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

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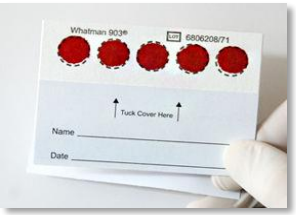
Dried blood spot

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



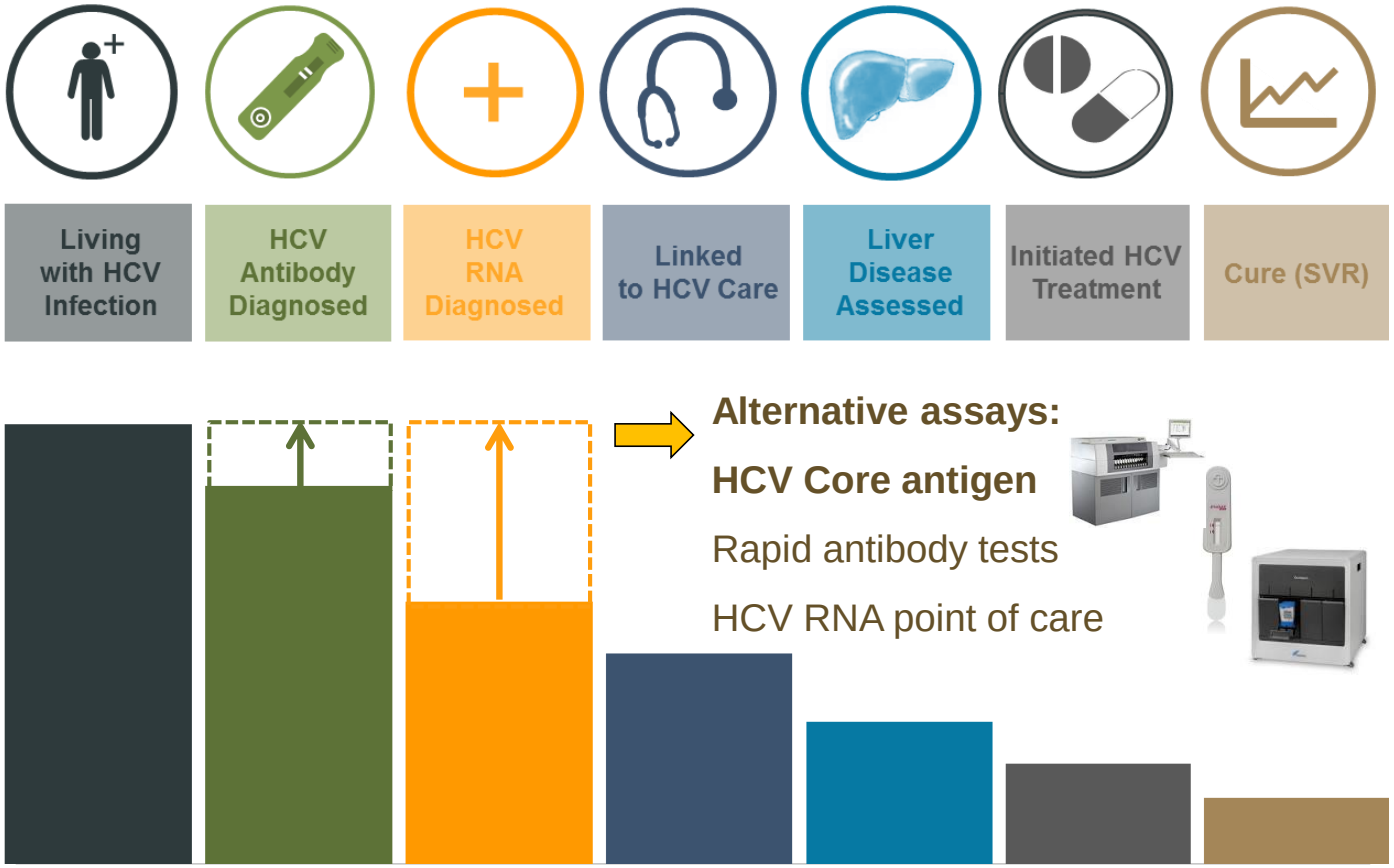



- Advantages
  - ✓ Easy and inexpensive
  - ✓ Painless / non-invasive
  - ✓ Less medical training
  - ✓ Easier access to high risk populations
  - ✓ Facilitate testing for remote areas

- Inconvenient
  - ✓ Low sample volume
  - ✓ Analyte degradation if storage condition is not respected[1]
  - ✓ Assay certification for clinical use – Research tool for specialized lab

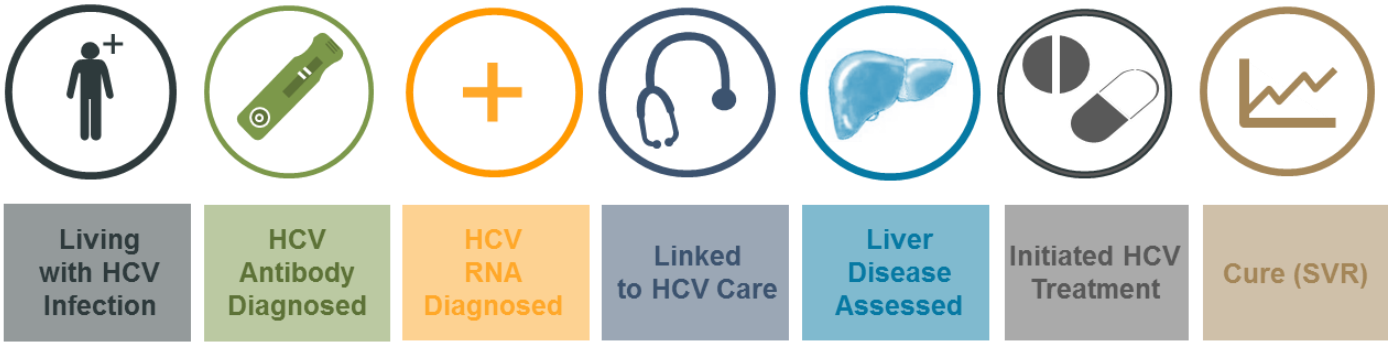
1 Cassol S et al. J Clin Microb 1992

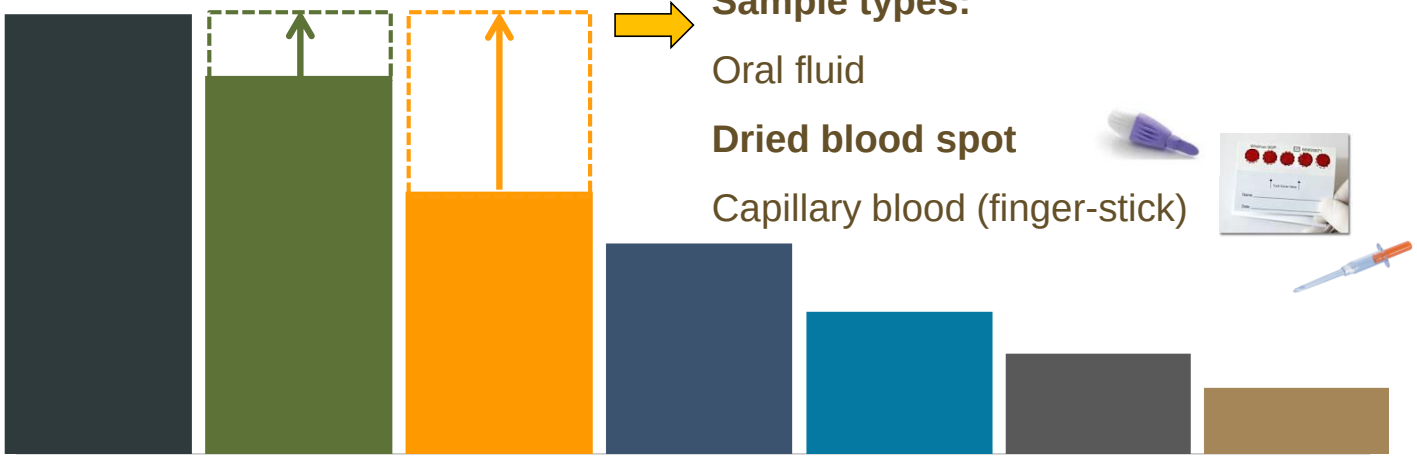
Strategies to enhance linkage to HCV care



Grebely J et al. *Int J Drug Policy* (2015)

Strategies to enhance linkage to HCV care





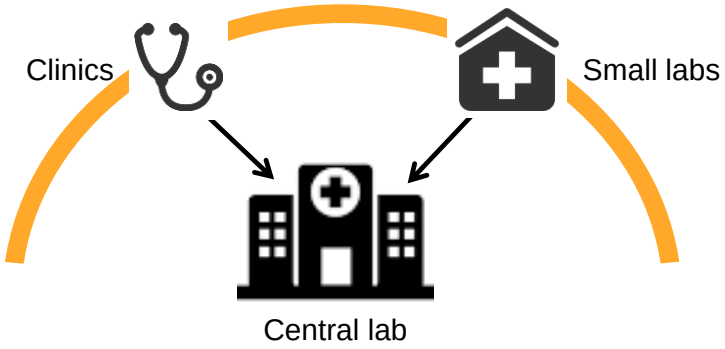
Sample types:

Oral fluid

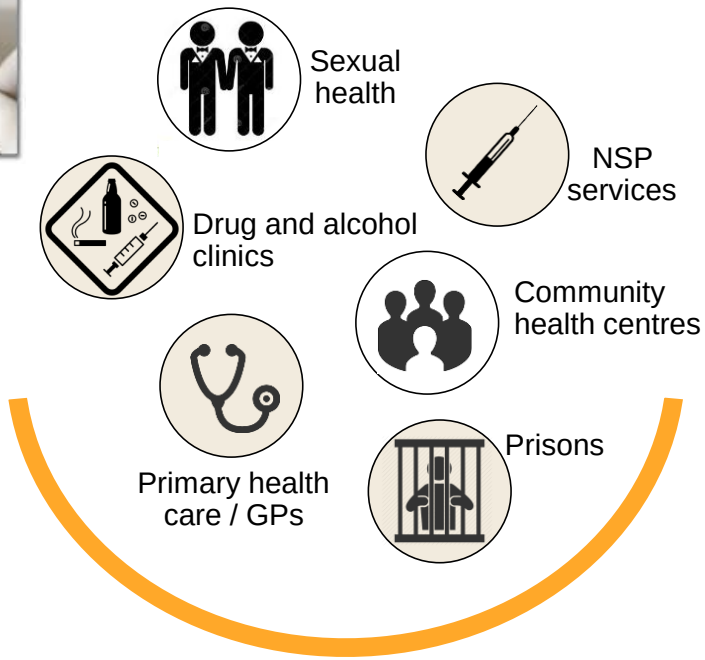
Dried blood spot

Capillary blood (finger-stick)

Diagnostic models of care



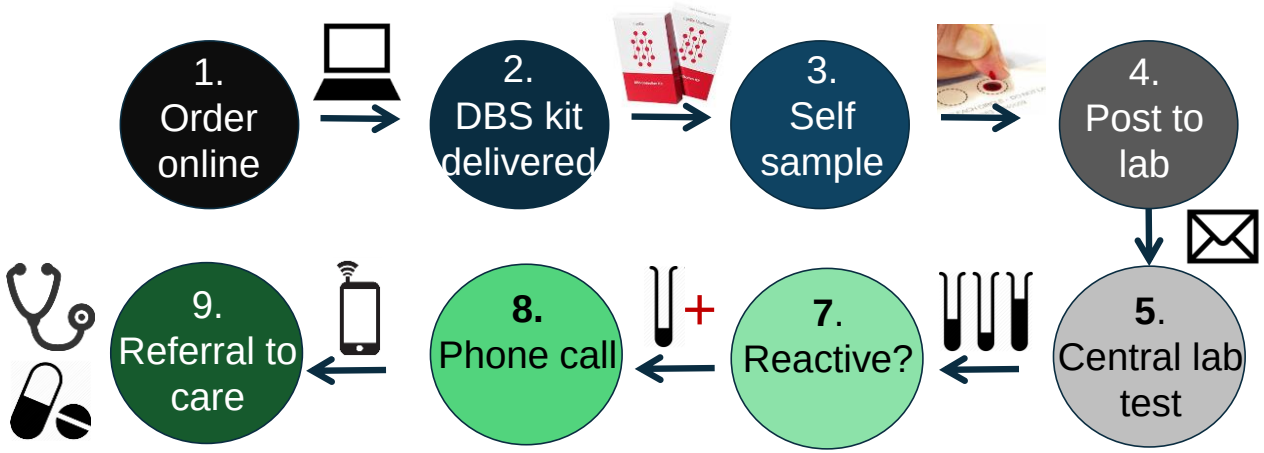
Centralised



Decentralised

Diagnostic models of care

On-line, self collected DBS for HIV testing

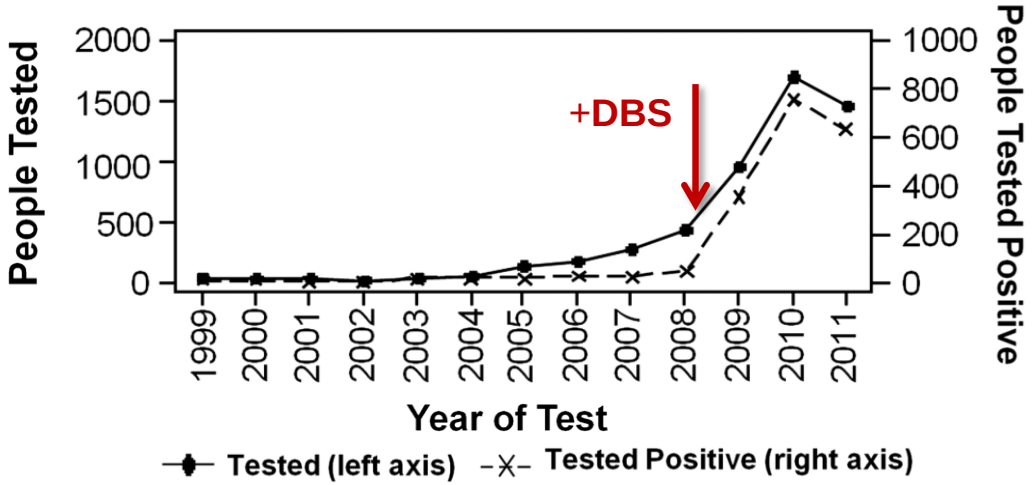


HIV testing in comparison with STI clinics (UK)

- 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)

McLeod A et al. J Epidemiol Community Health 2014

Aim

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

Methodology – sample processing

10mL
EDTA blood
Collection

Plasma collection

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

Plasma and DBS Storage in -80C freezer

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

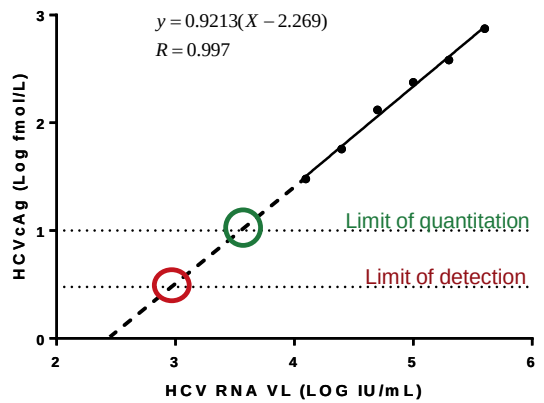
DBS elution
2 x 10mm spot
Eluation in 400µL
PBS-0.25% Triton-X100
for 1h, RT

HCV core antigen Architect assay
ARCHITECT-i2000R Immunoassay Analyser

HCVcAg sample volume:
Plasma: 108µL
DBS eluate: equivalent to 13.5µL plasma

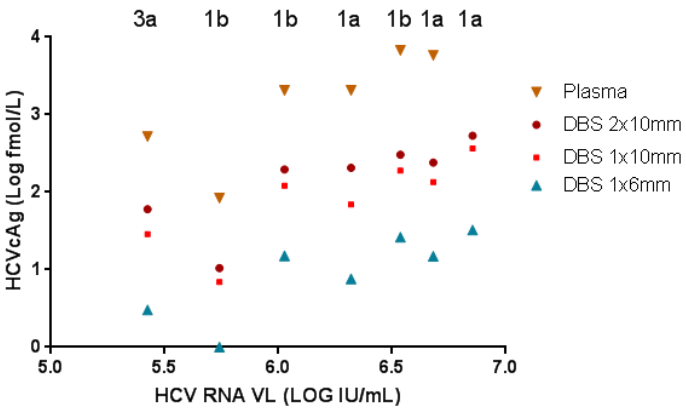
Result – Setting up Core antigen conditions

Plasma dilutions



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

Plasma samples



2 x 10mm DBS	-1.1 log fmol/L
1 x 10mm DBS	-1.4 log fmol/L
1 x 6mm DBS	-2.4 log fmol/L

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

References for studies

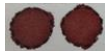
Ross et al., J Clin Microbiol 2010.	500-3,000 IU/ml
Ottiger et al., J Clin Virol 2013	3,467 IU/ml

2 Ross et al., J Clin Microbiol 48:1161. 2010; 3 Murayama et al., J. Clin. Microbiol. 50: 1943. 2012; 4 Ottiger et al., J Clin Virol 58:535-540. 2013; Medici et al., J Clin Virol 51: 264. 2011

Result – Specimen characteristics

Characteristics of the paired plasma and venous DBS sample population

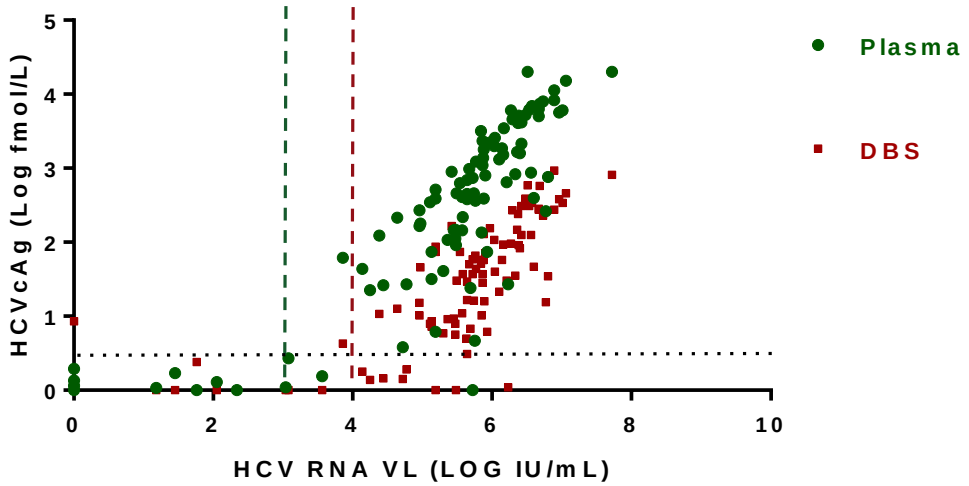
		Total (n=120) n(%)
PLASMA HCV RNA detected	HCV +	95 (79.2)
PLASMA HCV RNA non detected	HCV -	25 (20.8)

	Median concentration (n=120) (IQR)
LOG HCV RNA IU/mL in PLASMA  	5.57 (2.52-6.16) 
LOG HCVcAg fmol/L in PLASMA  	2.29 (0.07-3.13)
LOG HCVcAg fmol/L in DBS  	1.14 (0.00-1.91)

Δ 1.15 log

Result – correlation between plasma and DBS

Correlation between HCVcAg in plasma and DBS, with HCV RNA plasma



Correlation coefficient

Plasma samples (r=0.89, 95% CI: 0.85 to 0.92, p<0.0001)

DBS samples (r=0.81, 95% CI: 0.73 to 0.86, p<0.0001).

Result – Sensitivity and specificity for paired plasma and DBS

HCV RNA > 15IU/mL

HCVcAg **plasma**	Roche HCV RNA **+**	**-**
+	87	0
-	7	26
Sensitivity	**92.6%** (95%CI, 85-97%)	
Specificity	**100%** (95%CI, 84-100%)	

Conclusion

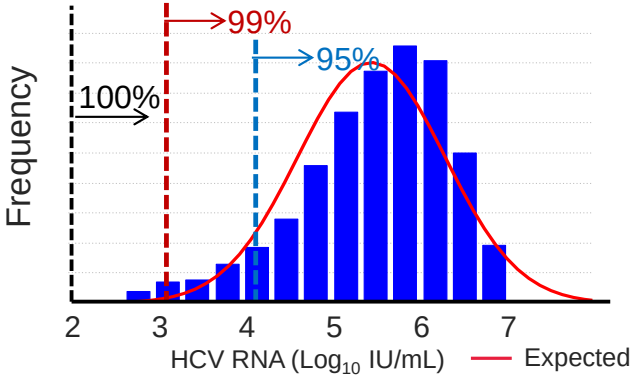
- DBS HCVcAg detection showed over one log reduction compared to plasma
- Correlation of HCVcAg compared to plasma HCV RNA satisfactory in plasma when VL >3 log IU/mL and DBS when VL >4 log IU/mL
- Further work is required to understand potential mechanism of reduced sensitivity in those undetected by 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:

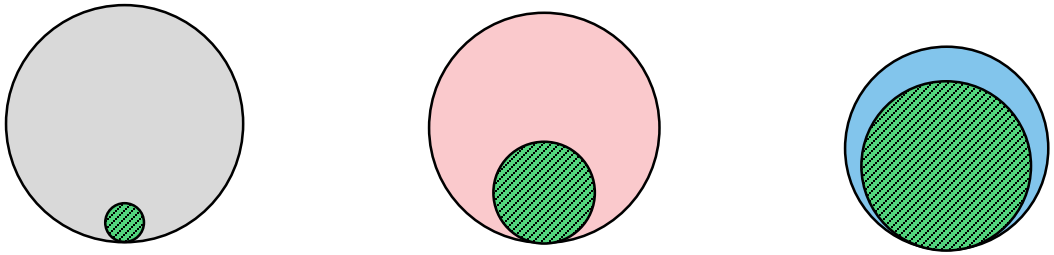
- ✓ Epidemiology
- ✓ Infrastructure
- ✓ Easy to use
- ✓ Cost
- ✓ Analytical sensitivity
  - ➢ Nucleic acid testing
  - ➢ Immunoassay
  - ➢ DBS
  - ➢ Rapid diagnostic testing

Distribution of HCV RNA in chronic HCV infection



- ➢ 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

1: Hepatitis C Diagnostic Technology Landscape 1st Edition 2015, Unitaid

Acknowledgments

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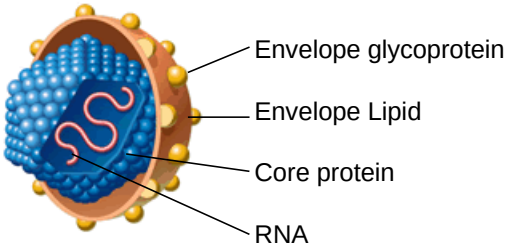
HCV Core antigen (HCVcAg)

- Can detect active infection
- Easy to perform
- Less expensive
- More stable
- Expressed as fmol/L (10^{-15} mol/L)
- Measured with the HCV Ag ARCHITECT assay on the ARCHITECT-i2000R Immunoassay Analyser.

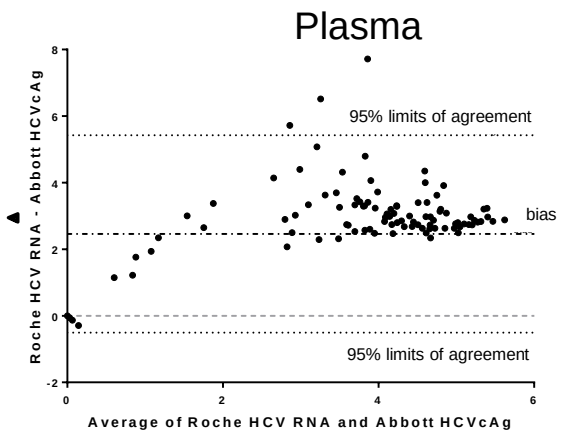
  Range: 0 – 20000fmol/L

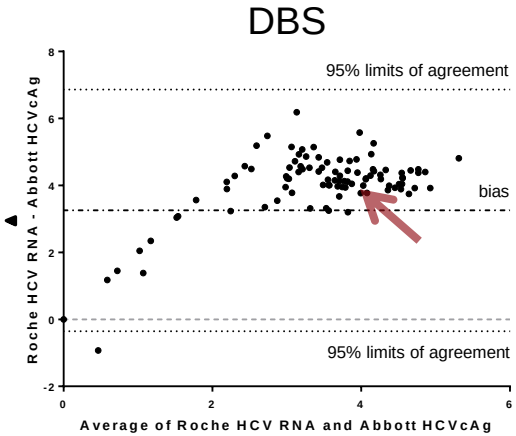
  Reactive > 3fmol/L. Quantified > 10fmol/L



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[1,2,3,4,5]

	Bland-Altman Bias (95% limits of agreement)	mean difference (95%CI)
Plasma	2.46 log IU/mL (-0.50, 5.42)	2.46 log IU/mL (2.19-1.51)
DBS	3.26 log IU/mL (-0.35, 6.86)	3.25 log IU/mL (2.92-3.59)

1 Chevaliez S et al. Antiviral therapy 2016; 2 Ross et al., J Clin Microbiol 48:1161. 2010; 3 Murayama et al., J. Clin. Microbiol. 50: 1943. 2012; 4 Ottiger et al., J Clin Virol 58:535-540. 2013; Medici et al., J Clin Virol 51: 264. 2011

13 DBS HCV RNA detected / HCVcAg non-detected

ID:	Gt	VL (IU/mL):	Plasma Core Ag fmol/L	DBS Core Ag fmol/L
8888-61231-339	ND	27	0.7	0
8888-61231-313	1a	57	0	1.4
8888-61231-386	1a	110	0.28	0
8888-61231-388	3a	220	0	0
8888-61231-387	3a	1100	0.09	0
8888-61231-466	1a	1200	1.67	0
8888-61231-317	1a	3659	0.54	0
8888-61231-430	3a	17600	21.38	0.38
8888-61231-444	1b	58600	25.79	0.91
8888-61231-420	3a	153800	5.12	0
8888-61231-304	1b	302200	89.44	0
8888-61231-433	3a	436800	697.04	2.06
8888-61231-407	1b	1686900	26.06	0.1

9 plasma HCV RNA detected / HCVcAg non-detected

ID:	Gt	VL (IU/mL):	Plasma Core Ag fmol/L	DBS Core Ag fmol/L
8888-61231-339	ND	27	0.7	0
8888-61231-313	1a	57	0	1.4
8888-61231-386	1a	110	0.28	0
8888-61231-388	3a	220	0	0
8888-61231-387	3a	1100	0.09	0
8888-61231-466	1a	1200	1.67	0
8888-61231-317	1a	3659	0.54	0