Dried Blood Spot Valid Real-life Screening

Dried Blood Spot, Valid Screening Method for Viral Hepatitis and HIV in Real-Life

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Aim

1) To investigate the performance and feasibility of Dried Blood Spot (DBS) sampling in a real-life setting.
2) To compare the sensitivity and specificity of DBS on capillary blood to whole plasma when analysed at a modern high throughput diagnostic laboratory using an automated analytic platform (Abbott Architect).

Materials & Methods

The study was conducted in the Region of Southern Denmark.

To be eligible for inclusion, patients should have known HBV, HCV or HIV infection, and attend one of the Outreach clinics in either Odense Drug treatment center or Nyborg Stateprison, or the Outpatient clinic at the Hospital.

To prepare the DBS, five spots on a Whatman® 903 protein saver card (Sigma-Aldrich, Copenhagen, Denmark) were each covered with drops of capillary blood, and eluted with 1000 µl of a buffer (PBS/0.05% Tween 20/0.08% sodium azide) overnight at room temperature on a shaker, followed by centrifugation. The average estimated DBS sample dilution was 1:23 compared to plasma. The DBS eluates as well as the corresponding plasma were tested for the presence of anti-HIV, anti-HCV, hepatitis B surface antigen, anti-HBc and anti-HBs with use of the ARCHITECT system (Abbott Diagnostics, Delkenheim, Germany). In addition all samples were tested by the Procleix Ultrio Elite assay, a qualitative nucleic acid amplification test (NAT) for the simultaneous detection of HIV-1/2 -RNA, HCV RNA and HBV DNA, using the Procleix Panther System (Grifols Diagnostic Solutions, Allschwil, Switzerland)

Disclosures

The study was made possible from an unrestricted grant from Abbvie.
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Results

Among 404 included persons corresponding plasma and DBS samples were obtained. Descriptive summary and characteristics of viral infections shown in table:

Viral infection	N total	n Mono- + n Co-infected	N with quantitative analysis	Median Viral load	IQR (25-75) Viral load
Blood donors	99	0	n.a.		
HBV	85	78 + 5 HIV + 2 HCV	78	144	19-2780
HCV	116	111 + 3 HIV + 2 HBV	105	225000	4300-1110000
HIV	114	106 + 5 HBV + 3 HCV	112	0	0-19

N; total number, IQR; interquartile range (expressed as IU/ml (HBV and HCV) and copies/ml (HIV))

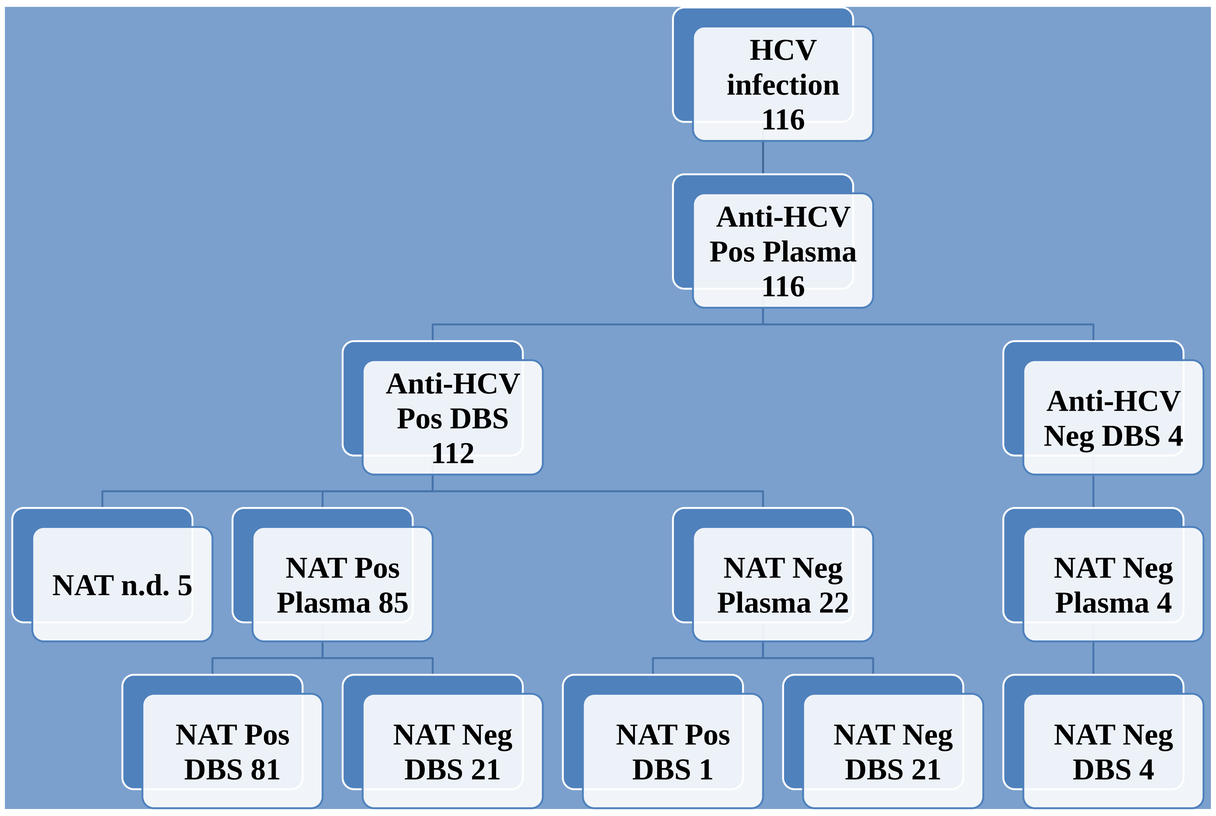
DBS had a sensitivity >96% and a specificity >98% for detection of all three infections. Anti-HBs and anti-HBc showed low sensitivity in DBS (42% and 68%) , see table below:

	Plasma		DBS		Sensitivity		Specificity		ROC	PPV	NPV
	Neg (N)	pos (N)	Neg (N)	Pos (N)	%	(C.I 95%)	%	(C.I 95%)		%	%
Anti-HCV	288	116	292	112	96.6	(91.4;99.1)	100	(98.7;100)	0.98	100	98.6
HBsAg	318	86	319	85	96.5	(90.1;99.3)	99.4	(97.7;99.9)	0.98	97.6	99.1
Anti-HBc	232	172	286	118	68	(60.5;74.9)	99.6	(97.6;100)	0.84	99.2	80.8
Anti-HBs	246	158	337	67	42.4	(34.6;50.5)	100	(98.5;100)	0.71	100	73
Anti-HIV	289	115	290	114	98.3	(93.9;99.8)	99.7	(98.1;100)	0.99	99.1	99.3

ROC: area under the Reciever Operating Curve, PPV: positive predicting value, NPV: negative predicting value

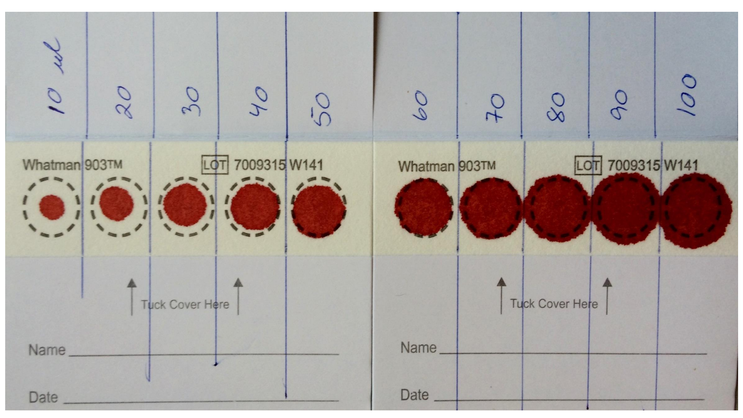
Flowcharts for DBS detecting chronic viral infection was constructed, here chronic hepatitis C shown as example: NAT testing did not improve sensitivity, but classified 95% of anti-HCV positive samples correctly in chronic and past infection.

Supplemental flowcharts for HBV and HIV are available at request



Comments

Sensitivity for anti-HBc and anti-HBs was low, most likely due to the dilution factor, in real-life a possible pitfall is whether enough blood is added to the DBS. An indicator ensuring this should be developed (e.g. weight, haemoglobin, visual guidance, see below).



Conclusion

Our study confirms that DBS is a feasible screening method in outreach clinics and confirms the high sensitivity and specificity of previous laboratory based studies for detection of **chronic infection**.
Careful attention on sampling volume for anti-HBs and anti-HBc markers
Offering easy access to DBS testing enables large-scale implementation in difficult to reach populations.

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