# Optimisation of a Microsampling Device for Collection of $HbA_{1C}$ Samples

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## INTRODUCTION

Glycated haemoglobin ($HbA_{1C}$) is routinely tested to diagnose and monitor diabetes mellitus[1]. In the presence of high blood glucose levels, haemoglobin undergoes an irreversible non-enzymatic glycation at the β-chain to form $HbA_{1C}$[2].

Diabetes mellitus (DM) is a disorder of glycaemic regulation. In 2011, the World Health Organisation (WHO) recommended that $HbA_{1C}$ can be used for the diagnosis of DM[3].

A variety of methods are currently commercially available for $HbA_{1C}$ analysis, including High Performance Liquid Chromatography (HPLC), affinity chromatography, immunoassay and enzymatic methods[4].

In 2019 the WHO identified a new disease, COVID-19. This went on to become a global pandemic with a small but significant mortality rate[5]. Patients with DM are more susceptible to COVID-19 and have worse associated disease outcomes[6].

Volumetric Absorptive Microsampling (VAMS) is a technique for whole blood collection and storage, where whole blood from a finger prick is collected and dried[7]. Samples can then be later be prepared by the laboratory for testing. This would negate the need for DM patients to enter the hospital environment and lower their risk of contracting COVID-19[8].

VAMS has previously been investigated for $HbA_{1c}$ analysis with HPLC methodology with unsatisfactory results[9].

The biochemistry department at the Princess Alexandra Hospital NHS Trust employs Abbott Alinity analysers in the automation section. Currently, HPLC methodology is used for HbA1c analysis, with variant haemoglobin populations sent for confirmation testing at another site.

Abbott recently released an enzymatic $HbA_{1C}$ method which would allow a single platform to be used for variant and non-variant population groups[10].

## AIMS

The aim of this project was to evaluate the new Abbott Alinity enzymatic method for $HbA_{1C}$ analysis against the currently in use HPLC method. Variant haemoglobins were evaluated against affinity chromatography. Following successful verification, VAMS sampling techniques were evaluated for suitability for at-home DM patient sampling.

## METHODS

Enzymatic method verification

The imprecision of the Abbott Alinity method was assessed by evaluating the within-run imprecision, between-run imprecision, and using Broughton's equation to calculate carryover.

The accuracy of the enzymatic method was assessed by running 1775 patient samples on the in-use HPLC method, followed by a repeat on the enzymatic method. This represents one-weeks worth of $HbA_{1C}$ requests received within the Trust. All samples were run on the same day they were received.

Passing Bablok and Bland-Altman statistical analysis was used to estimate method agreement and bias between the methods, for all patient populations and for specific target groups.

VAMS assessment

Neoteryx VAMS devices were used for this study. 20 samples were used for this part of the study. 20µL of EDTA persevered whole blood was applied to the sample tips and allowed to air dry at room temperature.

Once the tip was dried, the sample was reconstituted. 800µL of Abbott Alinity $HbA_{1C}$ diluent was applied to the tip and then vortexed for 10 minutes.

Samples were reconstituted on day 0, 3 and 7 to assess the impact of increased storage times on sample viability.

Reconstituted samples were analysed on both the in-use HPLC method and the new enzymatic method to compare differences in performance.

Again, Passing Bablok and Bland-Altman statistical analysis were used to estimate method agreement and bias.

## RESULTS

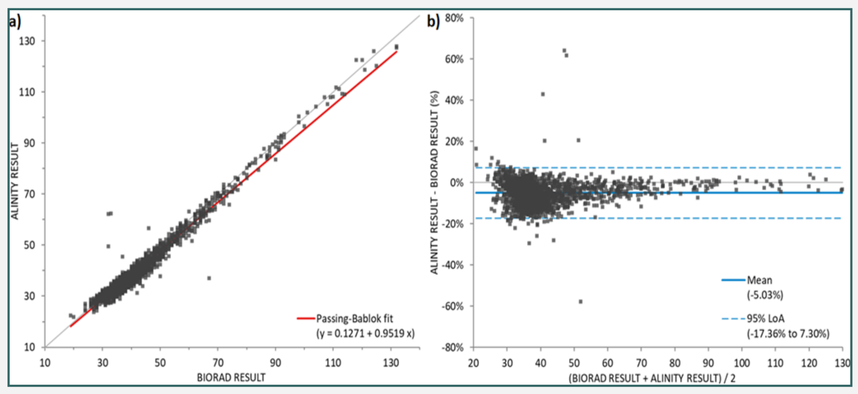
Enzymatic method imprecision

Abbott and third-party (Technopath) internal quality control (IQC) was used to assess imprecision. Both within-run and between run demonstrated an acceptable Coefficient of Variation (CV) <5%. Broughton's equation was used to calculate carryover; both manufacturer and third-party IQC demonstrated an I% value <5, indicating acceptable carryover.

Enzymatic method accuracy

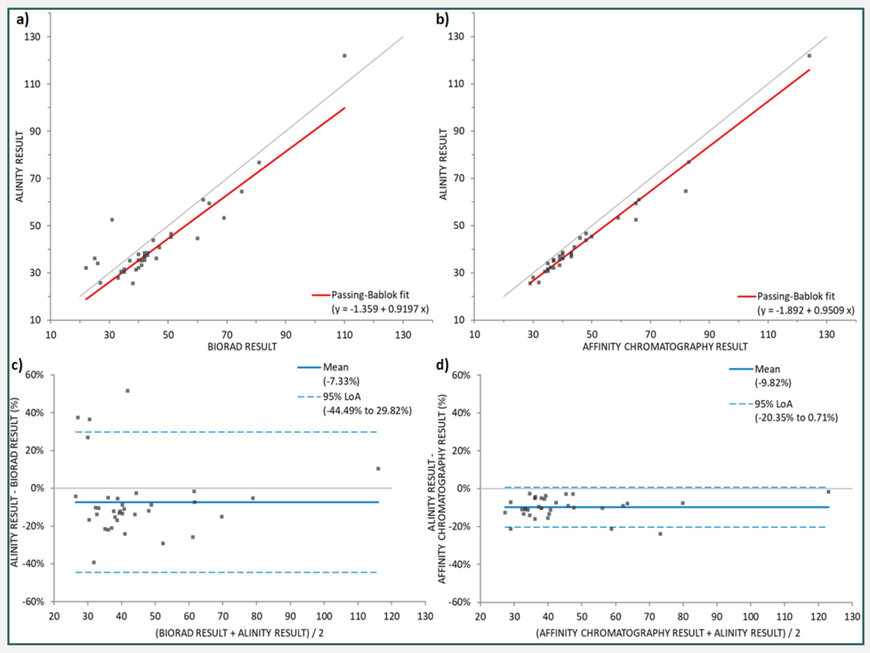
Passing Babok (Fig. 1a) and Bland-Altman (Fig. 1b) analysis showed that for all patient samples there was no constant error or proportional value. Similar findings were made when the data was compared for paediatric and antenatal populations.

**Figure 1: Results for enzymatic vs HPLC methods for all whole blood samples**




Enzymatic results were then compared against the HPLC method (Fig. 2a and 2c) and the referral affinity chromatography method (Fig. 2b and 2d)

**Figure 2: Passing Bablok and Bland-Altman analysis for variant populations for enzymatic vs HPLC and enzymatic vs affinity chromatography**




Both methods demonstrated agreement, however the accuracy was higher for variant results with significantly less bias against the affinity chromatography method.

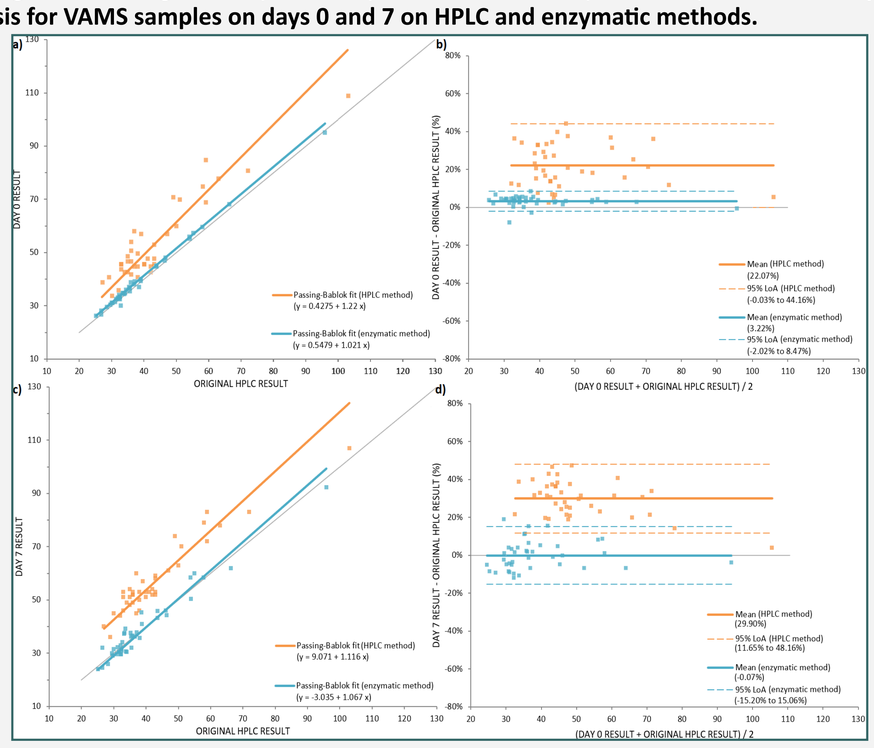
VAMS imprecision

Using the same 5% CV benchmark for acceptability as the enzymatic method verification, the VAMS imprecision was acceptable. However, %CV was higher on the HPLC method, at 4.6, than enzymatic, at 1.0.

VAMS accuracy

VAMS accuracy was assessed on day 0, day 3, and day 7 for both HPLC and enzymatic methodologies. Figure 3 shows the statistic analysis for day 0 and day 7 for HPLC methods and enzymatic methods.

**Figure 3: Passing Bablok (3a and 3c) and Bland-Altman (3b and 3d) statistical analysis for VAMS samples on days 0 and 7 on HPLC and enzymatic methods.**




Both methods showed linearity, however only the enzymatic method demonstrated sufficient agreement with limited bias, even after 7 days.

## CONCLUSIONS

The initial phase of this project was to perform an evaluative comparison of the new enzymatic method against the in-use HPLC method. Imprecision and accuracy assessments have demonstrated a good agreement between methods.

For variant populations, the enzymatic method displayed agreement with affinity chromatography, which negates the need to send samples to a referral laboratory. This offers the laboratory the opportunity to save time and money.

The second phase of this project was to assess the suitability of the VAMS devices. Confirming previous work, the VAMS devices showed poor agreement with original HPLC results. However, the newly validated enzymatic method showed a good agreement after 7 days of sample storage.

HPLC methodology generates a chromatogram which is dependent on the structure of the haemoglobin molecule[4]—it is likely that storage and reconstitution has an effect on haemoglobin structure. This is evident in Figure 4, which shows the chromatograms of one of the samples used in the project on days 0, 3 and 7:

**Figure 4: Chromatogram of VAMS samples on day 0, 3 and 7 post-extraction.**

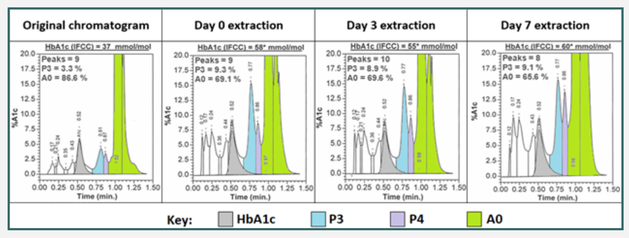



Figure 4 shows the increase in haemoglobins P3 and P4 which are closely related to sample age.

The enzymatic method, however, does not rely on haemoglobin structure to produce a result. Instead the haemoglobin is lysed to release fructosyl dipeptide fragment, which can then react with a fructosyl peroxidase enzyme which generates hydrogen peroxide and produces a colour change which can be measured spectrophotometrically[11]. This also explains the limited impact of haemoglobin variants on the enzymatic method.

This project used EDTA preserved whole blood—in a real world setting patients would use a finger prick sample to apply to the VAMS device. Further investigation into the effect this may have on patient results to ensure it does not negatively impact the quality of results generated is warranted.

This project has demonstrated that VAMS can be used with the new enzymatic method to give acceptable results up to 7 days post-collection, allowing vulnerable patients to minimise social contact in the hospital setting.

## REFERENCES

1. Khokhar A, Naraparaju G, Friedman M, Perez-Colon S, Umpaichitra V, Chin VL. Comparison of A1C to oral glucose tolerance test for the diagnosis of prediabetes in overweight and obese youth. Clinical Diabetes. 2017;35(3):133-40.
2. John W. Glycated Haemoglobin Analysis. Annals of Clinical Biochemistry: International Journal of Laboratory Medicine. 1997;34(1):17-31.
3. National Institute for Health and Care Excellence. Type 2 diabetes in adults: management, NICE; 2015(Clinical guideline [NG28]).
4. Bird IM. High performance liquid chromatography: principles and clinical applications. BMJ: British Medical Journal. 1989;299(6702):783.
5. Esakandari H, Nabi-Afjadi M, Fakkari-Afjadi J, Farahmandian N, Miresmaeili SM, Bahreini E. A comprehensive review of COVID-19 characteristics. Biological procedures online. 2020;22:1-0.
6. Pugliese G, Vitale M, Resi V, Orsi E. Is diabetes mellitus a risk factor for COrona-VIrus Disease 19 (COVID-19)?. Acta diabetologica. 2020:1-1.
7. Volani C, Caprioli G, Calderisi G, Sigurdsson BB, Rainer J, Gentilini I, Hicks AA, Pramstaller PP, Weiss G, Smarason SV, Paglia G. Pre-analytic evaluation of volumetric absorptive microsampling and integration in a mass spectrometry-based metabolomics workflow. Analytical and bioanalytical chemistry. 2017;409(26):6263-76.
8. Nys G, Kok MG, Servais AC, Fillet M. Beyond dried blood spot: current microsampling techniques in the context of biomedical applications. TrAC Trends in Analytical Chemistry. 2017;97:326-32.
9. Verougstraete N, Lapauw B, Van Aken S, Delanghe J, Stove C, Stove V. Volumetric absorptive microsampling at home as an alternative tool for the monitoring of HbA1c in diabetes patients. Clinical Chemistry and Laboratory Medicine (CCLM). 2017;55(3):462-9.
10. Weykamp C, John W, English E, Erasmus R, Sacks D, Buchta C et al. EurA1c: The European HbA1c Trial to Investigate the Performance of HbA1c Assays in 2166 Laboratories across 17 Countries and 24 Manufacturers by Use of the IFCC Model for Quality Targets. Clinical Chemistry. 2018;64(8):1183-1192.
11. Yonehara S, Inamura N, Fukuda M, Sugiyama K. Use of fructosyl peptide oxidase for HbA1c assay. Journal of diabetes science and technology. 2015;9(2):200-5.

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