

using remote blood collection with VAMS to accurately measure HbA1c in diabetes

James Rudge, PhD, Technical Director, Neoteryx



Abstract

This technical brief presents methods for measuring accurate HbA1c in diabetic patients from blood samples collected remotely using Mitra[®] microsampling devices, which are based on volumetric absorptive microsampling (VAMS[®]) technology. Measurement of HbA1c from samples taken by patients at home and sent to a laboratory for analysis is an ideal solution for remote patient monitoring of diabetic patients. This is especially true during a viral pandemic, when vulnerable patients cannot safely travel to the clinic for routine blood draws. Using examples from studies that have compared blood sampling using DBS and other techniques versus VAMS, we show that with more research focused on stabilizing blood microsamples, remote blood collection with VAMS for accurate HbA1c measurement is feasible.

Introduction

What is diabetes? Understanding the Disease Process

It is estimated that 415 million people worldwide are living with diabetes, which means that 1 in 11 people in the world have this chronic condition. This figure is expected to rise to 642 million by 2040¹. The main types of diabetes are type 1 and type 2. Type 1 diabetes, which accounts for approximately 10% of all diabetes diagnoses, is caused by an autoimmune attack on the β -cells of the pancreas. This attack shuts down production of the hormone insulin, which is needed to allow glucose to be absorbed into cells.

Type 2 diabetes, in contrast, is attributed to lifestyle and other factors, where both a diet rich in refined carbohydrates, and a lack of exercise leads to insulin resistance. Insulin resistance is where the hormone, released from a working pancreas, becomes progressively less efficacious because of altered insulin receptors of peripheral tissues, thus preventing optimal uptake of glucose into these cells. Over time, the pancreas becomes overstimulated, which is further exacerbated by medication, such as metformin, designed to treat the illness. Eventually, the pancreas will shut down insulin production, reducing glucose clearance in the blood. The result of reduced insulin production, and/or insulin resistance, is that glucose levels



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in the blood rise (hyperglycemia), leading to several serious complications. These include heart disease, blindness, kidney failure and capillary vascular disease, which can lead to ulceration, amputation and, in some cases, sepsis. In addition to hyperglycemia, diabetics can also experience hypoglycemia, which can lead to coma and, eventually, death.

For those living with diabetes, good control of blood sugar (glucose) through lifestyle modifications and medication is critical to preventing complications. There are two common blood tests which diabetics and their medical practitioners rely on to monitor and manage the condition. The first is measurement of blood glucose. This measurement is transient, but important information for the diabetic to have, as it indicates if they are presently hypo- or hyperglycemic. It is critically important for diabetics to measure their blood glucose on a daily basis to avoid dangerous complications, such as diabetic coma, which can sometimes be deadly. Blood sampling and glucose testing is often conducted by the diabetic patient or a caregiver. Advances in medical technologies have led to the development of point-of-care devices that can be used to collect and measure capillary blood from a fingertip. However, to help physicians and the medical team diagnose and measure the progression of the disease over time, it is also common practice to schedule routine clinic visits for the measurement of glycated hemoglobin, known as HbA1c.

What is HbA1c?

It is common for proteins in our bodies to have some degree of glycation. Sugars on the surfaces of proteins confer a plethora of cellular and metabolic functions. However, glucose can also covalently bond, non-specifically, to proteins. This causes some of the complications seen with diabetes. Indeed, the non-specific glycation of the gas exchange molecule hemoglobin in the blood acts as an ideal biomarker. Glucose, when present in the blood, will react nonenzymatically with a N-terminal valine residue of one or both beta chains of the protein through a Schiff base reaction, forming an aldimine. This then rearranges irreversibly to the more stable ketoimine adduct. Simply put, the more sugar in the blood (averaged out over a period of time), the more glycation will be observed on the Hb molecule. Red blood cells have a lifetime of 100-120 days. Thus, measuring A1c concentrations during this timeframe gives the physician a snapshot of glucose levels maintained by the diabetic. The A1c measurement allows for further intervention, if necessary, such as an adjustment of insulin dose or a change in diet.

At the time of writing, the World Health Organization (WHO) suggests the following guidelines for the diagnosis of type 2 diabetes: an HbA1c measurement that falls below 42 mmol/mol (6%) is non-diabetic. HbA1c levels of 42-47 mmol/mol (6.0-6.4%) are prediabetic, and a level of 48 mmol/mol (6.5%) or over is seen as developed type 2 diabetes².



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Measuring HbA1c

There are a variety of methods employed that can be used to measure HbA1c. These include chromatography, immunoassay, capillary electrophoresis and enzymatic. The most popular of these used in clinical chemistry laboratories are chromatography (either cation exchange or affinity) and immunoassay, such as immunoturbidimetry. Typically, when testing for HbA1c, venous blood is used. However, for some clinics, especially those focused on pediatrics, testing capillary blood can be used as it has been shown to be interchangeable to venous blood³. Furthermore, although typically up to 7 mL of blood is taken, only a small drop is needed for the analysis, which makes it ideal for patient-centric microsampling. Indeed, there are several point-of-care devices available that use a number of different methods and, although some of these devices are excellent, there has been slow adoption of their use. Moreover, a review and meta-analysis of devices published in 2017 suggested that some of these devices showed a negative bias when compared to conventional lab methods, plus larger standard deviations⁴.

The clinical acceptance criteria for HbA1c is based on those recommended by The Royal Australasian College of Physicians (RACP), which state that if the target HbA1c result is ≤ 86 mmol/mol, an allowable error should be within ± 4 mmol/mol. If the target HbA1c result is > 86 mmol/mol, the measured value should be within $\pm 5\%$ limits of the target value⁵. Such requirements make development of new HbA1c assays challenging, but appealing. Remote patient-centric sampling for HbA1c analysis would be ideal for monitoring diabetes without requiring the patient to go to clinic for a standard HbA1c blood test. Pairing the advantages of dried blood microsamplers such as the Mitra with VAMS devices for at-home blood collection, with validated testing in a controlled laboratory would provide an ideal solution.

Using Microsampling to Evaluate Patient-Collected Samples for Measuring HbA1c

First published online in 2016, Stove *et al* investigated the possibility of using Mitra with VAMS for at-home blood collection. Their paper discussed the challenges of developing a dried blood assay using both DBS and VAMS⁶. When comparing dried extracts from both DBS and VAMS to fresh venous blood samples, even analyzing extracts after just 1-3 days of drying (under desiccant), neither microsampling technique met the RCPA criteria. Furthermore, after 4-6 days in storage, the situation for both sampling techniques was further degraded, showing poor agreement compared with fresh samples. It must be noted, however, that under these conditions, DBS samples showed greater acceptance criteria than Mitra with VAMS. Nevertheless, neither technique met the criteria, for a reliable HbA1c measurement, with at-home sample collection and drying under



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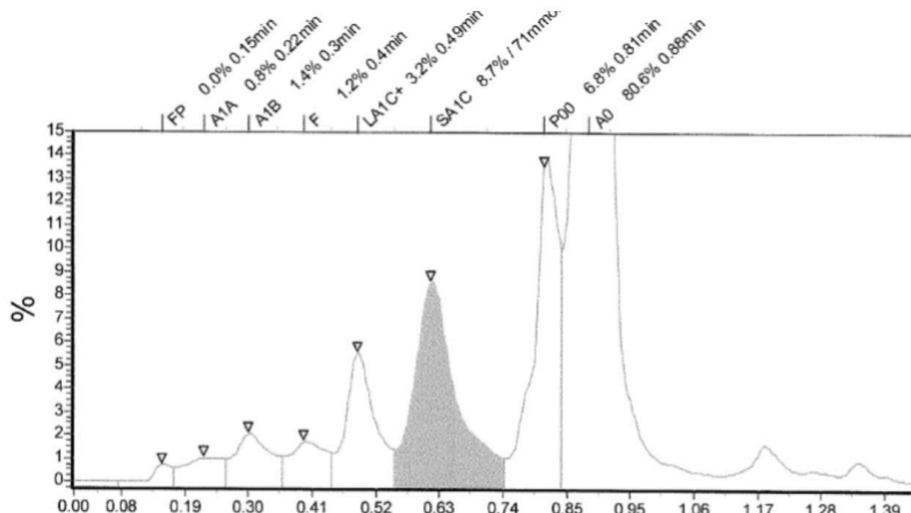
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desiccant.

Interestingly, when measuring HbA1c from wet Mitra tips (within one hour of sampling), the results were well within the RCPA quality requirements ($\rho = 0.995$ and $CCC = 0.996$). This indicates that the drying of the tips appeared to cause changes in blood, rather than the tips themselves. One of the conclusions drawn from the paper is that the use of chromatography, which was the chosen analytical technique, may have contributed to poor results from aged dried blood samples. This is due to the method's limits imposed regarding separation and integration of the different hemoglobin peaks. Any shifts in these chromatographic results would potentially cause the autoanalyzer to measure peaks at the wrong retention time and so 'fail' the result. Moreover, work that Stove and group subsequently conducted, shows that oxidation of hemoglobin occurs soon after drying, which seems to suggest that redox affects the chromatography⁷. Indeed, this temporal stability effect on the chromatography from dried blood spot samples has been reported elsewhere in the literature⁸. Furthermore, work conducted by Taylor and Davison in 2018 (unpublished) investigated the effect of aging Mitra tips on the chromatographic retention times using a similar instrument to that used in the Stove study on HbA_{1c}⁹. They confirmed that the chromatography is unstable when comparing dried blood samples extracted and analyzed on day 0 compared to day 7. It can be seen from [Figure 1](#) and [Figure 2](#) that as dried blood ages on the Mitra tips, the chromatograms from the extracts are completely different. On extended drying, the retention times seem to have shifted later, and the resolution is reduced. The A1c peak has possibly moved from around 0.63 to around 0.87 minutes, though it is very hard to tell without a confirmatory analysis. Either way, the Tosoh G8 instrument (the type used in this experiment and by Stove *et al*) would need to be reprogrammed to handle such changes (if technically possible). In this instance, it would have to be reported that the A1c peak disappeared and the method failed.

Figure 1. HbA1c sample of a fresh blood extract from Mitra (Day 0)



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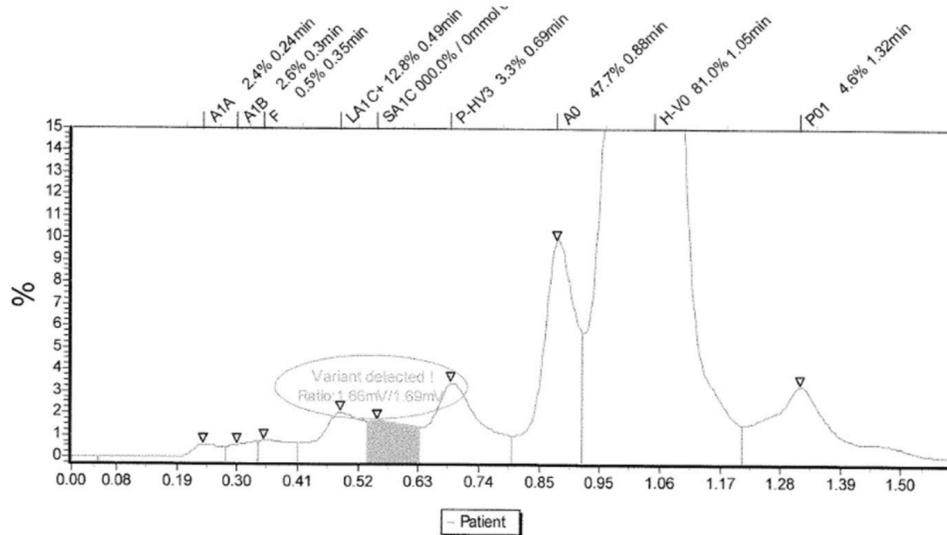
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Figure 2. HbA1c sample of an aged dried blood extract from Mitra (Day 7)



Another interesting observation in the paper from Stove *et al*, was that patient surveys indicated that both microsampling techniques were found to be convenient. Furthermore, VAMS clearly stood out to be the preferred sampling technique. The authors of the paper stated, “Responses to questionnaires (for detailed results, see Supplemental data, [Table 1](#)) indicated that most participants experienced the VAMS and DBS sampling technique as very convenient to use. When asked for the preferred sampling technique, VAMS clearly stood out, in both adults and children.”

To explore the practicality of wet VAMS microsampling further, another paper (published in 2018) from the same group looked at testing the Mitra devices with VAMS technology for at-home blood collection for testing of diabetic children¹⁰. The group needed to find a way to stabilize the wet blood so that the samples would survive the three days in transit between home sampling and the lab for analysis. The group found that if sampled tips were added to Eppendorf tubes prefilled with 800 μ L of sterilized water within 30 minutes of sampling, they saw very good stability, even after six days. The results from this test of healthy cohorts (n=37), showed a reported concordance correlation coefficient [CCC] of 0.994. The authors noted that this fulfilled the RCPA requirements. They then tested a group of pediatric patients (n=76) who were able to dispense the at-home sampled Mitra tips into the Eppendorf tubes with water and mail samples back to the lab in appropriate packaging for sending wet clinical samples. Although not quite as good as the pilot group, results showed that 80% met the RACP quality criteria. This was, in fact, double the value when compared to dried samples stored over a few days from the previous study. The authors commented that the real-life scenario and environmental conditions may have accounted for the difference. They concluded that more patient education may help with improved results. The participants were also asked to fill in a questionnaire about their experience with at-home sampling. Below is what they reported in the paper.

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Most (96%) found the demonstration leaflet for sample collection very or fairly clear. VAMS sampling at home was considered as very convenient (49%), fairly convenient (29%) or cumbersome (1%); 20% had no opinion on this matter. More patients (49%) preferred VAMS sampling at home than capillary sampling in the hospital (39%); 12% had no preference¹⁰.

Using Immunoturbidimetry to Measure HbA1c from Dried Blood Samples

The two papers from Stove's group, as discussed above, demonstrated the following important points. The first was that microsampling, and especially Mitra microsampling, was a preferred technique for capillary blood collection for HbA1c samples. The second point was that at-home sampling was convenient and, with the right training, sampling and sending wet samples back for analysis would be feasible. Finally, dried blood, possibly due to oxidative effects over time, alters chromatography in detectors that use this technique.

It must be noted that there would be big advantages to utilizing Mitra with VAMS if there was a way to measure stable A1c from dried devices that had been shipped from patients managing their own at-home collection. The first challenge to this is that the more steps to a process, the greater the chances of errors. By eliminating tip removal into a collection tube by the patient, we could eliminate a few key steps, thus preventing the possibility of such errors. The second challenge to overcome is that dried blood is more straightforward to ship compared to wet matrices¹¹.

Unfortunately, without a means to stabilize blood (possibly oxidatively), then development of a stable A1c method would be unlikely using chromatographic detectors. Indeed, Stove *et al* propose that the more open structure of Mitra may possibly expose blood to more oxidation than the flatter fibrous surface of DBS paper. This could explain why DBS fared better in their experiments. Although it must be noted that neither microsampling technique gave the results necessary to pass RACP criteria.

As mentioned above, there are other methods to test A1c. For example, immunoturbidimetry shows promise. A paper by Fokkema *et al* (published in 2009), used immunoturbidimetry on extractions from paper that had been dried for 5 days. Although there was a small increase in A1c values, the results highly correlated with routine HbA1c tests ($r = 0.987$)¹². When patients were surveyed in this study, 83% said they would like the filter method to be brought into practice. Given that Mitra has since been reported to be the preferred sampling technique over DBS and/or venous sampling, it makes sense to explore immunoturbidimetry as an alternative detection method for dried Mitra extracts^{6,13,14,15}. Moreover, many



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companies offer validated immunoturbidimetry methods for measuring A1c in standard clinical chemistry laboratories.

Using Immunoturbidimetry to Measure HbA1c from Dried Mitra Samples

To investigate the stability of dried Mitra samples using immunoturbidimetry, we conducted a study at Neoteryx comparing wet samples to dried Mitra samples using a Roche Cobas C111 system using a Tina-quant HbA1c assay. The Tina-quant assay is a turbidimetric inhibition immunoassay (TINIA) for the *in vitro* determination of hemoglobin A1c in whole blood or hemolysate¹⁶. The main advantage of this assay is that it requires only small amounts of sample, for example 10 μ L of fresh blood (diluted 1:101 in homolyzing reagent), so is an ideal for analyzing microsamples.

This report outlines the method development and method validation results.

Materials and Methods

Overview

To study whether extracts from dried Mitra tips could be interfaced with the Roche Tina-quant(R) A1c assay (Gen.2). A comparison was made between dried Mitra samples (10 μ L) and 10 μ L of fresh blood (K2EDTA) under various conditions outlined below. First, an extraction optimization was carried out. Following this, Mitra samples were tested for temporal stability and various stabilization conditions were examined. A sample 5% assay bias criteria, compared to the wet sample, was applied as a pass/fail for each test.

Samples and Sampling

For both matrices, 1 mL of hemolyzing reagent (Tina-quant A1c Gen.2) was used for each sample as a diluent. The wet samples were then analyzed per the manufacturer's instructions for wet A1c blood analysis. Mitra tips were sampled into the tubes where care had been made to ensure the blood was homogeneous by gently inverting the closed tube before each collection. Mitra sampling followed Neoteryx instructions, where tips were allowed to touch the blood, but not fully immerse it, and were to be sampled by pointing the sampling tips downwards toward the floor. The blood was allowed to saturate the device tip. The tip was



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held in this position for another two seconds, and then gently removed from the blood. Drying times varied depending on the experiment, and then samples were exposed to various extraction conditions. Once the extracts were prepared, they were treated the same as wet samples on the analyzer, and always analyzed within 60 minutes of extraction.

Optimization and Verification of Extraction Methodology

Extraction of the dried blood on Mitra tips (pre-dried for 2 hours on Neoteryx 96-Autoracks™) was performed by placing the tip of the Mitra device in a Sarstedt microtube (2 mL) and then adding the hemolysate to each tube. The tips were then exposed to several extraction conditions: Vortexing (Heidolph Vibramax 100 at 600 RPM) versus sonication (Branson 2510) for 30 minutes at room temperature (initial batch temperature 22.9 °C). The result obtained showed better extraction when sonication was employed, so sonication was selected for the remainder of the experiments. To optimize extraction time, tips (N=6 replicates per condition) were sonicated for 5, 10, 15, 20 and 30 minutes.

The optimized conditions were then tested by comparing fresh EDTA venous blood to dried blood samples on Mitra from 3 volunteers (N = 6 per condition). The results were converted to a Bland Altman plot (see [Figure 3](#) in “Results and Discussion”). To make this plot, an average ‘X’ was calculated. This was calculated by summing the results of the EDTA fresh blood data and the Mitra tip extracted data, and then dividing by 2. The difference between the two measurements was calculated as follows:

$$\frac{\text{Blood (Fresh)} - \text{Mitratip (extracted)}}{X} * 100\%$$

Accuracy of the Assay

To determine the accuracy of the assay, Roche QC blood samples with target values of HbA1c 5.73% (Ref: 05479207) and HbA1c 11.0% (Ref: 05912504) were used to produce Mitra tips with defined HbA1c concentrations. These QC controls are not certified reference materials and the target values can therefore not be used for precision measurements. The Roche recommended concentration interval for QC HbA1c 5.73 is 4.71 – 6.75% and for the HbA1c 11.0 at 11% and the recommended concentration interval is 8.9 – 13.1%. From each QC blood sample, 5 Mitra tip QC blood samples were prepared, as discussed earlier. The Mitra tip QC blood samples were dried for two hours in the 2-sampler Cartridges, which were placed in a 3x5 inch sealed aluminium pouch together with 6g silica gel desiccant. Sample preparation was performed according to the protocol earlier described. This procedure was performed six times on six different days with a new calibration



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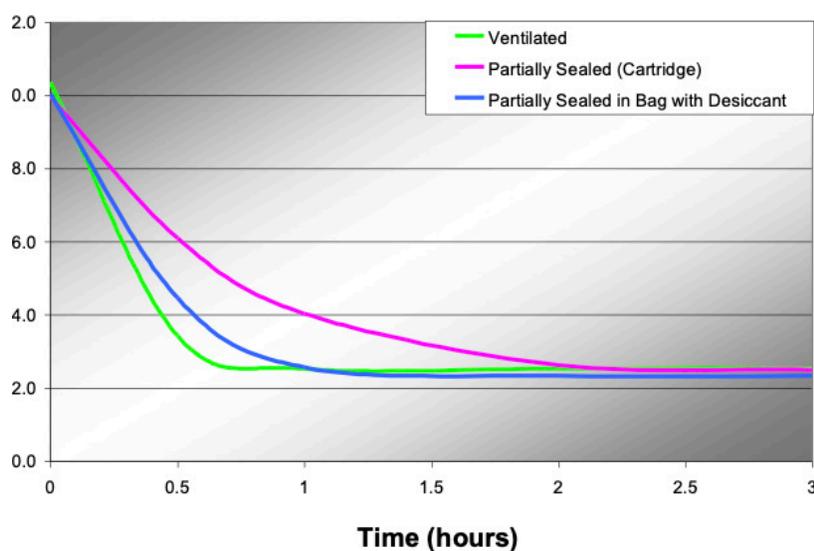
curve every time. [Table 2](#) shows the summary of the results (see “Results and Discussion”).

Evaluating the Effect of Rapid Drying and Elevated Temperature on the Stability of Dried Mitra Samples

As highlighted in the first paper by Stove *et al* and reported elsewhere, the stability of HbA1c in dried blood changes over time^{6,12}. Although Fokkema *et al* reported good stability when using immunoturbidimetry, they also commented that other researchers had seen poor stability and had resorted to using pre-treatments, such as Glucose Oxidase to attempt to stabilize the blood spot cards¹². It was, therefore, decided that they should test the temporal stability of HbA1c peaks using Mitra. Although investigating pre-treatments of stabilizers on Mitra tips may convey stability, it was decided that this might be impractical to implement, as there could be concerns raised about the stability of the stabilizers.

One simple experiment, which was straightforward to conduct, was to investigate the effect of a desiccant on stability, as the Mitra samples are available sealed in a bag with desiccant as an option. It is well known that proteins are generally more stable in their dry form and the mechanisms of stabilization are reasonably well understood¹⁷. Also, there are some examples where the rapid drying of Mitra samples have conveyed greater stability for certain analytes (unpublished data). Indeed, when Mitra was being developed, speed of drying under different conditions was investigated. It was found that when Mitra tips were fully ventilated under low humidity conditions, and when they were sealed in a bag with desiccant, drying times were vastly reduced compared to samplers being partially sealed in a cartridge without desiccant (see [Figure 3](#)).

Figure 3. Time Required to Dry Mitra Samples Under Various Conditions



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It was hoped that rapidly drying blood on the Mitra tips by enclosing the samplers in the presence of desiccant may aid in stabilizing HbA1c. Volunteers (N = 6) were asked to sample their own capillary blood following the manufacturer's instructions¹⁸. For each volunteer, 24 Mitra tips were sampled (6 x 4pk clamshells 10µL tips). Each clamshell was exposed to different conditions and compared, and these comparisons are summarized below. For each condition, one clamshell was dried enclosed in a foil bag with 5 grams of silica gel desiccant (Neoteryx part number 10110) and the second clamshell was closed but dried without the presence of desiccant (Neoteryx part number 10004). After each storage condition experiment, the samplers were extracted and analyzed using the optimized extraction conditions.

- Condition 1 - analyzed immediately (between 10 and 30 min after sampling) as a control.
- Condition 2 – dried for 2 hours at room temperature and stored for 3 days at 37 °C, analyzed at the end of day 3.
- Condition 3 – dried for 2 hours at room temperature then stored for 3 days at 37 °C, followed by 4 days at room temperature, analyzed at the end of day 7.

Results and Discussion

Optimization and Verification of Extraction Methodology

When comparing vortexing to ultrasonication, usage of the ultrasonic device led to better extraction of hemoglobin from the tips, as could be seen by the naked eye. Additionally, this comparison showed a higher yield of HbA1c and Hb concentration (data not shown). Ultrasonication was chosen as the extraction method for the remainder of the study. Optimizing extraction times (see Table 1) yielded broadly similar results. Fifteen minutes was the timing selected as the best balance between complete extraction (based on tip coloration) and the ratio between fresh blood and Mitra extract.

Table 1. A Comparison of Mitra Extraction Times to Fresh Blood for the Measurement of HbA1c

Sample	HbA1c (%)	HbA1c (mmol/mol)	CV (%)	Ratio Mitra data/fresh blood data (%)	Remark*
Fresh blood	5.30	34.43	1.10		
Mitra 5 **	5.32	34.67	1.25	100.7	++
Mitra 10	5.30	34.45	2.73	100.1	+
Mitra 15	5.30	34.39	1.25	99.9	-
Mitra 20	5.18	33.14	1.58	96.3	-
Mitra 30	5.24	33.75	1.87	98.0	-

* Naked eye observation: ++ Mitra tip red all over; + Mitra tip moderately red; - Mitra tip white

** Extraction time in minutes in ultrasonic bath



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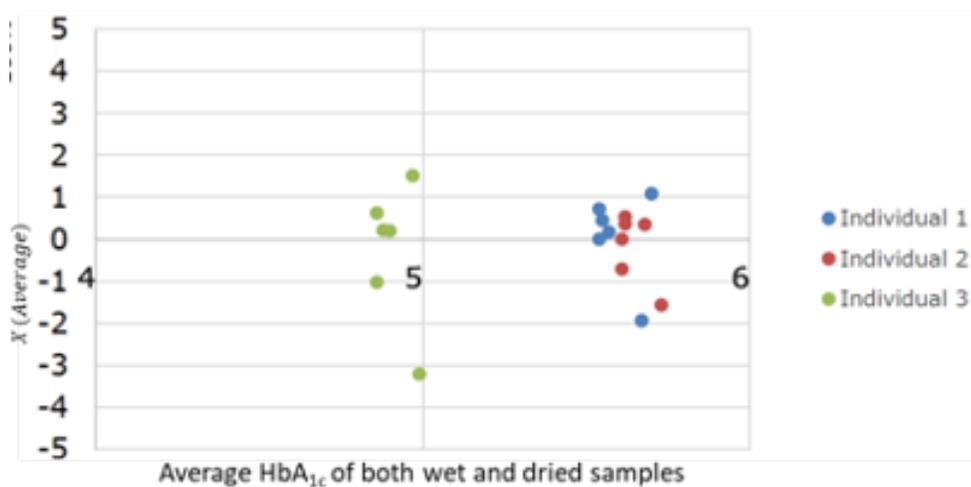
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Shorter extraction times were insufficient for a more complete extraction of blood from the tip, and longer extraction times showed less concordance to wet blood. It is postulated that this was due to a possible breakdown of the hemoglobin molecule due to the extended extraction times. This could have been due to temperature build-up in the baths, as it was observed that bath temperature increased from 22.9 to 33.3 °C over a period of 30 minutes. It is unlikely that ultrasound was solely responsible for the potential degradation, but the effect of ultrasonic heating of the bath water that caused thermal degradation. Further, a brief literature search did not yield any suggestion that ultrasound would directly cause the hypothesized degradation of the protein.

The extraction methodology was then tested against wet and dried blood samples from three volunteers. [Figure 4](#) shows a Bland-Altman plot that highlights the similarities of the results between the EDTA fresh blood and the Mitra tip dried blood measurements. The HbA1c results of dried blood Mitra tip samples are within 5% of the results from the fresh blood samples, which complies with the demands of the assay. It was therefore decided to use 15 minutes sonication in hemolysis buffer as an optimized extraction method.

Figure 4. Bland-Altman plot of HbA1c data from 10µL fresh blood versus 10µL Mitra tip dried blood samples.



Accuracy of the Assay

All results of HbA1c assessments ([shown in Table 2](#)) were within the Roche recommended concentration interval. Repeatability CV was lower than 2.5%, indicating that sample measurements could be performed in a single measurement. Intermediate CV of QC norm was 1.97%, which is comparable with Roche intermediate CV of 2.36%. The intermediate CV of the QC path is 5.18%, which is not comparable with the Roche intermediate CV of 1.58%. The reason for the



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high intermediate CV is the difference between the HbA1c measurements of calibrations 1-3 and 4-6, indicating a lower sensitivity (delta signal/delta concentration) at the higher end of the calibration curve.

Table 2. Results of accuracy measurements.

Calibration nr	QC HbA1c = 5.73%		QC HbA1c = 11.0%	
	Mean (N=5) (%)	CV repeatability (%)	Mean (N=5) (%)	CV repeatability (%)
1	5.64	1.6	9.97	0.4
2	5.59	1.6	9.73	1.6
3	5.56	0.7	9.73	1.2
4	5.86	0.7	10.96	0.7
5	5.77	0.9	10.89	0.6
6	5.80	0.7	10.73	1.1
Mean intermediate precision (N=30)	5.70%		10.34%	
SD Intermediate precision (N=30)	0.11%		0.53%	
CV intermediate precision (N=30)	1.97%		5.18%	

Evaluating the Effect of Rapid Drying and Elevated Temperature on the Stability of Dried Mitra Samples.

[Table 3](#) shows that without the presence of desiccant, there is an apparent increase of HbA1c in the sample, which seems to suggest potentially increased glycation events occurring post-drying. Indeed, the successful use of glucose oxidase as a stabilizer (impregnated on DBS disks), as discussed in the paper by Fokkema *et al*, seems to provide evidence for this¹². Moreover, LC-MS work published by Davison *et al* suggests that glycation events can occur post drying, on both the alpha and beta chains, including formation of dimers and trimers^{19,20}. It can be seen from the data, that all but one sample stored in desiccant passed the 5% inclusion criteria for the assay. Furthermore, this outlier only just failed the inclusion criteria and, interestingly, recorded the lowest A1c value.



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Table 3. Results of enhanced drying of finger prick blood in Mitra tip of 6 volunteers on day 0, 3 and 7 (N=4).

Volunteer	With/ without silicagel	Day 0	Day 3	HbA1c %	Day 7	HbA1c %
		HbA1c %	HbA1c %	percentage increase	HbA1c %	percentage increase
1	With	5.33	5.46	102.4	5.53	103.8
	Without	5.39	6.10	113.2	6.32	117.3
2	With	5.18	5.40	104.2	5.42	104.6
	Without	5.13	6.28	122.4	6.24	121.8
3	With	5.79	5.90	101.9	5.90	101.9
	Without	5.76	6.60	114.7	6.71	116.6
4	With	4.85	5.10	105.2	5.12	105.6
	Without	4.97	5.84	117.5	5.92	119.0
5	With	5.61	5.71	101.2	5.49	97.9
	Without	5.64	6.55	116.1	6.37	112.9
6	With	5.31	5.44	102.4	5.13	96.6
	Without	5.28	5.98	113.3	5.87	111.2

Elevated temperatures were employed in conditions 2 and 3 in order to mimic the ‘journey of a sample’ from the point when home sampling is posted through the mail to the point when it arrives at a laboratory for final analysis. The results showed that when dried in the presence of desiccant, temperature did not seem to have a deleterious effect on the results.

Such experiments have been performed before on Mitra for the analysis of proteins, and the results have also been favourable. One study investigated stability of the IgG antibody, to look at immunity to 30 strains of Influenza. Volunteers were sampled with both venous blood (to obtain plasma) and capillary blood on Mitra devices by phlebotomists at a clinic. Volunteers then sampled themselves at home with Mitra devices and sent their samples back to the laboratory. This experiment was conducted at the height of the summer in New York (where temperatures can reach >30 °C or more in mail vans that were not air conditioned). The blood samples typically arrived at the lab three days later for analysis. Very high concordance was observed when comparing samples collected by a healthcare professional and samples collected by volunteers at home²¹.

The data seems to suggest that, for samplers with or without desiccant, no further elevation of A1c peaks were observed after day three. It should be noted that a limitation of this experiment was that the HbA1c values reported



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were well within the normal range, so it is conceivable that higher levels of glycation may affect the result. However, a study commissioned by Neoteryx and conducted in 2017 by Bayshore Clinical Laboratories using a Beckman-Coulter immunoturbidimetric assay, reported a one-month stability for Mitra at room temperature using very similar storage conditions as discussed in the previous study²². The samples analyzed were patient samples, so this gives promise that use of desiccant stabilizes HbA1c. More work is needed to confirm this. These two studies seem to back up the stability shown by Fokkema *et al* using immunoturbidimetry on dried blood.

Conclusion

As the world remains in the grip of a COVID-19 pandemic, more flexible ways are being sought to monitor and treat patients remotely and safely. Diabetes is a condition that has a very high morbidity, mortality, and economic impact. Measurement of HbA1c from samples taken by patients at home and sent to a laboratory is an ideal solution for remote patient monitoring of diabetes. However, as discussed, development of a robust HbA1c assay on dried blood is not straightforward. Oxidative effects on analyte stability have a deleterious effect on chromatographic analysis, as demonstrated by Stove *et al*. Wet microsampling, however, seems to overcome these issues as a potential but partial solution^{6,10}. On the one hand, keeping the tips wet significantly improves the concordance between traditional samples and samples collected on Mitra. However, more steps are needed for a successful at-home sample collection.

Use of immunoturbidimetry, coupled with in-pouch drying of Mitra samples in desiccant shows great promise as a means to stabilize blood samples collected at home. More work needs to be conducted to fully show clinical utility. Nevertheless, the use of Mitra VAMS samplers is on the rise. Mitra devices and VAMS technology are being used for a wide range of applications, including immunosuppressive therapy monitoring and large serological studies^{23,24}. Mitra has proven repeatedly to be a popular platform for a remote patient-centric sampling option, so it may be only a matter of time before Mitra with VAMS is routinely utilized to monitor HbA1c in diabetes, as well as for many other conditions.



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References

- ¹Diabetes Prevalence: Guides and Information
<https://www.diabetes.co.uk/diabetes-prevalence.html#:~:text=World%20diabetes%20prevalence,with%20diabetes%20worldwide%20by%202040.>
- ²Diabetes: HbA1C Testing in Diagnosing Diabetes
[https://www.diabetes.co.uk/hba1c-test.html#:~:text=The%20World%20Health%20Organisation%20\(WHO,or%20over%3A%20Type%20%20diabetes](https://www.diabetes.co.uk/hba1c-test.html#:~:text=The%20World%20Health%20Organisation%20(WHO,or%20over%3A%20Type%20%20diabetes)
- ³Evaluation of Capillary Collection System for HbA1c Specimens
<https://pubmed.ncbi.nlm.nih.gov/1516490/>
- ⁴Performance of point-of-care HbA1c test devices: implications for use in clinical practice – a systematic review and meta-analysis.
https://www.degruyter.com/view/journals/cclm/55/2/article-p167.xml?tab_body=pdf-74962
- ⁵Royal College of Pathologists of Australasia. RCPA quality requirements.
<https://www.westgard.com/rcpa.htm>. (Accessed: 13 March 2017).
- ⁶Volumetric Absorptive Microsampling at Home as an Alternative Tool for the Monitoring of HbA1c in Diabetes Patients.
<https://pubmed.ncbi.nlm.nih.gov/27732552/>
- ⁷A Novel, Non-destructive, Dried Blood Spot-Based Hematocrit Prediction Method Using Noncontact Diffuse Reflectance Spectroscopy.
<https://pubmed.ncbi.nlm.nih.gov/27206105/>
- ⁸The use of dried blood spot sampling for the measurement of HbA1c: a cross-sectional study.
<https://bmcclinpathol.biomedcentral.com/articles/10.1186/s12907-015-0013-5>
- ⁹Davison A and Taylor J (RLUHT) unpublished work, chromatograms in figures 1 and 2 given with permission by the investigators.
- ¹⁰Wet absorptive microsampling at home for HbA1c monitoring in diabetic children
<https://www.degruyter.com/view/journals/cclm/56/12/article-pe291.xml>
- ¹¹Shipping Guidelines for Dried-Blood Spot Specimens
<https://hubs.ly/H0sciBd0>
- ¹²HbA_{1c} Measurements from Dried Blood Spots: Validation and Patient Satisfaction
<https://pubmed.ncbi.nlm.nih.gov/19751141/>
- ¹³Volumetric Microsampling of Capillary Blood Spot vs Whole Blood Sampling for Therapeutic Drug Monitoring of Tacrolimus and Cyclosporin A: Accuracy and Patient Satisfaction
<https://academic.oup.com/jalm/article-abstract/5/3/516/5815223>
- ¹⁴DIY Blood Sampling for Pediatric Clinical Trials—The Patient’s Perspective
<http://www.appliedclinicaltrialsonline.com/diy-blood-sampling-pediatric-clinical-trials-patients-perspective>
- ¹⁵Pharmacokinetics of albendazole, albendazole sulfoxide and albendazole sulfone determined from plasma, blood, dried blood spots and Mitra® samples of hookworm-infected adolescents.
<https://aac.asm.org/content/aac/63/4/e02489-18.full.pdf>
- ¹⁶Turbidimetric inhibition immunoassay (TINIA) for the *in vitro* determination of hemoglobin A1c in whole blood or hemolysate
<https://diagnostics.roche.com/global/en/products/params/tina-quant-hba1c-gen-3.html>
- ¹⁷Mechanisms of Protein Stabilization in the Solid State
[https://www.jpharmsci.org/article/S0022-3549\(16\)33070-2/pdf](https://www.jpharmsci.org/article/S0022-3549(16)33070-2/pdf)
- ¹⁸How to Take a Sample: The Dos and Don’ts of Using the Mitra Microsampler
<https://www.neoteryx.com/how-to-properly-take-a-blood-sample-using-the-mitra-microsampler-vams>
- ¹⁹Diabetes in Pregnancy: Effect on Glycation and Acetylation of the Different Chains of Fetal and Maternal Hemoglobin
<https://pubmed.ncbi.nlm.nih.gov/20883682/>
- ²⁰Fetal hemoglobin: assessment of glycation and acetylation status by electrospray ionization mass spectrometry
<https://pubmed.ncbi.nlm.nih.gov/18597590/>
- ²¹Application of volumetric absorptive microsampling (VAMS) to measure multidimensional anti-influenza IgG antibodies by the mPlex-Flu assay
<https://hubs.ly/H0nKnyj0>
- ²²Investigative work by Bayshore Clinical Laboratories (commissioned by Neoteryx,(2017) – short validation report available upon request.
- ²³VAMS Publication list
<https://www.neoteryx.com/hubfs/Content/Analytes%20-%20Publication%20list/publication%20list%20february%202020.pdf>
- ²⁴NIH Begins Study to Quantify Undetected Cases of Coronavirus Infection. Blood Samples from Healthy Volunteers Needed to Inform Public Health Decision Making
<https://www.niaid.nih.gov/news-events/nih-begins-study-quantify-undetected-cases-coronavirus-infection>



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