Original Article

Evaluation of the Dried Blood Spot (DBS) Collection Method as a Tool for Detection of HIV Ag/Ab, HBsAg, anti-HBs and anti-HCV in a Malaysian Tertiary Referral Hospital

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Abstract

Introduction: Dried blood spot (DBS) collection is an appealing alternative to whole blood or plasma sampling, as it has technical and economic advantages over the latter. Materials and Methods: A prospective cross-sectional study was conducted at a Malaysian tertiary referral hospital from November 2009 to March 2010. One hundred and fifty paired specimens of DBS and plasma were analysed by the standard assays for HIV Ag/Ab, HBsAg, anti-HBS and anti-HCV, separately (total 600 paired specimens). DBS sample titres were then compared to the results of plasma testing, which was used as the gold standard. Results: For the HIV Ag/Ab assay with a cut-off point of 0.35 Relative Light Units (RLUs), the sensitivity and specificity were both 100%. For the HBsAg assay, the sensitivity was 96.5% and the specificity was 97.8%, with a cut-off point of 1.72 RLUs. Sensitivity for the anti-HBs test was 74.2% and the specificity was 86.9%, using a cut-off point of 0.635 RLUs. For the anti-HCV assay, the sensitivity was 97.3% and the specificity was 100%, with a cut-off point of 0.10 RLUs. Conclusion: DBS is an ideal choice to be used as a screening tool for the detection of HIV, Hepatitis B and Hepatitis C virus infections. However, different cut-off values need to be used for the validation of test positivity in DBS samples because the small amount of blood in the DBS specimens leads to lower assay titres.

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Introduction

Dried blood spot (DBS) or dried plasma spot (DPS) collection offers to be a promising alternative to whole blood or plasma sampling, as it has technical and economic advantages when compared to the latter. In the HIV-1 setting, the feasibility of using DBS technology for human immunodeficiency virus (HIV) surveillance was first tested in several studies in Europe, United States and the rest of the world in the late 1980s.[1-4] Furthermore, studies conducted on DBS/DPS samples in resource-limited settings have proven the usefulness and accuracy of these samples for the serological and molecular diagnosis of HIV infection.[5-15] These studies prove that DBS/DPS sampling is effective for the detection of HIV-1 p24 antigen[5] and of HIV-1 DNA,[6,7] for serotyping,[8,9] for quantification of HIV-1 RNA,[6,10-12] for genotyping[13] and for the monitoring of drug resistance.[14-18]

DBS collection is easy-to-perform, requires minimal training, and it obviates the risks associated with the use and disposal of syringes and needles. Some viruses such as HIV-1, HIV-2, human T-cell lymphotropic virus (HTLV) and hepatitis C virus lose their infectivity upon drying, hence DBS represents a low infectious hazard.[19] DBS specimens cannot leak or be broken in transit, hence minimising the risks associated with shipping. The maintenance of cold chain and carriage on dry ice or in liquid nitrogen is also not required.[20] The process of centrifugation and separation of sera from blood samples is also not necessary, therefore eliminating the risks involved in the handling of potentially infected material.[21]

Apart from HIV infection, many studies using DBS collection and evaluation have also been conducted on Hepatitis B[22-24] and Hepatitis C.[25-27] Brown et al[28] in Manchester has recently described the DBS collection method and analysis as sensitive as well as specific and it

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would be ideal to be used for the screening of HIV, HBV, HCV and syphilis.

Many of these studies reporting on the use of DBS sampling have been conducted in remote and resource-limited settings, particularly in South Africa.[5,11,18,29,30] DBS collection methods have been tested in the Asian region and have proven to be useful in the early diagnosis of HIV-1 infection, although all of these studies were conducted mainly in Thailand.[6,31,32] Even though the use of DBS sampling in the diagnosis and monitoring of HIV-1 infection has been around for more than ten years, it has yet been used in our local setting in Malaysia, thus this research study was set up for the collection of local data.

Study Objectives

This study aims to:

1. Validate DBS sample collection and analysis as a tool for the detection of adult HIV infection, Hepatitis B and Hepatitis C in University Malaya Medical Centre, a tertiary referral hospital in Malaysia.

2. Determine the cut-off points, sensitivity and specificity of DBS samples for the detection of HIV Ag/Ab, HBsAg, anti-HBs and anti-HCV, compared to plasma as the gold standard.

Materials and Methods

Research Design

A prospective cross-sectional study was conducted at the University Malaya Medical Centre from November 2009 to March 2010. During this period, 600 samples of blood were tested in pairs, i.e. via DBS collection method and conventional plasma. These samples consisted of 150 blood specimens each for the testing of HIV Ag/Ab, HBsAg, anti-HBs, and anti-HCV, respectively. This study was conducted as the initial part of a research study to look at HIV drug resistance surveillance and monitoring in Malaysia, which has been approved by the Ethics Committee for Research, University Malaya Medical Centre.

Study Population

The study population who provided positive samples comprised of adults 18 years of age and above and were able to sign a consent form for enrolment into this research study. Negative samples were obtained and analysed from anonymous individuals who had donated blood to the University Malaya Medical Centre Blood Transfusion Unit.

For collection of HIV samples, HIV-positive patients undergoing treatment and follow-up at University Malaya Medical Centre HIV Clinic were randomly selected and recruited to enrol into the study, hence providing positive samples for the HIV Ag/Ab test.

For the testing of HBsAg and anti-HCV, samples were collected from patients attending the Hepatitis Clinic as well as in-patients at University Malaya Medical Centre during the study recruitment period. All consecutive patients who were known to have chronic Hepatitis B or Hepatitis C infection were selected and recruited into the study, providing positive samples for HBsAg and anti-HCV, respectively.

For the testing of anti-HBs, subjects were recruited from University of Malaya Year One medical students, who had completed Hepatitis B vaccination during infancy, as part of the national immunisation programme. Cluster sampling was done, whereby medical students from Multi-Disciplinary Labs (MDL) 2, 4, 6, 7, 8, 9, and 10 were recruited into the study.

Specimen Collection

A 3 mL blood sample was collected from each subject by venous puncture and placed into EDTA blood specimen tubes. From this 3 mL blood sample, 50 μL aliquots of whole blood were aspirated and spotted onto Guthrie card filter papers (Whatman no. 903 Protein Saver™ cards, formerly Schleicher & Schuell, Keene, USA). Five circles of whole blood spots measuring about 13 mm diameter (containing 50 μL of whole blood each) were prepared for every blood sample collected. The blood tubes were then centrifuged and the resultant plasma stored at -80°C in the HIV laboratory.

The Guthrie card filter papers with five circles of blood spots were left to dry overnight at room temperature on a 903 Dry Rack® (Whatman, USA) under a biohazard hood. These constituted the dried blood spot (DBS) samples and were stored at -20°C for various durations before further tests are conducted.

To prepare the DBS sample for analysis, a disc of dried blood was punched out from one blood spot circle using a hole puncher. The punched disc had a constant measurement of 5.5 mm diameter and placed into a microtitre tube using a forceps. Between each Guthrie card filter paper, the puncher was cleaned with 70% ethanol and allowed to air-dry before punching the next filter paper. Blood from the punched DBS disc was eluted out with 500 μL of miliQ water for at least one hour at 4°C. The eluate was then mixed before being transferred into an input tube.

Paired samples of DBS and plasma were analysed by the standard assays used in University Malaya Medical Centre laboratories, according to the manufacturer's instructions (Abbott).

Statistical Analysis

All statistical calculations were performed using Statistical Package for Social Sciences, SPSS (Version 17.0, SPSS Inc., Chicago) and the Microsoft Office Excel 2007 statistics function. Correlation was evaluated with Pearson's correlation coefficient. The level of significance was set at $P < 0.05$.

Receiver-operating characteristic (ROC) curve analysis was performed by using the SPSS statistical software, in order to determine the optimal cut-off points for each assay. The sensitivity, specificity, positive predictive value and negative predictive value were then calculated using the formula below in Figure 1.

Results

HIV Ag/Ab TEST

The area under the ROC curve (AUC) is 1.000, signifying high accuracy for the detection of HIV Ag/Ab using DBS samples, with an ideal cut-off point of 0.35 Relative Light Units (RLUs). The correlation between HIV Ag/Ab assay results obtained from DBS and plasma samples is shown in Figure 2, demonstrating a significantly good correlation (Pearson value, r = 0.824; $P < 0.001$).

		PLASMA SAMPLE		
		POSITIVE	NEGATIVE	TOTAL
DBS SAMPLE	POSITIVE	A	B	A+B
	NEGATIVE	C	D	C+D
	TOTAL	A+C	B+D	A+B+C+D

Fig. 1. Formula used to calculate sensitivity, specificity, positive predictive value and negative predictive value.
Sensitivity: A / (A+C); Specificity: D / (B+D); Positive Predictive Value: A / (A+B); Negative Predictive Value: D / (C+D)

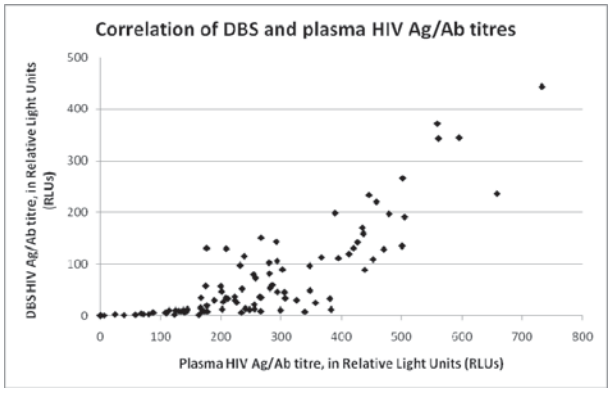

Fig. 2. Correlation of DBS and plasma HIV Ag/Ab titres.

HBsAg TEST

The AUC for the HBsAg ROC curve is 0.987, which is also highly accurate for the detection of Hepatitis B surface antigen (HBsAg) using DBS samples, with an optimal cut-off point of 1.72 RLUs. HBsAg assay results obtained from plasma compared to DBS samples showed a significant moderately good correlation (Pearson value, r = 0.432; $P < 0.001$), as demonstrated in Figure 3.

ANTI-HBs TEST

The AUC for the anti-HBs ROC curve is 0.841, which seems to be the worst out of all the assays tested. The optimal cut-off point appears to be 0.635 RLUs, giving a sensitivity of 74% and a specificity of 87%. The correlation between anti-HBs antibody assay results obtained from DBS and plasma samples is shown in Figure 4, demonstrating

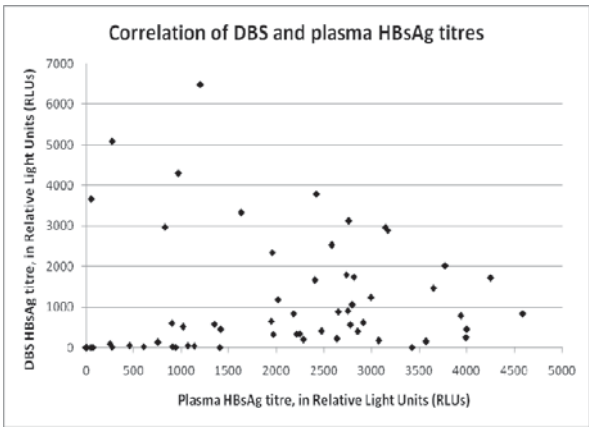

Fig. 3. Correlation of DBS and plasma Hepatitis B surface Ag (HBsAg) titres.

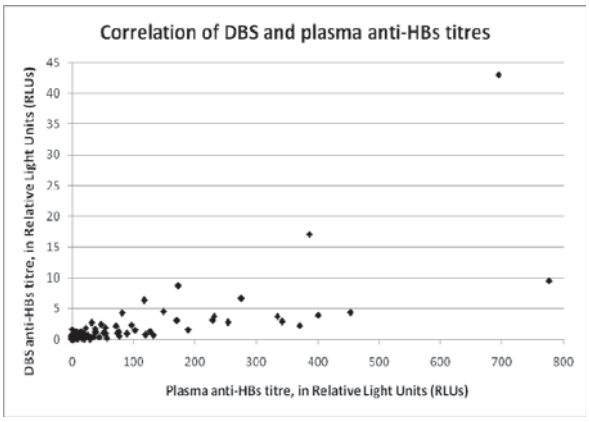

Fig. 4. Correlation of DBS and plasma anti-HBs (antibodies to HBsAg) titres.

a significantly good correlation (Pearson value, r = 0.721; *P* <0.001). However, there appears to be greater deviation among the few sample points at the very large RLU values.

ANTI-HCV TEST

Based on the anti-HCV ROC curve, the AUC is 0.999, signifying a high level of accuracy for the detection of Hepatitis C virus antibodies (anti-HCV) using DBS samples, with an ideal cut-off point of 0.10 RLUs. Figure 5 shows the correlation between anti-HCV antibody test results obtained from the DBS and plasma samples. Comparison of these results also demonstrated a significantly good correlation (Pearson value, r = 0.631; *P* <0.001).

Discussion

Dried blood spot (DBS) sampling involves the collection of whole blood samples by heel or finger-prick which is then dropped onto a filter paper, is a convenient method for obtaining and handling clinical samples intended for further analysis.[12] This type of sampling is useful in resource-limited environments especially in the developing countries where these procedures for obtaining and handling samples for laboratory analysis could possibly reduce costs.[21] It is a simple sampling method which requires minimal training and the risk of injuries is eliminated as needles and syringes were not used. Since DBS samples do not need special equipment for collection and transportation, costs are further reduced. This proves to be extremely advantageous for countries with limited health budgets.[21]

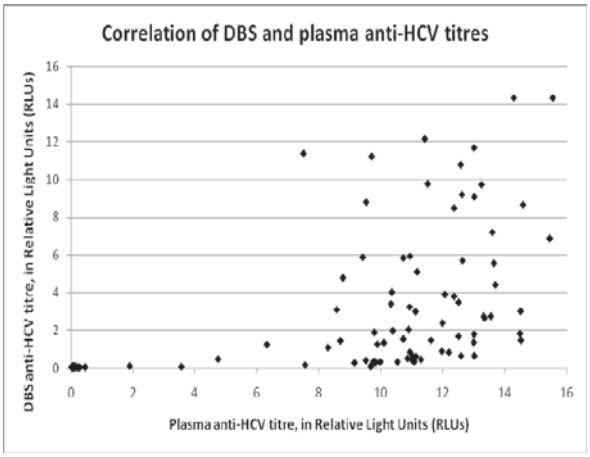

Fig. 5. Correlation of DBS and plasma anti-HCV titres.

Table 1. Summary of HIV Ag/Ab, HBsAg, Anti-HBs and Anti-HCV Detection Using DBS Samples

	HIV Ag/Ab	HBsAg	Anti-HBs	Anti-HCV
Pearson r	0.824	0.432	0.721	0.630
Cut-off values	0.35 RLU	1.72 RLU	0.635 RLU	0.10 RLU
Sensitivity	100%	96.5%	74.2%	97.3%
Specificity	100%	97.8%	86.9%	100%
Positive Predictive Value	100%	96.5%	81.7%	100%
Negative Predictive Value	100%	97.8%	81.1%	97.4%

Plots of correlation showed that the DBS and plasma results for all the tests conducted were significantly correlated by the Pearson test. The strength of this correlation between the DBS and plasma test results was best seen for the HIV Ag/Ab test (r = 0.824), followed by the anti-HBs assay (r = 0.721), and anti-HCV titres (r = 0.631). HBsAg titres obtained from DBS samples compared to plasma also showed moderately good correlation (r = 0.432). The level of significance for correlation by the Pearson test was less than 0.001 for all the assays tested. However, the absolute titres for each assay were mostly much lower for the DBS samples as compared to conventional plasma.

This discrepancy between the plasma and DBS titres is best shown in the correlation curve for the DBS and plasma anti-HBs titres, whereby the DBS anti-HBs titres do not exceed 45 RLUs even though the plasma anti-HBs titres have reached nearly 800 RLUs. This could be due to two reasons. Firstly, the amount of blood used for the preparation of the DBS samples was very miniscule, using aliquots of only 50 μL of whole blood. Secondly, blood from the DBS punched disc was then eluted with 500 μL of miliQ water, further diluting the sample. Therefore, the assay titres obtained from DBS samples were noted to be generally lower than the titres from plasma samples.

Thus, the argument is that the same cut-off values used for conventional plasma should not be used for the DBS samples. Based on the above observation, the receiver-operating characteristic (ROC) curve analysis was performed for all of the assays in question. This was done in order to determine the optimal cut-off points for each test which would determine whether the DBS result would be considered positive. According to the ROC curve analyses, the cut-off points for the tests were identified as 0.35 RLUs for the HIV Ag/Ab test, 1.72 RLUs for HBsAg, 0.635 RLUs for anti-HBs, and 0.10 RLUs for anti-HCV.

Using these new cut-off values, the sensitivity and specificity for the assays tested were calculated, taking conventional plasma as the gold standard. The data obtained shows very high specificities overall for all the assays run on DBS samples; 100% specificity for the detection of HIV Ag/Ab, anti-HCV, 97.8% specificity for HBsAg, and

86.9% specificity for the detection of anti-HBs. This means that the DBS sampling method is highly unlikely to give a false positive result. Sensitivity was also very good for the detection of HIV Ag/Ab (100% sensitivity), anti-HCV (97.3%), and HBsAg (96.5%). The sensitivity of anti-HBs detection from DBS samples was lower at 74.2%. These results of poor sensitivity warrant further investigation and analysis, i.e. whether increasing the number of samples, including more study centres or using different assays/ kits from various manufacturers for test analysis would improve the sensitivity results.

The sensitivity results presented in our study differed slightly from a recent study conducted by Brown et al[28] that evaluated the sensitivity and specificity of the DBS collection method with analysis for the screening of HIV, HBV, HCV and syphilis. One main difference in this research study was the use of the anti-HBs test instead of anti-HBc (antibody to Hepatitis B virus core antigen) which was used in Brown's study. The sensitivity results reported in their study were 98% for HIV Ag/Ab, 98% for HBsAg, 100% for anti-HBc and 100% for anti-HCV. Specificity was 100% for all their tests.

In the group of Year One medical students with positive anti-HBs antibodies, there were in fact 5 students with very high levels of anti-HBs antibodies (>1000 RLUs) in their blood. These antibodies were also detectable via DBS sampling as the levels in plasma were very high. However, the anti-HBs titres obtained by the DBS collection method were extremely low, ranging from 10.62 RLUs to 144.17 RLUs, when compared to the titres of >1000 RLUs obtained from plasma. These results were applicable for the calculation of the sensitivity and specificity of the assay via DBS sampling, but were omitted from the correlation testing because they caused marked skewing and hence inaccuracy in the figures.

Similar to many other studies prior to this,[6,8,11,29-31] DBS collection and processing were performed by controlled application of EDTA blood onto filter paper in laboratory conditions instead of whole blood collected directly from heel or finger-prick in a real-world situation. Although the method of DBS collection onto the five circles on the filter paper is easy and requires minimal training, the final appearances of the blood spots on the Guthrie card filter papers would still require standardisation. Few studies have been conducted so far regarding the effectiveness of this DBS collection method in the field, collecting samples via infant heel-prick.[32,33] Once validated, this method of blood sample collection should be able to be performed in communities in remote areas, without the need for advanced equipment, expensive transportation or shipping, maintenance of cold chain, risks associated with the handling of potentially infected material, and all the costs involved.

The purpose of conducting such a study in this centre was to initially validate DBS collection and analysis so that further studies and research can be performed on the field, i.e. rural communities in resource-limited areas. Further studies which would be of interest include HIV drug resistance surveillance and monitoring locally. The advantages of DBS sample collection will potentially allow health organisations and even governments to minimise health expenditure and better manage health resources.

Conclusion

DBS sampling would be an ideal choice to be used as a screening tool for the detection of HIV, Hepatitis B virus and Hepatitis C virus infections in the community. The tests showed high sensitivity and specificity for the detection of HIV Ag/Ab, HBsAg, anti-HCV, as well as anti-HBs in the DBS samples.

DBS samples provide very minimal blood available for testing as compared to conventional whole blood or plasma. This would lead to lower titres for the assays tested, hence the need for lower cut-off values for the validation of test positivity in these specimens.

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