University of the Highlands and Islands
Oilthigh na Gàidhealtachd agus nan Eilean

UHI Research Database pdf download summary

Haemoglobin A1c determination from dried blood spots prepared with HemaSpot blood collection devices

Hall, Jenny; Fowler, Claire; Pollock, Anne ; MacRury, Sandra

Published in:
Clinical Chemistry and Laboratory Medicine

Publication date:
2020

The Document Version you have downloaded here is:
Peer reviewed version

The final published version is available direct from the publisher website at:
10.1515/cclm-2020-0675

Link to author version on UHI Research Database

Citation for published version (APA):
Hall, J., Fowler, C., Pollock, A., & MacRury, S. (2020). Haemoglobin A1c determination from dried blood spots prepared with HemaSpot blood collection devices: comparison with fresh Capillary blood. *Clinical Chemistry and Laboratory Medicine*, *59*(2), e79-e82. https://doi.org/10.1515/cclm-2020-0675

General rights
Copyright and moral rights for the publications made accessible in the UHI Research Database are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights:

1) Users may download and print one copy of any publication from the UHI Research Database for the purpose of private study or research.
2) You may not further distribute the material or use it for any profit-making activity or commercial gain
3) You may freely distribute the URL identifying the publication in the UHI Research Database

Take down policy
If you believe that this document breaches copyright please contact us at RO@uhi.ac.uk providing details; we will remove access to the work immediately and investigate your claim.

Download date: 02. Oct. 2023

Full title: Haemoglobin A_{1c} determination from HemaSpot™ blood collection devices: comparison of home prepared dried blood spots with standard venous blood analysis.

Short title: HbA_{1c} determination using HemaSpot™ devices

J.M. Hall[1], C. F. Fowler[2], F. Barrett[3], R. W. Humphry[4], M. Van Drimmelen[2], S. M. MacRury[5]

[1] Division of Rural Health and Wellbeing, Institute of Health Research & Innovation, University of the Highlands and Islands, Centre for Health Science, Old Edinburgh Road, Inverness, IV2 3JH

[2]Department of Biochemistry, Blood Sciences, Raigmore Hospital, Inverness, IV2 3UJ, Scotland

[3]Highland Clinical Research Facility, NHS Highland, Centre for Health Science, Old Edinburgh Road, Inverness, IV2 3JH

[4] Epidemiology Research Unit, Scotland's Rural College, An Lòchran, Inverness Campus, Inverness' IV2 5NA

[5] Institute of Health Research and Innovation, University of the Highlands and Islands, Centre for Health Science, Old Edinburgh Road, Inverness, IV2 3JH

Corresponding author: Professor Sandra MacRury, Institute of Health Research and Innovation, University of the Highlands and Islands, Centre for Health Science, Old Edinburgh Road, Inverness, IV2 3JH. E-mail: sandra.macrury@uhi.ac.uk

Word Counts

Abstract: 248

Main body of text: 3160

Conflicts of interest: None

Number of tables: 3

Number of figures: 3

Supplementary materials: 2 tables

Novelty Statement

- HbA_{1c} determination from dried blood spots has been reported but results have been affected by stability issues, requiring methodologies which have included extended drying periods, and storage at low temperatures or for a limited period of time
- HbA_{1c} levels determined from HemaSpot™ blood collection devices show a strong correlation with venous HbA_{1c} results, with the potential for calibration against the venous method used.
- Patient acceptance of the blood collection method was high, with 61.7% of participants indicating that they would be more likely to have their testing carried out if this method of blood collection was available.
- By providing patients with an opportunity to increase compliance with regular HbA_{1c} testing, use of venous calibrated HbA_{1c} determination from HemaSpot™ blood collection devices provides the potential for improved glycemic control.

Funding

The study was supported by a grant from the Chief Scientist Office (CZH/4/1122)

Acknowledgements

The authors are very grateful to the people with diabetes who participated in the study

Abstract

Aims

HbA_{1c} monitoring in the Scottish Highlands is by HPLC analysis of GP collected venous samples at a centralised laboratory. Availability of HbA_{1c} results at clinic appointments is less than ideal due to patient reticence and availability/timeliness of appointments. This study assessed the clinical performance and patient acceptance of HemaSpot™ blood collection devices as an alternative blood collection method.

Methods

Adult men and women with any type of diabetes, routinely carrying out self-monitoring of blood glucose were recruited (n=128). Participants provided a venous blood sample and prepared two HemaSpot™ dried blood spots (DBS), one at clinics and one at home. HbA_{1c} analysis was by TOSOH G8 HPLC. Participants also completed a questionnaire.

Results

A strong linear relationships between HbA_{1c} levels in dried blood spots and venous blood were observed and a linear model was fitted to the data. Time between dried blood spot preparation and testing did not impact the model.

Participants were accepting of the approach, 69.2% would use this system if available and 60.7% would be more likely to use this system than going to their GP.

Conclusions

The combination of a robust desiccating dried blood spot device, home sample preparation and return by post produces HbA_{1c} data which supports use of a time-independent linear calibration of dried blood spot to venous blood HbA_{1c}. A robust remote sample collection service would be valuable to people living with diabetes in urban areas who are working or house-bound as well as those living in remote or rural locations.

Keywords

Diabetes mellitus, dried blood spot testing, glycated haemoglobin A, HPLC.

Introduction

The benefits of good blood glucose control in preventing long term complications of diabetes are well documented (1). Complications, arising from poor blood glucose control over extended periods, place an economic burden on health services and significantly reduce health-related quality of life in people with diabetes (2,3).

Ongoing blood glucose control is assessed by regular measurement of HbA_{1c}, with several laboratory methods available for use with fresh blood obtained using either venepuncture or by fingerprick with collection in capillary tubes. Point-of-care instruments are now available for measuring HbA_{1c} levels in fresh capillary blood (4).

In the Scottish Highlands (NHS Highland Health Board area) HbA_{1c} determinations are performed centrally in Inverness using ion-exchange HPLC analysis (TOSOH G8 HPLC analyser (TOSOH Bioscience, Tokyo, Japan)) on venous blood samples collected locally at

GP practices. If there is no recent HbA_{1c} result, DCA Vantage point-of-care instruments (Siemens Healthcare) are used at hospital appointments.

Current sampling methods are acceptable in terms of HbA_{1c} determination, however the use of venepuncture with centralised testing is not providing users in the NHS Highland area with an acceptable approach as evidenced by the frequency with which HbA_{1c} results are not available at clinical appointments.

Individualised HbA_{1c} targets should be agreed and regularly reviewed at diabetes appointments with clinicians, and lifestyle and/or medication changes discussed with the aim of optimising HbA_{1c} levels. The value of diabetes appointments where HbA_{1c} levels are not available is greatly diminished. For hospital based appointments, point-of-care instruments are available for immediate HbA_{1c} determinations. However the vast majority of people with diabetes (those with uncomplicated type-2 diabetes) are managed in the community by GPs where point-of-care instruments are not generally available. In our local Health Board area the cost of HbA_{1c} analysis is borne by secondary rather than primary care so the costs and maintenance implications of GP based point-of-care instruments are not straightforward.

Method and convenience of place of collection and timeliness of results are potential factors which impact the availability of HbA_{1c} results at appointments and consequently impact the opportunity for discussion of HbA_{1c} levels and targets.

In line with recognition by the UK and Scottish government that "patients need to be empowered to manage their care" (5.6), the long term aim of our approach is for people with diabetes to be able to take responsibility for sending off their own blood samples and for

results to be sent directly to them so that they can attend review appointments having had the opportunity to reflect on their latest HbA_{1c} result and what it might mean for them in terms of their individual HbA_{1c} target. To address this aim we have identified a robust blood sampling device (HemaSpot™, Spot on Sciences Austin, Texas, US) for preparing dried blood spots (DBS) which has the potential to fulfil the requirements of our overall approach.

HemaSpot™ blood collection devices comprise a robust plastic wallet enclosing an eight bladed filter paper surrounded by desiccant (Fig. 1). A protective cover allows blood application through a central hole. The device is designed to absorb two hanging drops of blood, equivalent to about 65-105µl blood.

The use of filter papers for collection of dried blood spots is accepted as an alternative method of blood collection for a variety of applications (7) as they are simple to prepare, have low costs of collection and transport, and are safer and more acceptable to study participants (8).

A systematic review identified 17 studies using dried blood spots for HbA_{1c} (9) with two extra studies recently published (10, 11). Variation and bias increase with increasing time between sample preparation and testing have been observed, meaning that even when HbA_{1c} can be calibrated against standard venous HbA_{1c}, calibration needs to consider the time between sample preparation and testing.

A laboratory based study previously carried out in our laboratory compared HbA_{1c} analysis of laboratory prepared HemaSpot™ dried blood spots (n=40) with HbA_{1c} values obtained from fresh capillary blood. A strong correlation between HbA_{1c} from dried blood spots and fresh capillary blood was observed, suggesting that HemaSpot™ devices have the potential to be

used as an alternative to current blood collection methods if they were well calibrated using a linear model and dried blood spots were tested within three days of preparation (see Supplementary Material Table 4).

The purpose of the current study was to assess the clinical performance and user acceptability of dried blood spots prepared using HemaSpot™ devices by people with diabetes at home, as an alternative blood sample collection method for HbA_{1c} determination.

Participants and Methods

Recruitment

Participants (128) were recruited when they attended their routine diabetes clinic appointments in Inverness. It was anticipated that this number of participants would provide at least the recommended 100 returned home dried blood spot samples for comparison (12).

Adult men and women, aged 18-75 years, with any form of diabetes and regularly carrying out self-monitoring of blood glucose (SMBG) were included in the study. Pregnant women and patients receiving renal replacement therapy were excluded.

All participants gave written informed consent. NHS research ethics approval was obtained (16/NW/0214).

While attending diabetes clinics, venous blood samples were taken for routine HbA_{1c} analysis. Under the guidance of a research nurse participants prepared dried blood spots from finger prick blood using HemaSpot™ blood collection devices by applying blood from a hanging

drop of blood until the filter paper in the device was visibly filled. HemaSpot™ devices were closed immediately after blood application and stored at 4°C until analysis.

Participants were given a home pack which included a dried blood spot preparation kit, questionnaire and information about HbA_{1c}. Free post envelopes were provided for return of home prepared dried blood spots and questionnaires. Participants were asked to post their dried blood spot on the day of preparation.

Questionnaire

A 5-point Likert scale 17 question questionnaire assessed participant experience of preparing dried blood spots, thoughts on a remote HbA_{1c} service and views on the information provided about HbA_{1c}.

HbA_{1c} analysis

HbA_{1c} analyses were performed using the Tosoh HLC 723-G8 (HPLC analyser (Tosoh Bioscience, Tokyo, Japan) using a cation exchange TSKgel variant HSi column. The column was calibrated to International Federation of Clinical Chemistry using Tosoh calibrators. Lyphocheck Diabetes bi-level controls (Bio-Rad Laboratories, California, USA) were used daily, with CVs of 2% for the low control (mean of 33mmol/mol (5.2%)), and 0.9% for the high control (mean of 78 mmol/mol (9.3%)).

Blood from dried blood spot samples was eluted by placing 1 HemaSpot™ filter blade in 1ml Hemolysis/Wash solution for 2 hours at ambient temperature. For analysis, 4µl of eluate were aspirated by the analyser before injection onto the column.

Dried blood spots were stored at 4°C in the laboratory. Processing and analysis was performed within four days of preparation.

Chromatograms were assessed visually using the G8 Operator's Manual specifications for results acceptability, with guidance from the software flags.

Data analysis

Data analysis was carried out using IBM SPSS versions 19/20 for Windows (IBM Corporation, NY, USA), R and Excel software (Microsoft Corporation, WA, USA). Correlation between sample collection methods was investigated using Pearson coefficient and regression analysis. Agreement and bias between sample collection methods was investigated using Bland-Altman plots. Statistical significance was determined at the 5% level.

Covariates that were considered to be potential confounders were tested separately to one another within the general linear model and as a full interaction with the main predictor variable (home result) to assess whether the confounder was affecting the relationship (i.e. the calibration) between the home result and the venous reading. Nested models (with and without the additional covariate) were tested for statistical significance using an F-test (using the function "anova()" in R)

Results

Participant Characteristics

Participant characteristics are presented by type and duration of diabetes (Table 1). More women (61.3%) than men with type 1 diabetes and more men (69.0%) than women with type

2 diabetes participated, however the overall numbers of men (N=53) and women (N=51) participants were similar.

Blood samples available

HbA_{1c} results were available for 127 venous blood samples, 125 clinic and 104 home prepared dried blood spots. Minimum and maximum times between home dried blood spot preparation and testing were 1 and 4 days, with 38.5%, 43.3%, 16.3% and 1.9% of samples tested on days 1, 2, 3 and 4 respectively.

HbA_{1c} analysis

HbA_{1c} from both home and clinic prepared dried blood spot types exhibit strong correlations with venous HbA_{1c} (R^2 values close to 0.98), (Fig. 2). There is a significant difference between the clinic and home dried blood spot relationships with venous blood. We have excluded the time between sample preparation and HbA_{1c} analysis as a potential source of the difference. Early transfer of clinic prepared dried blood spots to storage at 4°C differs from home dried blood spot treatment immediately after preparation, however we have not been able to confirm or exclude this as a reason for the difference observed.

The mean bias of home dried blood spots, compared with venous blood, across the measurement range was +4.27mmol/mol (+0.39 %). A plot of the absolute differences against the mean of venous and home dried blood spot HbA_{1c} indicates that the absolute difference increases with the mean of the pair (Fig. 3), suggesting that whilst home dried blood spot HbA_{1c} results may not be used directly as an equivalent to venous results they may be successfully calibrated against one another. A general linear model was used to establish a calibrating relationship between home dried blood spot results and venous results. Diagnostic

plots supported the assumption of normality amongst residuals and did not suggest heterogeneity of variance, supporting the use of this model.

The model fitted is:

$[HbA_{1c}]_{Venous} = 1.18[HbA_{1c}]_{HomeDBS} - 7.5$ (mmol/mol)

$[HbA_{1c}]_{Venous} = 1.18[HbA_{1c}]_{HomeDBS} - 0.69$ (%)

The model allows prediction of venous blood HbA_{1c} levels from dried blood spot HbA_{1c} results. Concordance values were 100%, 92.5% and 96.6% between venous and predicted values from home dried blood spots for HbA_{1c} values of <48 mmol/mol (<6.5%), 48-64 mmol/mol (6.5-8%) and >64 mmol/mol (>8%) respectively.

The model holds only for the range of HbA_{1c} values in the study sample (35-115 mmol/mole (5.4 – 12.7%)) and when HbA_{1c} analysis from HemaSpot™ is performed using a TOSOH G8 Analyser.

A number of covariates were individually tested to assess how they affected the calibration, using a set of nested linear models with each of the variables added separately to the main model (Table 2). Of the covariates tests, only a laboratory factor covariate (with lab staff carrying out the test as a proxy) statistically significantly affected the results.

Questionnaire

One hundred and ten (86%) questionnaires were returned. Of these, 101 had a corresponding home dried blood spot sample.

The majority (99.1%) of respondents found the instructions easy to use and 83.5% found it easy to get their HemaSpot™ in the post on time. Obtaining enough blood, blood application to the device and deciding when there was enough blood on the device was less easy (Table 3).

When asked if they would use the system if it was available, 69.2% of respondents agreed, while 61.7% agreed that they would be more likely to use a dried blood spot system than making an appointment with their practice nurse. A similar percentage (60.7%) agreed they would have a preference for using this system at home compared with having blood taken at their GP surgery.

Sixty per cent of respondents agreed that they would be more likely to have their HbA_{1c} test done if the system was available, while 50.0% of respondents felt that using a system like this would help them to feel more in control of their diabetes.

The majority (88.0%) of respondents found the information provided about HbA_{1c} interesting and 81.5% found the information useful.

Discussion

The principal finding from the study is that HemaSpot™ dried blood spots prepared by study participants at home and analysed using the TOSOH G8 system, produce clinically acceptable HbA_{1c} results when the dried blood spot method is calibrated to TOSOH G8 determined venous HbA_{1c} results using a general linear model. Time between sample preparation and testing did not significantly affect the calibration up to 4 days. For this analysis the unbalanced nature of the number of observations in each "bin" (i.e. number of days between

sampling time and testing) is not preferred but doesn't violate any of the assumptions in a general linear model such as this. It merely reduces the statistical power of the test.

A statistically significant effect on calibration was observed for the laboratory factor covariate tested (Table 2), where laboratory personnel has been used as a proxy. For example, as a consequence of logistics, there may have been a relationship between the personnel and the day, or time of day, on which a sample was tested. It is also possible that a type I error has occurred (given that 5 statistical hypotheses were tested, there is an estimated 23% chance of a type I error). In favour of the hypothesis that a type I error may have occurred is that for each of the particular proxies the additive and interaction terms affecting the calibration relationship all had confidence intervals encompassing zero– i.e. no individual proxy appears to be statistically or clinically significantly from the rest. For full details see Supplementary Material Table 5.

Several HbA_{1c} dried blood spot studies have been published (10,11,13,14,15) with analysis by turbidimetric inhibition immunoassay (13,16,17,18), HPLC analysis (10,11,15,19,20) and affinity chromatography (21,24). A systematic review and meta-analysis by Affan *et al* reported that, although results from venous and dried blood spots were different, there was close agreement (meta-analysis regression equation: $[HbA_{1c}]_{DBS} = 0.9553[HbA_{1c}]_{venous} + 0.2566$ (%)) between venous and dried blood spot samples, except when analysis was by affinity chromatography (9). Slope and intercept ranges in the meta-analysis were 0.88-1.25 and 0.002-1.8 (units: %) respectively. Our model slope and intercept fit within these ranges, however the model is only valid when used with the measurement method used in our study.

In vitro glycation of haemoglobin and degradation of HbA_{1c} during storage have been suggested as possible reasons for differences observed between dried blood spots and fresh blood sample (13, 22). Our results show increasing bias as HbA_{1c} increases which supports the *in vitro* glycation theory due to increased blood glucose levels (and hence *in vitro* glycation) associated with higher HbA_{1c} levels.

Precision of dried blood spot sample elution and analysis method was assessed by repeat analysis of high and low QC samples which had been applied to filter papers and eluted using the dried blood spot sample elution and analysis protocol described. CVs were comparable to CVs reported for fresh blood analysis suggesting that minimal error is introduced due to dried blood spot sample elution and analysis protocols..

Both venous blood collected in EDTA tubes (11,14,16,18,19,23) and finger prick capillary blood (10,13,15,17,20,21,24) have been used to prepare dried blood spots on filter paper with drying times ranging from 20 minutes (24) to overnight (28) reported, before placing the dried blood spots in a storage bag or envelope. The HemaSpot™ device has no requirement for a pre-drying step.

With the exception of two previous studies (10, 13), dried blood spots have been prepared by health care professionals or researchers. For routine, remote monitoring of HbA_{1c}, a dried blood spot approach needs to be evaluated in the hands of the end user. Our findings show acceptable clinical results when dried blood spots were prepared by users.

Stability of HbA_{1c} on dried blood spots has been a concern with an indication that a time dependent calibration for estimating venous HbA_{1c} levels from dried blood spots is required

(15,16). Our findings suggest that with the HemaSpot™ device a single calibration could be used for samples tested up to 4 days after preparation.

In agreement with our findings, high patient satisfaction with a potential dried blood spot HbA_{1c} monitoring system has previously been reported in The Netherlands (13).

Although participants in our study reported that instructions were easy to follow, several participants found difficulty with blood application and in deciding when there was sufficient blood on the device. Comments included "*I don't think I got enough blood into the device*", and "*does take a bit of work to "fill" the device to the edges*". However concern about sufficient filling was not reflected in the results, where a strong correlation between venous and dried blood spot HbA_{1c} results was observed. The difficulties reported could be overcome by inclusion of photographs of sufficiently and insufficiently filled devices in the instructions, to give patients confidence that they are providing an adequate amount of blood for analysis. Additional tips on blood application could also be incorporated.

A number of participants felt that they would still prefer to have an appointment at their GP practice both because they had other tests carried out at the same time and valued time spent with their health care professional. One participant wrote "*When visiting the nurse for a HbA_{1c} I also get my blood pressure, weight, and feet checked and the opportunity for questions and instructions/guidance from the nurse. I would miss this testing at home*". The introduction of a remote monitoring service for HbA_{1c} needs to consider not only what might be gained by this approach, but also what patients might lose through lack of contact with health care professionals.

Provision of information about HbA_{1c} was welcomed by participants. The opportunity to provide simple clear information about HbA_{1c} and its importance in management of diabetes should be considered in any remote HbA_{1c} monitoring approach.

The combination of a robust desiccating dried blood spot device, home sample preparation and return by post, to our knowledge, has not been reported for HbA_{1c}, and provides the opportunity to introduce a remote sample collection service which would be equally valuable to people living with diabetes in urban areas who are working or house-bound as well as those living in remote or rural locations.

The observation that the time between sampling and testing did not significantly affected the linear model indicates that use of the HemaSpot™ device might enable a single model to be used that is independent of the time between preparation of the dried blood spot and the day of testing.

Further studies are planned to assess the performance, acceptability and service delivery options of HemaSpot™ dried blood spots prepared by people with diabetes managed in the community.

The health economics of introducing a remote HbA_{1c} monitoring service using HemaSpot™ devices needs to be assessed, taking into consideration both the immediate impacts on costs and the longer term costs associated with complications.

Study Limitations

Some limitations of the study should be noted. First, participants were all regularly carrying out SMBG and so were familiar with obtaining drops of capillary blood. The wider diabetes community will have the same familiarity so this is a population in which the model derived in the study would need to be validated prior to practical application. Second, only one batch of HemaSpot™ devices was available to evaluate over the period of study so it was not possible to assess the impact of batch to batch variation. Third, the study focused only on analysis of HbA_{1c} using the TOSOH HbA_{1c} analyser, the method used in the laboratory where routine HbA_{1c} analyses for the region are performed. Fourth, the difference observed between clinic prepared and home prepared dried blood spots remains unexplained and warrants further investigation. Finally, further exploration of the observed laboratory factor effect is needed.

Clinical Implications

HbA_{1c} monitoring plays a pivotal role in preventing complications of diabetes and therefore in achieving as good a quality of life as possible. The HemaSpot™ blood collection device provides an opportunity for improvements in rates of HbA_{1c} monitoring and more effective consultations. Increased participation by the diabetes community in self-management would be anticipated.

References

1. Diabetes Control and Complications Trial (DCCT) Research Group The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent Diabetes Mellitus. N Eng J Med 329:977-998, 1993
2. Hayes A, Arima H, Woodward M, Chalmers J, Poulter N, Hamet P, Clarke P Changes in Quality of Life Associated with Complications of Diabetes: Results from the ADVANCE Study. Value Health 2016; 19:36-41.
3. Nielsen HB, Ovesen LL, Mortensen LH, Lau CJ, Joensen LE Type 1 diabetes, quality of life, occupational status and education level – A comparative population-based study. Diabetes Res Clin Prac 2016; 121:62-68.

4. Lenters-Westra E and Slingerland RJ Three of 7 hemoglobin A1c point-of-care instruments do not meet generally accepted analytical performance criteria. Clin Chem 2014; 60(8):1062-1072
5. Scottish Government Diabetes Action Plan. Scottish Government, 2010
6. Scottish Government Diabetes Improvement Plan. Scottish Government, 2014
7. McDade TW, Williams S. Snodgrass JJ What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography 2007; 44: 899-925.
8. Mei JV, Alexander JR, Adam BW, Hannon WH Use of filter paper for the collection and analysis of human whole blood specimens. J Nutr 2001; 131:1631s-6s.
9. Affan ET, Praveen D, Chow CK, Neal BC Comparability of HbA_{1c} and lipids measured with dried blood spot versus venous samples: a systematic review and metaanalysis. BMC Clin Pathol 014; 14:21-30.
10. Mastronardi CA, Whittle B, Tunningley R, Neeman T, Paz-Filho G The use of dried blood spot sampling for the measurement of HbA_{1c}: a cross-sectional study. BMC Clinical Pathology 2015; 15:13-19.
11. Maleska A, Hirtz C, Casteleyn E, Villard O, Ducos J, Avignon A, Sultan A, Lehmann S Comparison of HbA_{1c} detection in whole blood and dried blood spots using an automated ion-exchange HPLC system. Bioanalysis 2017; 9:427-434.
12. Clinical and Laboratory Standards Institute Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved. Guideline—Third Edition. CLSI document EP09-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2013
13. Fokkema M, Bakker A, de Boer F, Kooistra J, de Vries S, Wolthuis A HbA_{1c} measurements from dried blood spots: Validation and patient satisfaction. Clin Chem Lab Med 2009; 40:1259-1264.
14. Lakshmy, R and Gupta, R Measurement of Glycated Hemoglobin A_{1c} from Dried Blood by Turbidimetric Immunoassay. J Diabetes Sci Technol 2009; 3: 1203–1206.
15. Lomeo A, Bolner A, Scattolo N, Guzzo P, Amadori F, Sartori S, Lomeo L HPLC analysis of HbA_{1c} in dried blood spot samples (DBS): a reliable future for diabetes monitoring. Clin Lab 2008; 54:161-167.
16. Jones TG, Warber KD, Roberts BD Analysis of Hemoglobin A_{1c} from Dried Blood Spot Samples with the Tina-quant(®) II Immunoturbidimetric Method. J Diabetes Sci Technol 2010; 4:244-249.
17. Anjali, S Geethanjali F, Selva Kumar R, Seshadri M Accuracy of filter paper method for measuring glycated hemoglobin. J Assoc Physicians India 2007; 55:115-119.
18. Tabatabaei O, Heshmat R, Omidfar K, Pasalar P, Delavari A, Keshtkar A, Larijani B Glycated hemoglobin measurements from dried blood spots: Reliability and relation to results obtained from whole blood samples. J Diabetes Sci Technol 2011; 10:1-6.
19. Egier DA, Keys JL, Hall SK, McQueen MJ Measurement of Hemoglobin A_{1c} from Filter Papers for Population-Based Studies. Clinical Chemistry 2011; 57:577-585.
20. Lacher D, Berman L, Chen T-C, Porter K Comparison of Dried Blood Spot to Venous Methods for Hemoglobin A_{1c}, Glucose, Total Cholesterol, High-density Lipoprotein Cholesterol, and C-reactive Protein. Clin Chim Acta 2013; 422:54-58.
21. Little RR, McKenzie EM, Wiedmeyer HM, England JD, Goldstein DE Collection of blood on filter paper for measurement of glycated hemoglobin by affinity chromatogrphy. Clin Chem 1986; 32:869-871.
22. Eross J, Kreutzmann D, Crowell C, Silink M. Glycated haemoglobin measurement in dried blood on filter paper. Clin Chem 1986;32:2222.
23. Buxton OM, Malarick K, Wang W, Seeman T Changes in dried blood spot HbA_{1c} with varied post-collection conditions. Clinical chemistry 2009; 55:1034-1036.

24. Gay EC, Cruickshanks KJ, Chase HP, Klingensmith G, Hamman RF Accuracy of a filter paper method for measuring glycosylated hemoglobin. Diabetes Care 1992; 15:108-10.

Table 1. Description of study population for whom both venous and home DBS HbA_{1c} levels were available.

Type of diabetes		N	Age (years)			Duration of diabetes (years)		
			Mean	Minimum	Maximum	Mean	Minimum	Maximum
Type 1	Women	38	48.8	19	71	26.8	3	60
	Men	24	44.8	19	71	19.3	0.1	44
	Total	62	47.3	19	71	23.9	0.1	60
Type 2	Women	13	55.6	31	69	12.0	2	20
	Men	29	64.0	39	84	14.0	0.3	44
	Total	42	61.4	31	84	13.4	0.3	44
Total population		104	53.0	19	84	19.6	0.1	60

Table 2. The effect of the addition of potential covariates on top of the base model of the venous reading (dependent variable) regressed against the home result (the independent variable). Each additional covariate was added separately and as a full interaction with the home result. Each nested pair of models (without covariate and with) were compared using an analysis of variance. For each nested pairwise comparison of models the data set used was the full data set, less any observations that had missing data for either the home result or the added covariate. For further details see Supplementary Material Table 5.

Additional covariate	p-value for full interaction cf base model
Own Lancet (Y=27, N=74)	0.40
Type of Diabetes (Type 1=64, Type 2=42)	0.47
Sex (F=52, M=53)	0.11
Time between sampling & testing (N=105) (Days(n): 1(39), 2(47), 3(17) and 4(2))	0.30
Lab factor covariate (A=15,B=38,C=1,D=2,E=17,F=13,G=3,H=16)	0.016

Table 3. Analysis of questionnaire responses to questions relating to experience of using and potential use of the HemaSpot™, and the information provided about HbA_{1c}. The total number of responses to each question (n) is shown in the left hand column.

I found…..	Easy or very easy to use (%)	Neither easy nor difficult (%)	Difficult or very difficult (%)
..following the instructions (n=108)	99.1	0	0.9
..using the lancet provided (n=94)	89.4	8.5	2.1
..getting my sample in the post on time (n=109)	83.5	7.3	9.2
..getting enough blood (n=109)	50.5	21.1	28.4
..applying the blood to the device (n=108)	44.5	22.2	33.3
..deciding when I had applied enough blood (n=109)	56.9	20.2	22.9
	Agree or strongly agree (%)	Neither agree nor disagree (%)	Disagree or strongly disagree (%)
Would use if available (n=107)	69.2	11.2	19.6
More likely to use than making an appointment with practice nurse (n=107)	61.7	15.0	23.3
Prefer to use this system at home compared with having blood taken at GP (n=107)	60.7	17.8	21.5
More likely to have HbA_{1c} test done (n=107)	59.8	15.0	25.2
Help feel more in control of my diabetes (n=106)	50.0	22.6	27.4
Prefer to have pack sent to them by post (n=105)	66.7	15.2	18.1
Happy to collect pack from GP (n=104)	51.0	19.2	29.8
Found information sheet about HbA_{1c} interesting (n=108)	88.0	10.2	1.9
Found information sheet about HbA_{1c} useful (n=108)	81.5	16.7	1.8
HbA_{1c} information sheet detail about right (n=109)	83.5	14.7	1.8
Would like HbA_{1c} information sheet to be more detailed (n=107)	28.0	32.7	39.3

Legends for figures

Figure 1 The HemaSpot™ blood collection device showing the blades of the fan shaped filter paper (in red). Once blood has been applied through the hole in the application surface the device is folded over and snapped shut. *Image courtesy of Spot on Sciences.*

Figure 2**.** Home and clinic prepared DBS HbA_{1c} plotted against venous HbA_{1c}. Home DBS: solid symbols, dotted line. Clinic DBS: open symbols, dashed line.

Figure 3. Bland-Altman plot, the difference between venous and home DBS HbA_{1c} showing evidence of a straight line relationship between the absolute difference and the mean values for the pairs of scores. Solid line: mean of difference; dotted lines, upper and lower 95% confidence intervals.