HEPATITIS C VIRUS RNA QUANTIFICATION ON DRY CAPILLARY BLOOD SPOT

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Erika Castro[1], Rachel Mamin[2], Cyril André[2], Lorenza Oprandi[1].
[1]Policlinique d'addictologie. Service de Psychiatrie Communautaire and [2]Laboratoire de Diagnostic du Service d'Immunologie et Allergie . Centre Hospitalier Universitaire Vaudois (CHUV). Lausanne, Switzerland.
erika.castro-bataenjer@chuv.ch

BACKGROUND

In Western countries, new hepatitis C virus (HCV) infections mostly concern people-who-inject-drugs (PWID). A common obstacle for screening or monitoring of HCV in long-term intravenous drug users is peripheral vascular damage and difficult venous access. Recently, dried blood spots (DBS) collection in PWID has been successfully integrated to routine HCV screening strategies. This study evaluates the sensibility of an **HCV RNA quantitative protocol on dry capillary blood spot collected in PWID followed for a chronic hepatitis C** at the addiction medicine consultation from the CHUV.

PATIENTS AND METHODS

During 2015 we established a laboratory protocol for DBS elution with PBS for HCV RNA quantification (COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test, v2.0). DBS were obtained from PWID with a known hepatitis C infection (viremia >1.5 E+1 IU/mL within the last 12 months) during a medical visit for a routine control. All patients provided oral informed consent. Finger capillary puncture was spotted in saver protein™ 903 Whatman cards and dried according to manufacturer's recommendations at room temperature before storing at -20°C until DBS downstream analysis.

DBS Elution protocol

Each spot (12mm diameter) was eluted at room temperature in 800µl PBS during 30 minutes. Downstream analysis were performed on 650µl of eluted DBS, respectively. A negative control sample (PBS) was tested at each HCV RNA quantification

EDTA/DBS measurements "equivalence"

Conversion factor for comparison of DBS HCV RNA "raw" results to EDTA HCV RNA results was *x32* which stands for : *x16* of ~31.25µl fully tested DBS specimen vs 500µl EDTA plasma sample for standard PCR and *x2* => correction factor for hematocrit (mean/arbitrary).

RESULTS

Table 1.

DEMOGRAPHICS AND FOLLOW-UP CHARACTERISTICS
n = 16

Men (%)	12 (75.0)
Age; mean (range)	45.4 years (28-68)
Patient-years of follow-up (mean)	140 (8.7)
History of PVD* and external jugular vein venopuncture (%)	11 (68.7)
Years of hepatitis C diagnose; mean (range)	8.5 years (1-30)
HCV genotype (%)	
GT-1	10 (62.5)
GT-3	4 (25.0)
GT-4	2(12.5)
Liver fibrosis score (%)	
F0-F1	6 (37.5)
F2	2 (12.5)
F3-F4	8 (50.0)
HCV treatment naïve rate (%)	13 (81.2)
HIV coinfection rate	4 (25.0)

* PVD: peripheral vascular damage.

Figure 1.

CORRELATION OF PATIENT'S HCV VIREMIA ACCORDING TO BLOOD DRAW STRATEGY: DBS vs PLASMA/EDTA
n = 16

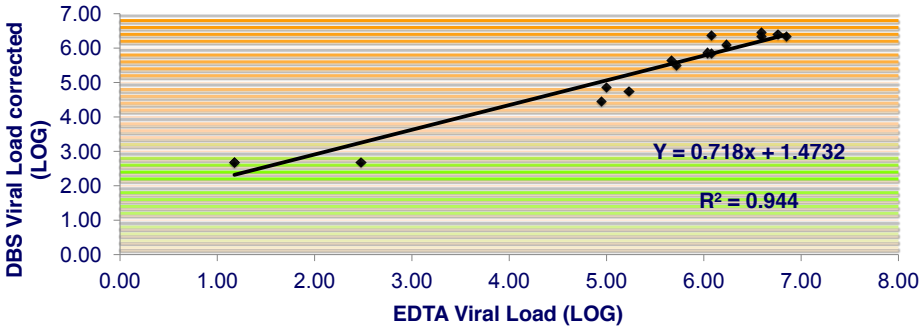
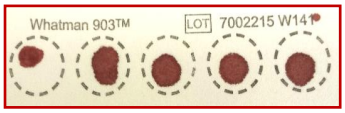


Figure 2.

DBS TROUBLESHOOTING



- We found a positive and strong correlation of patient's DBS and plasma/EDTA HCV viremia values (Fig.1)
- In this dataset EDTA plasma RNA average was 5.16 log (range: 1.17-6.85) with a limit detection value of 1.17 log (≤15 IU/mL).
- DBS RNA detection limit was 2.69 log (480 IU/mL).

DISCUSSION AND CONCLUSIONS

- This data shows a reliable and sensitive **quantitative protocol for HCV RNA screening of PWID with DBS in an addiction medicine setting** and feasible within a hospital routine diagnostic standard algorithm.
- Despite of these good results, DBS testing raised a common and important pre-analytic limitation: the heterogeneity of blood spots (Figure 2). To overcome this "drop variation" we plan to improve the current protocol with the use of capillary pipettes (50 µl) to collect blood drops as a systematic quality procedure.
- A high proportion of participants were known for history of PVD and external jugular vein venopuncture as standard blood draw procedure (Table 1). Which is a main obstacle for routine blood screening and HCV treatment follow-up in PWID.[1]

REFERENCES

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DISCLOSURE

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