-3.59).

Table 2: **Sensitivity and specificity of HCVcAg for paired plasma and DBS sample compared with Roche HCV RNA in plasma as the gold standard using HCV RNA thresholds of 15IU/mL or 1000IU/mL.**

Roche HCV RNA 15IU/mL threshold

Abbott HCVcAg plasma	Detected +	Undetected -	Total
Detected +	87	0	87
Undetected -	7	26	33
Total	94	26	120
Sensitivity	**92.6% (95%CI, 85-97%)**		
Specificity	**100% (95%CI, 84-100%)**		
Abbott HCVcAg DBS	Detected +	Undetected -	Total
Detected +	81	1	82
Undetected -	13	25	38
Total	94	26	120
Sensitivity	**86.2% (95%CI, 77-92%)**		
Specificity	**96.1% (95%CI, 78-100%)**		

1000IU/mL threshold

Abbott HCVcAg plasma	Detected +	Undetected -	Total
Detected +	87	0	87
Undetected -	3	30	33
Total	90	30	120
Sensitivity	**96.7% (95%CI, 90-99%)**		
Specificity	**100% (95%CI, 86-100%)**		
Abbott HCVcAg DBS	Detected +	Undetected -	Total
Detected +	81	1	82
Undetected -	9	29	38
Total	90	30	120
Sensitivity	**90.0% (95%CI, 81-95%)**		
Specificity	**96.7% (95%CI, 81-100%)**		

- Roche HCV RNA 15IU/mL threshold: The median viral load undetable by HCVcAg for DBS (n=13 / 94) was 3659 IU/mL (min 14 IU/mL / max log 6.2IU/mL).
- The false positive DBS (n = 1/26) detected HCVcAg at 7.52fmol/L.

Conclusion

These preliminary data indicate:

- **Despite reduced sensitivity compared to plasma, core antigen testing in DBS may provide a suitable screening and diagnostic tool for chronic HCV due high levels of HCV among this population (Hajarizadeh 2015; Hill AASLD, 2015).**
- **Further work is required to understand potential mechanism of reduced sensitivity in those undetected by HCVcAg.**
- **The feasibility of centralised Core antigen testing on DBS should be assessed as a diagnostic tool in remote settings, lower and middle-income countries.**

Acknowledgements:

We would like to acknowledge Hideaki Toji and Cristina Ferrarini from Sydpath for the DBS collection. We would like to acknowledge Joymarie Armstrong, Spyrros Repoussis, Mark Paul, Rebecca Collins and Lisa Stanton from Sydpath for their assistance for Core Ag testing. This study was funded by the Australian Government Department of Health and Ageing. The views expressed in this publication do not necessarily represent the position of the Australian Government. The Kirby Institute is affiliated with the Faculty of Medicine, University of New South Wales. The opinions expressed in this paper are those of the authors and do not necessarily represent that of Merck Sharp and Dohme. Support from Abbott Diagnostics for the supply of reagents is gratefully acknowledged.