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Performance of Dried Blood Spots as a Sample Type for HIV Ag/Ab Combo Assay

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Abstract

Background: In resource limited settings, the use of Dried Blood Spots (DBS) for HIV viral diagnosis and monitoring has become an important tool towards the WHO 90-90-90 targets to achieve the diagnosis of 90% of individuals infected with HIV and to improve access to care. In this study, we evaluated the performance of the Abbott ARCHITECT HIV Ag/Ab Combo assay using samples eluted from DBS.

Methods: Venous whole blood (WB) from matched pairs of HIV infected plasma/WB samples was spotted on Whatman 903 cards, dried overnight, eluted and tested with the HIV Ag/Ab Combo assay. To evaluate concordance with plasma results, DBS from 644 HIV- infected subjects (500 Ag/Ab-reactive from Cameroon and 144 HIV RNA-positive from UK) were tested and compared to matched plasma results. DBS stability was evaluated on samples stored for up to 14 days at -20ºC, room temperature (RT) and +37ºC. Reproducibility of the DBS results with low plasma signal to cutoff ratio (S/CO) levels was evaluated by testing 12 samples in 10-20 replicates. Reproducibility of the test results for high-reactive samples was assessed using triplicates of the 12mm and 6mm DBS from four subjects. Specificity testing was performed on 316 DBS from individuals with HIV Ag/Ab-nonreactive plasma.

Results: All 144 DBS from genetically diverse UK samples were HIV Ag/Ab-reactive. Of 500 samples from Cameroon, 471 DBS (94.2%) were reactive and 29 (5.8%) nonreactive. All samples with a plasma S/CO>23 (n=465) were reactive by DBS. Of the 35 samples with S/CO<23 only 6 (17%) DBS were reactive. The detection rate for samples with plasma S/CO range of 1-10, 10-23 and >23 was estimated as 4%, 56% and 100% respectively. DBS remained HIV-reactive for up 2 weeks of storage. We observed <7% drop in S/CO at -20ºC, <12% at RT and ≤20% at +37ºC on day 14. DBS results from samples with low S/CO (7-30) in plasma were reproducible for 11 samples; precision between replicates within reactive DBS was in a range of 2.52-19.87 CV%. DBS results were reproducible for high-reactive samples in both 12mm (CV 1-8.3%) and 6-mm (CV 1.9-5.8%). S/CO from 12mm DBS were up to 2-fold higher compared to 6mm.

Conclusions: The results indicate that Whatman 903 DBS is a suitable specimen type for the ARCHITECT HIV Ag/Ab Combo assay. The assay reliably detects HIV-1 p24 antigen/ anti-HIV-1/-2 antibodies in DBS with corresponding plasma S/CO>23. Data from the Abbott Surveillance Database would suggest that >95% of HIV positive specimens have HIV Ag/Ab results of S/CO>23 in plasma or serum.

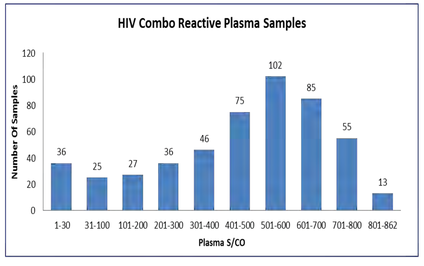
Background

- To increase access to diagnostic and treatment, the WHO/UNAIDS 90-90-90 initiative targets for 2020 are
  - 90% of all people living with HIV have been diagnosed
  - 90% of all people diagnosed with HIV receiving ART
  - 90% of all people receiving ART have undetectable viral load
- HIV diagnostic testing traditionally utilizes plasma specimens
- Dried blood spots (DBS) offers many advantages over plasma samples:
  - Cost-effective
  - No phlebotomy required
  - Deep freezer storage is not necessary
  - Samples can be shipped at ambient temperature

Methods

- Samples: Matched plasma and whole blood venous samples.
- General procedure:
  - Spotted 75 µl whole blood on 903 Whatman cards (12mm DBS).
  - Dried overnight at room temperature (RT).
  - Incubated in 400 µl PBS+ 0.25% Triton X-100 Buffer on a shaker/rotator for one hour at RT.
  - Transferred 300-350 µl into ARCH cup and tested with the ARCH HIV Ag/Ab Combo assay.
  - DBS not tested next day were stored in plastic bags with a desiccant at -70ºC.
- Concordance/Detection rate:
  - HIV RNA positive matched plasma and whole blood from UK (N=144): Plasma samples were pretested with the RealTi*m*e HIV-1 assay and had viral loads of 1.69 - 6.18 Log $_{10}$ copies/mL.
  - HIV Ag/Ab reactive matched plasma and whole blood from Cameroon (N=500): Plasma samples were prescreened for HIV Ag/Ab (S/CO 1.03-861.98; Fig.1).
- DBS Stability:
  - 12mm DBS from whole blood of 4 HIV positive subjects (ProMedDx) were stored in plastic bags with a desiccant at -20ºC, RT and +37ºC for up to 14 days.
  - Tested on day-3, day-7 and day-14.
- Reproducibility:
  - 12mm DBS in 5-10 reps X 2 tests for 12 low reactive samples
  - 12mm and 6mm DBS in 3 reps for 4 high reactive samples from ProMedDx.
- Specificity:
  - HBsAg reactive/HIV Ag/Ab-nonreactive in matched plasma (N=211).
  - Negative Panel (N=105), plasma samples were prescreened for HIV Ag/Ab, HBsAg, anti-HCV and HCV RNA.
- Phylogenetic analysis:
  - Subtypes for UK samples were classified based on gag p24, pol integrase and env IDR sequences from plasma samples by phylogenetic analysis using PHYLIP software (v3.5; J. Feisenstein, University of Washington, Seattle, WA

Figure 1. Sample distribution by HIV combo results in plasma (N=500)



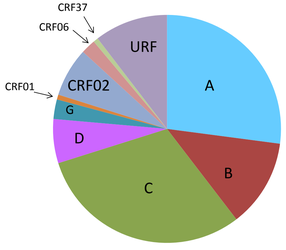
Results

Concordance between DBS and plasma results

Samples from UK (N=144)

- HIV RNA positive matched plasma
- Diverse HIV-1 strains
- 100% samples were HIV Ag/Ab reactive in DBS (11.5 – 853 S/CO.)

Figure 2. HIV-1 Subtype Distribution



Subtype	N=144	%
A	39	27
B	18	13
C	44	31
D	9	6
G	4	3
CRF01_AE	1	<1
CRF02_AG	10	7
CRF06	3	2
CRF37	1	<1
URF	15	10

- The diverse panel consists of 5 subtypes, 4 CRFs and 15 URFs.

Samples from Cameroon (N=500)

- Concordance with plasma results: 94.2 %
  - 471 reactive
  - 29 nonreactive

Figure 3. Correlation of HIV Ag/Ab results in plasma and DBS

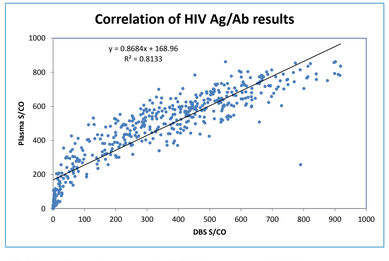


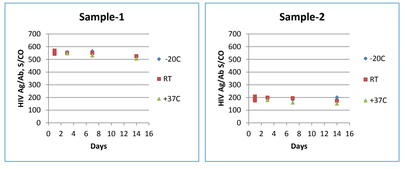
Table 1. Detection rate In DBS by plasma S/CO intervals

Plasma S/CO	N=	Reactive	Nonreactive	% Reactive
1-10	26	1	25	**4**
10-23	9	5	4	**56**
>23	465	465	0	**100**

- Overall concordance of DBS/plasma results from two studies is 95.5%

DBS stability

Figure 4. DBS Stability Data



S/CO Change (%) from Day-1

	D1	D3	D7	D14
-20ºC		+0.7	1.9	-6.2
RT	0	-0.6	-0.5	-5.2
+37C		-1.3	-4.2	-8.8

	D1	D3	D7	D14
-20C		-1.6	-4.0	5.4
RT	0	4.4	2.4	-8.7
+37C		-5.4	-15.6	-20.2

Data shown for 2 samples. Samples 3-4 had stability profile similar to sample 1

- <7% drop in S/CO at -20ºC, <12% at RT and ≤20% at +37ºC on day 14

Reproducibility in low reactive samples

Table 2. Reproducibility of DBS results for samples with plasma S/CO 7-30 (N=12)

ID	Plasma HIV Combo 2P36, S/CO	DBS # of Rep	DBS HIV Combo Mean S/CO	SD	% CV	Number of Reactive Reps	% Reactive Reps
S4603	7.18	10	0.34	0.05	13.55	0/20	0
K4867	9.46	10	0.53	0.04	7.80	0/20	0
M71	11.46	8	1.13	0.09	7.71	16/16	100
S4711	13.40	5	0.56	0.04	7.72	0/10	0
D6377	16.45	10	0.39	0.06	15.85	0/20	0
N1266	17.09	5	2.03	3.50	172.26	2/10	20
N1337	18.43	10	0.57	0.06	11.33	0/20	0
D5839	19.15	10	9.92	0.25	2.52	20/20	100
D6210	19.27	10	2.95	0.59	19.87	20/20	100
S5066	21.72	10	7.32	0.39	5.31	20/20	100
F1151	22.76	10	0.69	0.03	4.99	0/20	0
A218	29.87	10	14.35	0.64	4.44	20/20	100

2 tests were done from each replica of DBS

- CV 2.52-19.87% for reactive samples

Reproducibility in high reactive samples

Table 3: Reproducibility for 12-mm DBS

ID	Plasma	DBS reps 12 mm					
HIV smp	HIV Ag/Ab, S/CO	a	b	c	Mean	SD	% CV
1	731.73	569	549.1	543.1	553.73	13.56	2.45
2	515.95	207.23	186.5	176.21	189.98	15.80	8.32
3	807.35	618.11	612.13	605.35	611.86	6.38	1.04
4	680.71	486.43	455.34	470.99	470.92	15.55	3.30

Table 4: Reproducibility for 6- mm DBS

ID	Plasma	DBS reps 6 mm					
HIV smp	HIV Ag/Ab, S/CO	a	b	c	Mean	SD	% CV
1	731.73	431.64	461.32	410.92	434.63	25.33	5.83
2	515.95	96.27	96.19	92.43	94.96	2.19	2.31
3	807.35	486.51	500.19	504.97	497.22	9.58	1.93
4	680.71	350.23	375.07	362.99	362.76	12.42	3.42

- HIV combo results were reproducible in both 12mm (CV 1-8.3%) and 6-mm (CV 1.9-5.8%)
- S/CO results from 12mm DBS were 1.2-2 fold higher compared to 6mm.

Specificity

- 99.53% (210/211) in HBV-infected samples (HBsAg reactive/ HIV Ag/Ab nonreactive in plasma)
- 100% (105/105) in the Negative Panel (prescreened for HIV Ag/Ab, HBsAg, anti-HCV and HCV RNA)

Conclusions

- Concordance between DBS and plasma results was 95.5% (615/644).
  - Concordance for the 144 HIV-1 RNA positive genetically diverse samples from UK was 100%.
  - Overall DBS/plasma concordance for 500 HIV samples from Cameroon was 94.2%.
  - Concordance in samples with plasma S/CO >23 was 100%.
- According to Abbott Surveillance Database, 95.5% of HIV specimens from Cameroon have S/CO > 23.
- DBS samples were stable at +37ºC, RT and -20ºC for up to 2 weeks.
- The results were reproducible with CV<9% for highly reactive samples and ≤20 % for low-reactive samples.
- S/CO results from 12mm were up to 2-fold higher than from 6mm DBS.
- Specificity was 99.7% (315/316).
- Results demonstrated that Whatman 903 DBS is a suitable sample type for the Architect HIV Ag/Ab Combo assay.