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

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Dried blood spot

• Biosampling where blood samples are blotted and dried on filter paper

• Advantages
  ✓ Easy and inexpensive
  ✓ Painless / non-invasive
  ✓ Less medical training
  ✓ Easier access to high risk populations
  ✓ Facilitate testing for remote areas

• Inconvenient
  ✓ Low sample volume
  ✓ Analyte degradation if storage condition is not respected¹
  ✓ Assay certification for clinical use – Research tool for specialized lab

¹ Cassol S et al. J Clin Microb 1992
Strategies to enhance linkage to HCV care

Alternative assays:
- HCV Core antigen
- Rapid antibody tests
- HCV RNA point of care

Strategies to enhance linkage to HCV care

Sample types:
- Oral fluid
- Dried blood spot
- Capillary blood (finger-stick)
Diagnostic models of care

Centralised

Small labs

Central lab

Clinics

Drug and alcohol clinics

Primary health care / GPs

Community health centres

NSP services

Sexual health

Prisons

Decentralised
Diagnostic models of care

On-line, self collected DBS for HIV testing

1. Order online
2. DBS kit delivered
3. Self sample
4. Post to lab
5. Central lab test
6. Phone call
7. Reactive?
8. Referral to care
9. Order online

HIV testing in comparison with STI clinics (UK)
- Equal recruitment, return results, and reactivity
- DBS covered broader geographic area
DBS and HCV: the Scotland’s action plan (2009)

• DBS testing introduced into specialised drug services during 2009

• Test for HCV Antibody testing

• Drug services referred 16% of new HCV diagnosed in Scotland during 2009-13 (compared to <1% during 2003-08)
Aim

*Evaluate the diagnostic performance of HCV core antigen detection in plasma and DBS*
Methodology – sample processing

**DBS preparation**
50µL whole blood per spot
Whatman 903 Protein saver card

**10mL EDTA blood Collection**

**Plasma collection**

**Plasma and DBS Storage** in -80°C freezer

**HCV RNA levels testing**
AmpliPrep/COBAS Taqman assay (Roche)

**DBS elution**
2 x 10mm spot
Elution in 400µL PBS-0.25% Triton-X100 for 1h, RT

**HCV core antigen Architect assay**
ARCHITECT-i2000R Immunoassay Analyser

**HCVcAg sample volume:**
Plasma: 108µL
DBS eluate: equivalent to 13.5µL plasma
Result – Setting up Core antigen conditions

Plasma dilutions

Plasma samples

Limit of detection 3fmol/L 612IU/mL
Limit of quantitation 10fmol/L 2261IU/mL

Cutoff value of HCVcAg test
in terms of HCV RNA levels (IU/ml)

References for studies

Ross et al., J Clin Microbiol 2010. 500-3,000 IU/ml
Ottiger et al., J Clin Virol 2013. 3,467 IU/ml
## Result – Specimen characteristics

### Characteristics of the paired plasma and venous DBS sample population

<table>
<thead>
<tr>
<th></th>
<th>Total (n=120) n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLASMA HCV RNA detected</td>
<td>HCV + 95 (79.2)</td>
</tr>
<tr>
<td>PLASMA HCV RNA non detected</td>
<td>HCV - 25 (20.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Median concentration (n=120) (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOG HCV RNA IU/mL in PLASMA</td>
<td>5.57 (2.52-6.16)</td>
</tr>
<tr>
<td>LOG HCVcAg fmol/L in PLASMA</td>
<td>2.29 (0.07-3.13)</td>
</tr>
<tr>
<td>LOG HCVcAg fmol/L in DBS</td>
<td>1.14 (0.00-1.91)</td>
</tr>
</tbody>
</table>
Correlation between HCVcAg in plasma and DBS, with HCV RNA plasma

Correlation coefficient
Plasma samples \( (r=0.89, \text{ 95\% CI: 0.85 to 0.92, } p<0.0001) \)
DBS samples \( (r=0.81, \text{ 95\% CI: 0.73 to 0.86, } p<0.0001) \).
## Result – Sensitivity and specificity for paired plasma and DBS

### HCV RNA > 15IU/mL

<table>
<thead>
<tr>
<th>HCVcAg plasma</th>
<th>Roche HCV RNA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>87</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>7</td>
<td>26</td>
</tr>
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</table>

- **Sensitivity**: 92.6% (95% CI, 85-97%)
- **Specificity**: 100% (95% CI, 84-100%)

**False positive**: HCVcAg: 7.5 fmol/L

**False negative**: HCV RNA: 1100 / 1200 / 3659 IU/mL

HCV RNA > 15 IU/mL
Conclusion

- DBS HCVcAg detection showed over one log reduction compared to plasma.

- Correlation of HCVcAg compared to plasma HCV RNA satisfactory in plasma when VL >3 log IU/mL and DBS when VL >4 log IU/mL.

- Further work is required to understand potential mechanism of reduced sensitivity in those undetected by HCVcAg.

- The feasibility of testing Core antigen on DBS should be further assessed as a diagnostic tool in remote settings, lower and middle-income countries.
Discussion – increasing HCV testing in LMIC

Choice of HCV test:

- Epidemiology
- Infrastructure
- Easy to use
- Cost
- Analytical sensitivity
  - Nucleic acid testing
  - Immunoassay
  - DBS
  - Rapid diagnostic testing

- HCV RNA Threshold > 25IU/mL
- Chronic HCV population 100%
- Assay cost $$$$

Access to testing

1 A. Hill AASLD 2015, Adapted from Bowden, 2007
Other and future application

• Core antigen as point of care testing\(^1\)

**Daktari™ system**

- HCVcAg point of care instrument released in 2018
- Need a drop of blood to the cartridge
- Result in 30 minutes
- Cost US$ 15-20 per assay, US$ 8000 for the instrument

• Other use of DBS

- HCV RNA testing
- Sequencing for genotyping, phylogenetic and resistance studies
Acknowledgments

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HCV Core antigen (HCVcAg)

- Can detect active infection
- Easy to perform
- Less expensive
- More stable
- Expressed as fmol/L \((10^{-15} \text{ mol/L})\)

Measured with the HCV Ag ARCHITECT assay on the ARCHITECT-i2000R Immunoassay Analyser.

- Range: 0 – 20000 fmol/L
- Reactive > 3 fmol/L. Quantified > 10 fmol/L
Result – Comparison between plasma and DBS

Bland-Altman Bias plot: HCVcAg vs Roche HCV RNA for plasma

HCVcAg levels were converted to log IU/mL based on a conversion factor of 1fmol/L = 500IU/mL

<table>
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<tr>
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<th>Bland-Altman Bias (95% limits of agreement)</th>
<th>mean difference (95%CI)</th>
</tr>
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<tbody>
<tr>
<td>Plasma</td>
<td>2.46 log IU/mL (-0.50, 5.42)</td>
<td>2.46 log IU/mL (2.19-1.51)</td>
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<tr>
<td>DBS</td>
<td>3.26 log IU/mL (-0.35, 6.86)</td>
<td>3.25 log IU/mL (2.92-3.59)</td>
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### 13 DBS HCV RNA detected / HCVcAg non-detected

<table>
<thead>
<tr>
<th>ID:</th>
<th>Gt</th>
<th>VL (IU/mL):</th>
<th>Plasma Core Ag fmol/L</th>
<th>DBS Core Ag fmol/L</th>
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<tr>
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<td>8888-61231-313</td>
<td>1a</td>
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<td>0</td>
<td>1.4</td>
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<td>8888-61231-386</td>
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<td>0.28</td>
<td>0</td>
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<tr>
<td>8888-61231-388</td>
<td>3a</td>
<td>220</td>
<td>0</td>
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<tr>
<td>8888-61231-387</td>
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### 9 plasma HCV RNA detected / HCVcAg non-detected

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