Evaluation of various methods of point-of-care testing of haemoglobin concentration in blood donors

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Background. Point-of-care testing (POCT) devices for determining pre-donation haemoglobin (Hb) concentrations mark the advent of advanced technology for blood banks. POCT devices have undergone several improvements including changes in testing methodology and size of device, befitting the needs of blood donors and blood banks in terms of safety and quality of blood components. This study was planned to evaluate the suitability of non-invasive and invasive POCT devices for blood donor Hb screening.

Material and methods. Pre-donation Hb in apparently healthy blood donors was measured by a non-invasive spectrophotometric based method (NBM-200, OrSense) and an invasive method utilizing reagent free cuvettes (DiaSpect) along with a device using sodium azide-coated cuvettes (HemoControl, EKF diagnostic GmbH). The performance of the devices was evaluated by comparison with the reference method, i.e. an automated cell counter (KX-21).

Results. Hb was measured in 485 prospective blood donors. DiaSpect hemoglobin T system was found to be the most sensitive method of POCT for Hb (sensitivity 98.1%) followed by HemoControl (sensitivity 86.8%). NBM-200 was the least sensitive method (sensitivity 71.7%). The intraclass correlation coefficient was highest for DiaSpect (0.78), followed by HemoControl (0.77) and NBM-200 (0.43). The variation of results on repeat testing was high for NBM-200 with a coefficient of variation of 4.28%, compared to 2.19% for DiaSpect. On comparing the mean testing time, DiaSpect (1.9 seconds) was found to be significantly quicker than the other two POCT devices ($p<0.001$).

Discussion. NBM-200 has the apparent advantage of eliminating pain but also a substantial possibility of causing ineligible donors to be accepted. DiaSpect was fast and accurate, with its results showing perfect agreement with those of the standard method. It is, therefore, aptly suited for screening donors in blood banks.

Keywords: point-of-care testing, haemoglobin, blood donors, non-invasive.

Introduction

Determination of the haemoglobin (Hb) concentration in prospective blood donors is a well-established initial screening test routinely performed before donation, which protects both donors' and recipients' health. The rationale for performing this test is to ascertain that the loss of a unit of blood would not lead to symptoms of anaemia in the donor following the blood donation, and to ensure that the unit of packed red blood cells collected for the patient meets or exceeds an objective standard in terms of Hb content. In India, the Drugs and Cosmetics Act 1940 made pre donation Hb estimation mandatory. According to this Act, both male and female blood donors should have a Hb of at least 12.5 g/dL and a haematocrit of 38% to be accepted for donating blood[1].

The estimation of Hb in blood donors is traditionally performed by invasive finger prick methods. The copper sulphate gravimetric method[2] is the most common method of Hb estimation in Indian blood banks because of its rapidity and low cost. Being a qualitative method, the copper sulphate method is vulnerable to subjective errors and its quality control is also difficult[3], so more and more blood banks are switching over to point-of-care testing (POCT) devices for Hb testing. Currently, several invasive POCT devices are available on the market for Hb estimation. One such device (HemoControl, EKF Diagnostic GmbH, Barleben, Germany) works on the principle of the photometric azide-methaemoglobin method using sodium azide-coated cuvettes[4]. Another modified POCT device (DiaSpect hemoglobin T system, DiaSpect Medical GmbH, Sailauf, Germany) utilises a broad-spectrum, multichromatic sensor which measures the absorbance of unaltered whole blood in reagent-free cuvettes over a wide spectral range simultaneously; this device has the added advantage of providing results within 2 seconds[5]. However, both these methods require

finger pricking, exposing health care personnel to the risk of needle sticks and donors to infection. Recent additions to POCT devices for Hb estimation are non-invasive methods which have the potential to improve donor screening by eliminating pain and reducing infection risks. These devices are based on a spectrophotometric method to determine Hb concentration. The commercial device (NBM-200 OrSense, Nes Ziona, Israel) works by temporarily occluding the blood flow through a pneumatic finger cuff and reading the transmitted light by a multi-wavelength sensor[6].

With further advances in the dynamic POCT technology, the analysers are becoming compact, faster, and more user-friendly and have demonstrated accuracy with smaller blood sample requirements. The availability of numerous POCT devices has posed a dilemma in selecting an appropriate device for blood bank. It is prudent to evaluate a POCT device against a reference method before introducing it for donor screening. We did a prospective study to evaluate the accuracy and precision of DiaSpect and NBM-200 along with HemoControl, which is the currently used method of donor screening at our centre. The reference method for comparison was an automated cell analyser (KX-21, Sysmex Corporation, Kobe, Japan).

Material and methods

This prospective study was conducted on normal healthy blood donors at a blood donation centre in a tertiary care institute by trained health care personnel. Approval from the ethical committee of the institute was obtained before performing the study. A non-invasive POCT device (NBM-200), an improvised invasive POCT device (DiaSpect) and the currently used invasive POCT method of Hb testing (HemoControl) at our centre were compared. A total of 534 prospective blood donors, after obtaining their consent were included in the study. Out of these, 485 donors were subjected to one-time Hb testing by all four methods in order to compare the performance of the devices. The other 49 donors were tested in duplicate to compare the precision of the various techniques.

All the devices were well calibrated and the manufacturers' instructions were followed strictly while performing the Hb estimation. The donors were tested by DiaSpect and HemoControl using capillary blood obtained from pricking the index finger of the right hand. Simultaneously, Hb testing by NBM-200 was done on the thumb of other hand. Time taken and the Hb result values for each device were recorded. Blood donors with the predefined level of Hb for blood donation by HemoControl were accepted for blood donation. Just after phlebotomy, a 2 mL blood sample was collected in an EDTA vial from the diversion pouch of the blood bag and subjected to Hb estimation by the automated cell analyser KX-21. Blood samples of the donors who were deferred from blood donation on the basis of low Hb level by HemoControl were also collected and tested on the KX-21.

In order to estimate the precision of the devices, Hb was measured in duplicate on EDTA venous samples by KX-21, DiaSpect and HemoControl from a separate set of 49 donors. The same donors were tested twice with NBM-200 in order to determine its reproducibility. Variation between capillary and venous blood Hb levels measured by DiaSpect and HemoControl was also checked. As the volume of capillary blood obtained was very small, it could not be tested on KX-21. Using the KX-21 values as the reference, the data were split into the following three groups since the eligibility criterion for whole blood donation is Hb≥12.5 g/dL and the World Health Organisation definition for anaemia is Hb≤11 g/dL[7]: group 1: Hb≤11.0 g/dL; group 2: Hb 11.1-12.4 g/dL; and group 3: Hb ≥12.5 g/dL.

Donors whose Hb value was ≤11g/dL were recommended iron supplementation and referred for complete work-up of their anaemia.

Data were maintained on SPSS software version 17 (IBM corporation, Chicago, USA) and Medcalc software version 2013 (MedCalc Software bvba, Ostend, Belgium) for statistical analysis. The sensitivity, specificity, positive predictive value and negative predictive value of each method were calculated. The concordance with the reference method was established by plotting Bland Altman graphs and the estimation of the accuracy of results was evaluated by calculating bias values along with intra-class correlation coefficients (ICC). The ICC were interpreted as follows: <0: poor; 0.01-0.20: slight agreement; 0.21-0.40: fair agreement; 0.41-0.60: moderate agreement: 0.61-0.80, substantial agreement; and 0.81-1.00: almost perfect agreement[8]. The precision of the three methods was analysed by calculation of the coefficient of variation (CV) on repeat testing by a particular method.

Results

The study population comprised 534 prospective blood donors. Out of 485 blood donors subjected to one-time Hb testing by all the devices, 456 were male (94%) and 29 were female (6%). Table I shows the distribution of the blood donors in the three groups based on Hb level detected by the various devices. The reference method detected 11 (2.26%) anaemic donors (Hb<11 g/dL), whereas NBM-200 detected only two (18.2%), DiaSpect nine (81.8%) and HemoControl seven (63.6%) of the truly anaemic donors. Based on HemoControl values, 47 (9.69%) donors were deferred from blood donation, whereas the reference method detected Hb values less

than 12.5g/dL in 55 (11.34%) donors and NBM-200 in 31 donors (6.39%).

As shown in Table II, the mean values of Hb measured using DiaSpect, NMB-200 and HemoControl were 14.3g/dL, 14.8 g/dL and 14.4 g/dL, respectively, which are values close to that of reference method (14.1 g/dL). DiaSpect was found to be the most sensitive method (sensitivity 98.1%) followed by HemoControl (sensitivity 86.8%). NBM-200 was found to be the least sensitive method (sensitivity 71.7%). In terms of mean time taken per test, Diaspect (1.9 seconds) was significantly faster ($p<0.001$) compared to other devices, whereas the slowest test method was NBM-200 (81.7 seconds).

Bland-Altman plots were drawn to describe the concordance between DiaSpect, NBM-200 and HemoControl (Figure 1). The value of two standard deviations of difference of Hb measurement was <2.0 g/dL between KX-21 and DiaSpect as well as between KX-21 and HemoControl; however, it was >2.0 g/dL between KX-21 and NBM-200. For DiaSpect the absolute mean difference was −0.18 g/dL (95% confidence interval [CI]: −0.27 to −0.09) with an upper agreement limit at 1.68 g/dL (95% CI: 1.54 to 1.83) and lower agreement limit at −2.05 (95% CI: −2.19 to −1.19). For NMB-200 and HemoControl the absolute mean differences were −0.66 (95% CI: −0.78 to −0.53)

Table I - Division of subjects according to the Hb levels obtained by DiaSpect, NBM-200 and HemoControl against the reference (n=485).

Reference values		DiaSpect			NBM-200			HemoControl		
		≤11	*11.1 to 12.4*	*≥12.5*	*≤11*	*11.1 to 12.4*	*≥12.5*	*≤11*	*11.1 to 12.4*	*≥12.5*
≤11	11	9	2	0	2	5	4	7	4	0
11.1 to 12.4	44	5	18	21	1	11	32	7	21	16
≥12.5	430	3	7	420	2	10	418	3	5	422
Total	485	17	27	441	5	26	454	17	30	438

Table II - Comparison of the performance characteristics of DiaSpect, NBM-200 and HemoControl (n=485).

Result	DiaSpect	NBM-200	HemoControl
Sensitivity (%)	98.1	71.7	86.8
Specificity (%)	78.4	79.5	94.7
Positive predictive value (%)	35.9	30.2	66.9
Negative predictive value (%)	99.7	95.8	98.3
Time taken in sec. (mean±SD)	1.9 (±0.3)	81.7 (±9.9)	45.5 (±5.9)

SD: standard deviation.

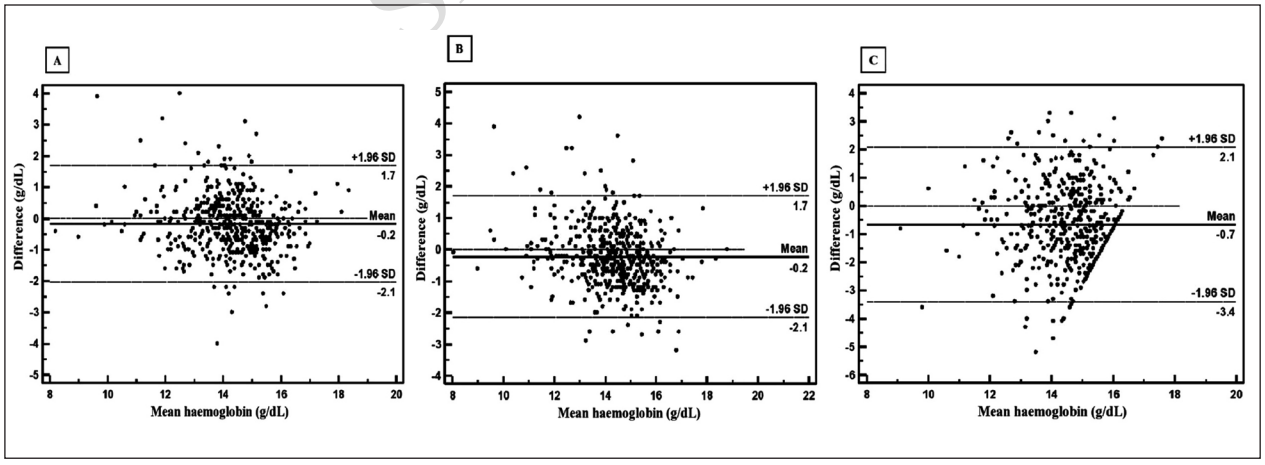

Figure 1 - Bland-Altman plot showing concordance of the tested methods with the reference method.

Panels A, B and C present results for DiaSpect, Hemocontrol and NBM-200, respectively. Each dot represents the mean of difference in Hb values between the reference method and the test method with the x-axis showing the Hb values of donors measured with the reference method and y-axis showing the variation of the mean difference in Hb with standard deviation. The two extreme lines mark two standard deviations from the central line at level 0. The line adjacent to the 0 line represents the mean of difference in Hb values. Hb: haemoglobin.

and −0.22 (95% CI: −0.31 to −0.13), respectively, with upper agreement limits at 2.09 g/dL (95% CI: 1.88 to 2.30) and 1.70 (95% CI: 1.55 to 1.85) and lower agreement limits at −3.39 g/dL (95% CI: −3.16 to 3.19) and −2.15 (95% CI: −2.30 to −1.99) respectively. The intraclass correlation co-efficient (ICC) was calculated to determine the accuracy of the testing method in comparison to the standard test method. The highest ICC of about 0.78 was found for DiaSpect, followed by HemoControl (0.77) and NBM-200 (0.43).

The precision of the test methods was determined by calculating the variation on repeat testing on the same donor and is represented as percentage coefficient of variation. Low variation was seen with DiaSpect, which had a CV of 2.19%. The CV for HemoControl and NBM-200 were 2.51% and 4.28%, respectively (Table III).

A mountain plot (folded empirical cumulative distribution plot) was created by computing a percentile for each ranked difference of results between the test method and the standard method. A broad-based mountain with a left or right skewed position indicated poor agreement with the standard method (Figure 2). The least difference from the standard was seen with DiaSpect and HemoControl.

The comparison of mean Hb measured in capillary and venous blood is presented in Table IV. No statistically significant difference was observed between the mean Hb in capillary and venous blood measured by DiaSpect and HemoControl (p=0.841 and p=0.68, respectively). The mean Hb of venous blood measured by DiaSpect and HemoControl was significantly higher than that by measured by KX-21 (p<0.001). The difference between the mean Hb in capillary blood measured by DiaSpect

Table III - Concordance parameters of the three POCT methods in relation to the reference method.

	DiaSpect	NBM-200	HemoControl
Bias (g/dL) (95% CI)	−0.18 (−0.27 to −0.09)	−0.66 (−0.78 to −0.53)	−0.22 (−0.31 to −0.13)
Upper limit of agreement (g/dL) (95% CI)	1.68 (1.54 to 1.83)	2.09 (1.88 to 2.30)	1.7 (1.55 to 1.85)
Lower limit of agreement (g/dL) (95% CI)	−2.05 (−2.19 to −1.91)	−3.39 (−3.61 to −3.19)	−2.15 (−2.30 to −1.99)
ICC coefficient (g/dL) (95% CI)	0.78 (0.74 to 0.82)	0.43 (0.27 to 0.55)	0.77 (0.73 to 0.81)
Coefficient of variation (%)	2.19	4.28	2.51

POCT: point-of care testing; CI: confidence interval.

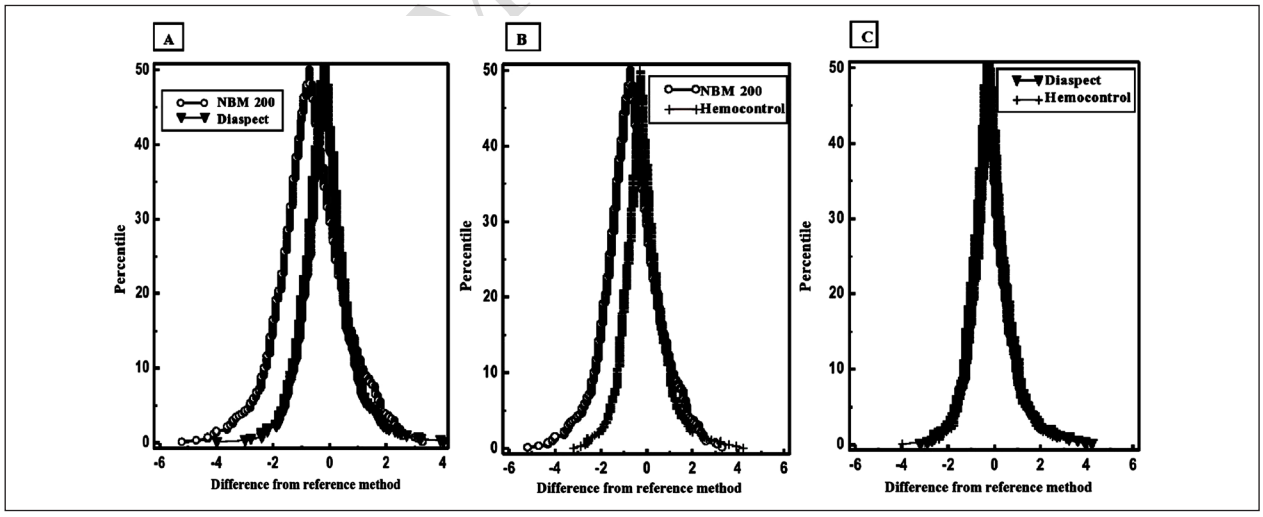

Figure 2 - Mountain plot (folded empirical cumulative distribution plot) showing the agreement of test results with those of the reference method.

Panels A, B and C present results for DiaSpect, Hemocontrol and NBM-200, respectively. The plots were created by computing a percentile for each ranked difference between the test method and the standard method. The y-axis shows percentiles up to the 50th. The x-axis shows differences between the two methods in relation to the reference method, with the 0 value representing perfect agreement.

Table IV - Comparison between capillary and venous Hb.

Equipment	Blood sample (n=43)	Mean Hb in g/dL (±SD)	p-value
DiaSpect	Venous	14.32 (±1.48)	0.841
	Capillary	14.36 (±1.61)	
HemoControl	Venous	14.40 (±1.54)	0.680
	Capillary	14.32 (±1.41)	
DiaSpect	Venous	14.32 (±1.48)	<0.001
KX-21		13.94 (±1.59)	
DiaSpect	Capillary	14.36 (±1.61)	0.053
KX-21	Venous	13.94 (±1.59)	
HemoControl	Venous	14.40 (±1.54)	<0.001
KX-21		13.94 (±1.59)	
HemoControl	Capillary	14.32 (±1.41)	0.018
KX-21	Venous	13.94 (±1.59)	

Hb: haemoglobin; SD: standard deviation

and that of venous blood measured by KX-21 was not statistically significant (p=0.053); however, the mean Hb of capillary blood measured by HemoControl was significantly higher than the venous blood Hb measured by KX-21 (p=0.018).

Discussion

Pre-donation Hb screening is one method to ensure the safety of donors and quality of blood. In India, the method for estimating Hb should be suitable for the set-ups of blood banks, which have operative time constraints and are overcrowded. Selecting a POCT device for measuring Hb in a blood bank can be difficult as many factors need to be addressed, such as donor acceptance policies, work load, competency of staff, frequency of outdoor blood donation camps and voluntary donations (implicating shorter waiting periods for pre-donation screening), *etc*. Recent advancement in POCT devices for Hb screening include devices using a non-invasive spectrophotometric method and newer invasive methods. The former inflict no pain to the donor and generate no bio-hazardous material. The latter use reagent-free cuvettes and generate results quickly. However, any new method should be validated against the reference method before being introduced into blood banks for donor screening.

Validating the device for a blood bank is important only when tested in ideal blood bank settings; we, therefore, measured the Hb of donors in capillary blood simultaneously on the new POCT device (DiaSpect) and the currently used device (HemoControl). In the present study, there were 55 ineligible donors (Hb <12.5 g/dL) according to the reference method. DiaSpect detected 80% (44/55) and HemoControl detected 85.4% (47/55) out of these. Thus a decrease in deferral rate could be expected in our centre if we introduce DiaSpect for screening blood donors in place of HemoControl. In a study done by Canadian blood services on the validation and implementation of DiaSpect as a replacement of the copper sulphate gravimetric method, it was found that the deferral rate decreased from 10.1% to 8.1% in females which may have resulted from a shift of the Hb measurement distribution curve[5]. We are not able to comment on deferral rates based on gender of blood donors as there were very few females in our study (6%). In our study, NMB-200 did not detect 45.5% of the ineligible donors, which was a finding similar to that of Kim MJ, *et al*.[9] who conducted a study in which NBM-200 failed to detect over half (61.4%) of the ineligible donors. Those authors also found that measurements obtained using the NBM-200 showed a left-skewed distribution and tended to be greater than those obtained using the reference method, which could be a threat to the protection of donors' health. In a recent study, it was seen that non-invasive methods do not ameliorate the percentage of donors correctly screened for blood donation and this was 88% for NBM-200[10]. The results given by NBM-200 have been found to be influenced by a variety of factors, including skin colour[11], perfusion index of the finger[12], structure of the thumb, type of finger used, finger temperature and position of donors (standing or sitting)[13]. These factors may account for the poor agreement of NBM-200 results with those of the standard method.

An important aspect of blood donor safety is to detect donors who are anaemic or likely to become anaemic after blood donation which expectedly lowers the Hb level by 1-1.5 g/dL. As the symptoms of anaemia start manifesting at Hb levels of 9-10 g/dL in a person with a healthy cardiovascular system, it is important that no donor with a Hb level below 11 g/dL is accepted for blood donation[14]. Important factors in achieving this objective are the accuracy and precision of the screening method used to determine the donors' Hb concentration. In the present study, DiaSpect was able to detect 82% (9/11), HemoControl 64% (7/11) and NBM-200 only 18% of the donors who had a Hb below 11 g/dL. However, a recent study found that NBM-200 was more precise than the routine finger stick technique in use with regards to preventing donation by anaemic donors, since the median of percentage error observed was below that observed with the reference method[15].

The ideal Hb screening method for blood collection centres should have high specificity and sensitivity, with low false failure (deferral) rates and low false pass rates; approaching zero for donors with true Hb levels under 11 g/dL[14]. In this study, no donor with a Hb level <11.0 g/dL was passed by DiaSpect or HemoControl whereas NBM-200 passed four such donors. DiaSpect was found

to be the most sensitive (98.1%) and NBM-200 the least sensitive (71.7%) of all the devices compared. However, in an Italian study, the authors found a higher sensitivity for the latter device (98%) accompanied by a higher rate of screening failure[10].

An invasive type of POCT device for Hb testing uses finger pricking to obtain capillary blood. There are reports indicating that Hb values are higher in capillary blood than in venous blood[16]. The use of capillary blood for Hb testing is associated with certain drawbacks such as variation with the sequence of drop of blood used, contamination with tissue fluid and the sedimentation of red cells in large drops of blood[17,18]. Testing venous blood Hb in a blood bank is impractical as it is time-consuming and leads to double phlebotomy for one unit of donated blood[19]. In our study, using a particular device, we found that the mean capillary Hb was higher than the venous Hb, but that the difference was not statistically significant. Hb values measured in venous blood by HemoControl and DiaSpect were significantly higher than corresponding values on the standard device which may be due to different testing principles used in the POCT devices and automated cell counter. On comparing the venous blood Hb values determined by the reference method with capillary Hb values determined by POCT devices, the difference was significant with HemoControl but not with DiaSpect. This may be due to differences in cuvette design and testing methodology of the two devices.

The accuracy of a device improves the reliability of the testing performed and thus helps to assure quality. Our study showed a bias greater than 0.5 g/dL with limits of agreement more than 3 g/dL for NBM-200 and a bias of less than 0.25 g/dL with limits of agreement less than 2 g/dL for HemoControl and DiaSpect. A previous study of NBM-200 done in France[12] found a bias of less than 0.25 g/dL. However, the authors used a different reference method (ADVIA 2120) and their subjects were mainly patients whereas our study was performed on apparently healthy blood donors. The ICC for NBM-200 showed only fair agreement whereas that DiaSpect and HemoControl showed moderate agreement with that of the standard. In addition, the results obtained with NBM-200 were found to be more variable, with a CV of 4.3%. DiaSpect and HemoControl showed slightly lesser variation (CV of 2.2% and 2.5%, respectively).

On comparing the rapidity of testing, the mean testing time for DiaSpect was 1.9 seconds which is significantly shorter ($p<0.01$) than that of the other available POCT devices. This makes DiaSpect perfectly suitable for screening purpose in a blood bank and in outdoor camps, helping to shorten the waiting period for donors and to facilitate efficient crowd management.

Conclusion

To conclude, POCT devices which are becoming increasingly popular for screening Hb in blood banks are time-saving and easy to operate but lack reliability in many circumstances. It is very important to check the precision and accuracy of a device before its use blood banks. The non-invasive NBM-200 device has the advantage of inflicting less pain and hence being more acceptable to donors, but it lacks the desired sensitivity. This test is not effective in excluding ineligible donors which may pose a threat to donors' health. DiaSpect gives fast and accurate results and is, therefore, suited for screening donors in blood banks.

Authorship contributions

AS and AS contributed to research, design, acquisition, analysis and interpretation of data. AD drafted the paper and revised it critically. RC approved the final version.

The Authors declare no conflict of interest.

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