SIEMENS Healthineers

B-042

Performance evaluation of Atellica IM infectious disease assays with dried blood spot sample

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Background

Dried blood spots (DBS), prepared from fingerstick or capillary blood samples dried on filter paper, are a simple and convenient method of sample collection compared to venipuncture. The use of DBS as a sample type became widespread in the 1960s when it was implemented during neonatal screening via heel prick for phenylketonuria.[1] As early as the 1950s, DBS were used for syphilis testing as well as serological surveillance of infectious diseases.[1] In the US today, the newborn Recommended Uniform Screening Panel (metabolic and genetic disorders) is often completed via DBS. In high resource countries, DBS are increasingly used for screening of infectious disease (HIV, Hepatitis virus) in key hard to reach populations in remote areas and populations such as intravenous drug users, homeless, and sex workers due to ease of sample collection and transportation.[1] DBS are useful in low resource countries for diagnosis and therapeutic monitoring of infectious diseases as collection and transportation are less resource intensive.[2]

Siemens Healthineers Atellica IM CHIV, HBsII, and HBcT2 assays are commercially available fully automated assays for the diagnosis of HIV and HBV infection in serum and plasma. Globally it is estimated there are 240 million chronic HBV infections of which only 27% have been diagnosed; in 2024, there were 900,000 people newly infected.[3] As of 2024, there were an estimated 40.8 million people living with HIV, with 1.3 million new infections.[4] To maximize utility of Atellica IM CHIV, HBsII, and HBcT2 assays in widespread detection of HIV and HBV infected individuals, performance of these assays is evaluated with DBS.

Methods

DBS Sample Preparation

Whole blood was collected from 30 normal healthy donors and spiked with high positive samples for CHIV and HBcT2 and recombinant HBsAg for HBsII. The samples were prepared by diluting positive sample or recombinant antigen in whole blood to achieve multiple levels of analyte. A Whatman 903 filter card, an FDA approved filter paper, was spotted with 50 µL of spiked or unspiked blood from each donor. The remaining blood was centrifuged to obtain plasma for use as matched control.

The optimal conditions for blood spot drying time, punch size, and elution conditions (buffer, time, and mixing method) were determined with dried plasma spots. The selected conditions were verified with dried blood spots. The final method used for DBS sample preparation is depicted in Figure 1.

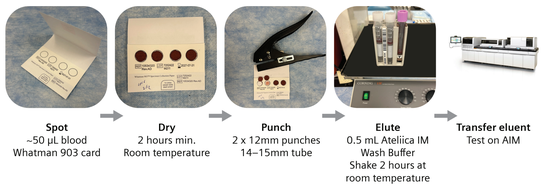

Figure 1. DBS sample preparation method

Testing of DBS with Atellica IM Assays

The DBS eluates and matched plasma were tested with Atellica IM CHIV, HBsII, and HBcT2 assays using standard assay parameters. The results were analyzed using current assay cut-off as well as a DBS specific cut-off derived from results obtained with unspiked DBS samples.

Results

DBS Optimization

Elution Buffer:

Multiple elution buffers containing detergents were tested. None of the elution buffers showed significantly improved elution over Atellica IM Wash Buffer (Table 1).

Table 1. Elution buffers evaluation

Sample	Elution Buffer	Atellica IM HBsII Index
Plasma: HBsAg Positive Sample 1	N/A	12.68
DBS: HBsAg Positive Sample 1	Atellica IM Wash Buffer	2.76
	PBS+0.05% Tween 20	3.08
	PBS+0.25% TritonX-100	3.17
Plasma: HBsAg Positive Sample 2	N/A	1.22
DBS: HBsAg Positive Sample 2	Atellica IM Wash Buffer	0.24
	PBS+0.05% Tween 20	0.19
	PBS+0.25% TritonX-100	0.20
	PBS+0.1% SDS	0.28
	PBS+0.1% TMNX 100	0.20
	PBS+0.1% Tergitol 15-S-9	0.16
	PBS+0.1% Brij35	0.34
	PBS+1M Guanidine Thiocyanate	0.13

Blood Spot Drying Time:

Compared to overnight drying of blood spot, 2 hours drying at room temperature showed greater analyte recovery indicating drying time of at least 2 hours is sufficient (Table 2).

Table 2. DBS drying time evaluation

Sample	Dry time	Atellica IM HBsII Index
HBsAg Positive Sample	2 hr	364.24
	Overnight	336.92
HBsAg Negative Sample	2 hr	0.06
	Overnight	0.05

DBS Elution Time:

Eluting DBS for at least 2 hours with shaking resulted in increased analyte recovery. Longer elution times did not significantly increase elution in tested samples (Table 3).

Table 3. DBS Elution time and method evaluation

Sample		Elution time	Atellica IM HBsII Index
HBsAg Positive Sample1	Plasma	N/A	11.70
	DBS	10 min on shaker	0.71
		30 min on shaker	0.99
		1 hr on shaker	1.26
		2 hr on shaker	1.68
		4 hr on shaker	2.16
		6 hr on shaker	1.76
		O/N on shaker	1.77
		2 hr, still	0.96
		2 hr on shaker with centrifugation	0.86
		2 hr on shaker with syringe elution	1.95
HBsAg Positive Sample 2	Plasma	N/A	1.11
	DBS	10 min on shaker	0.35
		30 min on shaker	0.10
		1 hr on shaker	0.34
		2 hr on shaker	0.23
		4 hr on shaker	0.23
		6 hr on shaker	0.20
		O/N on shaker	0.22
		2 hr, still	0.12
		2 hr on shaker with centrifugation	0.16
		2 hr on shaker with syringe elution	0.11

DBS Size and Number:

An increase in analyte recovery was seen when increasing the number of DBS eluted from 1 to 3 spots in 500 µl elution buffer (Table 4). However, addition of 3 spots resulted in reduction in eluted sample volume required for testing.

The optimal analyte recovery was observed with 12 mm punch size from 2 spots in 500 µL buffer (Table 4).

Table 4. DBS number and size optimization

DBS Sample	# of DBS (Punches)	Elution Buffer Volume	Atellica IM HBsII Index
HBsAg Positive Sample 1	1 (3 x 6mm)	500 µL	0.45
	2 (6 x 6mm)	500 µL	2.53
	3 (9 x 6mm)	500 µL	5.77
HBsAg Positive Sample 2	1 (1 x 12mm)	300 µL	1.84
	2 (2 x 12mm)	500 µL	2.39

Based on these studies, elution from 2 x 12mm punches from 2 spots in 500 µl of Atellica IM Wash buffer with shaking for 2 hours at room temperature was selected as optimal condition for sample elution to evaluate performance of Atellica IM assays.

Atellica IM CHIV, HBsII, and HBcT2 performance with DBS

Specificity:

DBS and plasma prepared from unspiked blood of 27–30 normal healthy donors were tested with Atellica IM CHIV, HBsII, and HBcT2 assays.

The current assay cut-off, 1.00 Index, was used to calculate specificity. Additionally, DBS specific assay cut-offs were calculated from results obtained with normal healthy donors (Table 5).

Table 5. DBS specific cut-off

	Average Index	SD	Cut-off
Atellica IM CHIV	0.22	0.041	0.51 Index (Avg+7SD)
Atellica IM HBsII	0.01	0.030	0.31 Index (Avg+10SD)
Atellica IM HBcT2	0.28	0.025	0.46 Index (Avg+7SD)

With both current assay and DBS specific cut-off, all 3 assays showed 100% specificity (Figure 2).

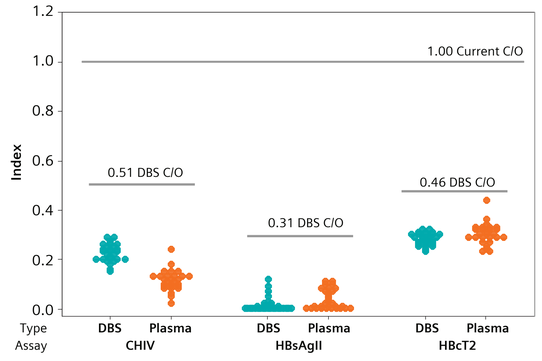

Figure 2. Reactivity of DBS and Plasma samples from Normal Healthy Donors

Dilutional Sensitivity:

• Atellica IM CHIV

When DBS and plasma of HIV-1, HIV-2, Group 'O' and p24 spiked blood were tested with CHIV assay, DBS were reactive in 1 to 3 dilutions lower compared to plasma using current assay cut-off (Table 6).

With DBS specific cut-off, dilutional sensitivity was improved and showed one dilution difference compared to plasma (Table 6).

Table 6. Atellica IM CHIV dilutional sensitivity in DBS

Sample	Dilution Factor	Atellica IM CHIV	
		Plasma* Index	DBS** Index
HIV 1	1	>12.00	2.42
	2	7.33	1.45
	5	3.69	0.81
	10	2.28	0.54
	20	1.33	0.36
	40	0.83	0.27
HIV 2	1	>12.00	2.23
	2	10.53	1.42
	4	5.75	0.94
	20	1.28	0.38
	40	0.80	0.31
	80	0.50	0.23
HIV O	1	11.52	1.58
	2	4.26	1.00
	10	1.17	0.35
	15	0.89	0.31
	20	0.69	0.30
	30	0.52	0.28
HIV P24	1	>12.00	9.30
	2	>12.00	4.64
	10	10.30	1.45
	20	5.34	0.88
	40	2.97	0.69
	80	1.89	0.46
	200	0.27	0.24

**Plasma cut-off: 1 Index; **DBS specific cut-off: 0.51 Index*

• Atellica IM HBsII

The HBsII assay showed reactivity at 2 dilutions lower in DBS compared to matched plasma samples when current assay cut-off was used (Table 7).

With DBS specific cut-off, HBsII dilutional sensitivity was improved and showed one dilution difference compared to plasma (Table 7).

Table 7. Atellica IM HBsII dilutional sensitivity in DBS

Sample	Dilution Factor	Atellica IM HBsII	
		Plasma* Index	DBS** Index
HBsAg	1	60.17	7.24
	2	28.13	3.60
	4	13.47	1.55
	16	3.21	0.34
	32	1.58	0.18
	64	0.76	0.06
	126	0.37	0.03

Plasma cut-off: 1 Index,DBS specific cut-off: 0.31 Index*

• Atellica IM HBcT2

With the current assay cut-off, HBcT2 assay was reactive at 3 dilutions lower in DBS compared to plasma samples (Table 8).

Using DBS specific cut-off, HBcT2 assay exhibited one dilution difference with DBS compared to plasma (Table 8).

Table 8. Atellica IM HBcT2 dilutional sensitivity in DBS

Sample	Dilution Factor	Atellica IM HBcT2	
		Plasma* Index	DBS** Index
HBcT	1	59.45	4.95
	2.5	19.52	2.44
	12.5	4.58	0.79
	25	2.12	0.51
	50	1.38	0.39
	100	0.47	0.29

Plasma cut-off: 1 Index,DBS specific cut-off: 0.46 Index*

Conclusion

- The DBS can be eluted efficiently for testing with Atellica IM system using common laboratory methods.
- The specificity of Atellica IM CHIV, HBsII and HBcT2 assays was 100% in tested DBS samples from normal healthy individuals using respective current assay and DBS specific cut-off.
- The dilutional sensitivity of all tested assays (CHIV, HBsII, HBcT2) was observed lower with DBS compared to plasma samples using current assay cut-off.
- With DBS specific cut-off, the dilutional sensitivity of all tested assays (CHIV, HBsII, HBcT2) was improved.
- These studies demonstrate that DBS can be implemented to achieve performance comparable to plasma with Atellica IM CHIV, HBsII, and HBcT2 assays.

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References

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In Development. Not commercially available. Future availability cannot be guaranteed.

Published by Siemens Healthcare Diagnostics Inc.
07-2026