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A Simplified Expedited Screening of Haemoglobinopathy and Pre-Diabetes in Whole Blood Using MALDI MS

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Introduction

- Clinical MALDI-TOF-MS requires not only accurate mass determination but also quantitative measurement e.g. as used in the direct identification of haemoglobinopathy and diabetes from blood prick sample. This has been demonstrated by us and Dr Marvin Vestal's group (1,2,3).
- Two aspects are known to affect the reliability of quantification result in MALDI: (i) number of shots averaged and (ii) optimal dilution of the sample.
- Here, we examine the effect of the acquisition strategy (number of laser shots, sampling method) for achieving optimised but rapid data for qualitative and quantitative blood globin analysis from pin prick blood.

Methods and Materials

Eight blood samples (see table 1) were collected from pin prick blood collected on Guthrie cards and eluted in mass spec grade distilled and deionized water. Diluted to 1 in 2000, a 1ul samples of each were sandwich layered between matrix of sinapinic acid (SA) and analysed on a linear MALDI-TOF mass spectrometer (AXIMA-CFR, Shimadzu, Manchester, UK) see table 1: spectra. To demonstrate the robustness of the method, and evaluate optimal speed of sample repeat spectral accumulation, subsequent analysis was performed on a new, benchtop linear MALDI-TOF instrument (MALDI-8020, Shimadzu, Manchester, UK).

Process experiments: The blood samples were grouped into 6 experimental conditions as shown in table 2 and run in triplicate.

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Table 1. Samples and MALDI-ToF Spectra

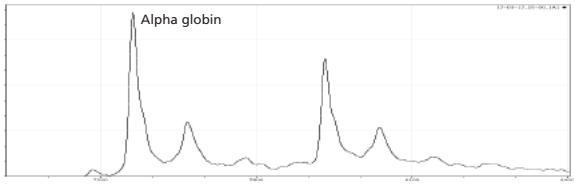
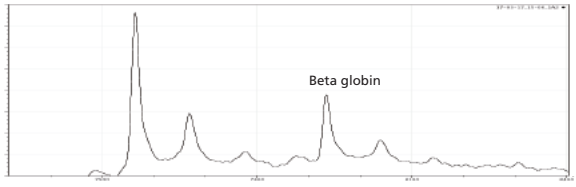
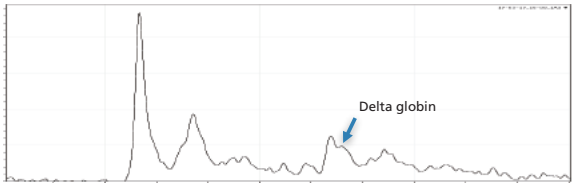
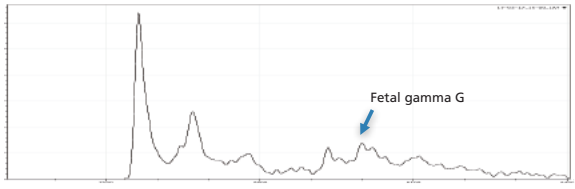
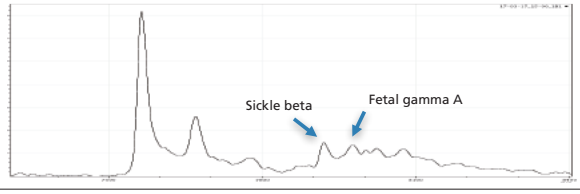
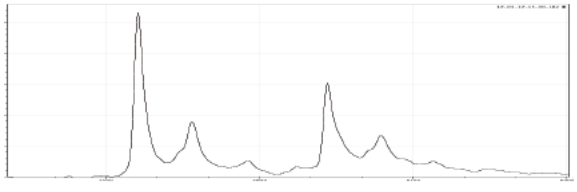
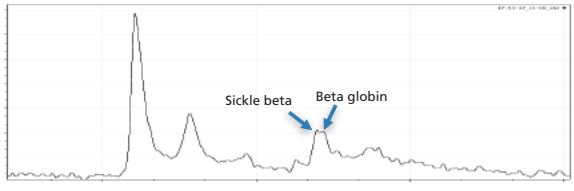
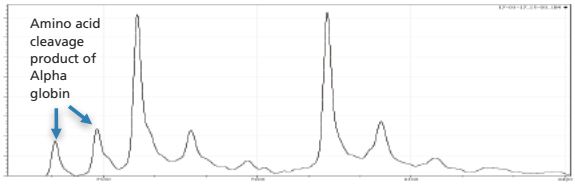
Sample No	Description	Spectra
1	Non-haemoglobinopathy control	
2	Non-haemoglobinopathy control	
3	Beta – thalassemia expressing reduced beta globin and increased delta globin	
4	Beta-thalassemia expressing reduced beta globin and increased fetal globin gamma G. Also increased glycation	
5	Sickle cell beta-globin with increased expression of fetal globin gamma-A	
6	Non-haemoglobinopathy control	
7	Sickle cell haemoglobinopathy – Heterozygous for sickle plus reduced beta expressing betathalassemia	
8	Unstable alpha globin heterozygote leading to reduced pseudo alpha-thalassemia HbH trait	

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Table 2. Specimen sampling and shot repeat experimental format

Simple Raster pattern (10 spectra per position)	Blast Raster (10 shot burn pattern then 10 spectra per position)
1000 shots (100 positions)	1000 shots (100 positions)
3000 shots (300 positions)	3000 shots (300 positions)
5000 shots (500 positions)	5000 shots (500 positions)

Data processing and analysis: Spectra were acquired between 2,000 and 100,000m/z. Doubly charged blood globins (7300 to 8500 m/z) were analysed both as raw plotted data and minimally processed plots: Gaussian smoothing (50mz), and linear base line correction.

Assessment of spectra was by peak identification (3 times base line) in the triplicate samples, Coefficient of variation (CV) of absolute intensity of alpha globin, and coefficient of variation (CV) of beta globin to alpha globin ratio of absolute intensities (a.i).

Result

The ability to recognize peaks of the blood globin proteins, including clinically important post translation modification such as glycation (i.e. HbA1c equivalents), is dependent on relative concentration and number of spectra accumulated and averaged (Figure 1).

The reproducibility (CV) of absolute intensity (a.i.) measures correlates with both the intensity of sample signal and the number of repeat shots (Figure 2). However, absolute intensity measures are not sufficiently reproducible here for a.i to be used as a measure for quantification (Table 3). Dr Marvin Vestal's group have demonstrated excellent a.i. CV on replicate sample when 100,000 spectra or more are averaged. This is not necessary for quantification of relative signal intensity between peaks within a sample. As demonstrated the alpha and beta globin ratio was substantially more reproducible with CVs lower than 0.05 when only 5000 spectra are collected and averaged per sample (Table 3 and Figure 3).

For the linear ToF MALDI-8020 instrument, the minimal optimal sampling for reproducible peak identification and relative quantification of blood globins is 5000 shots, under blast raster format. This was achieved in the instrument under an average of less than 1 minute per sample.

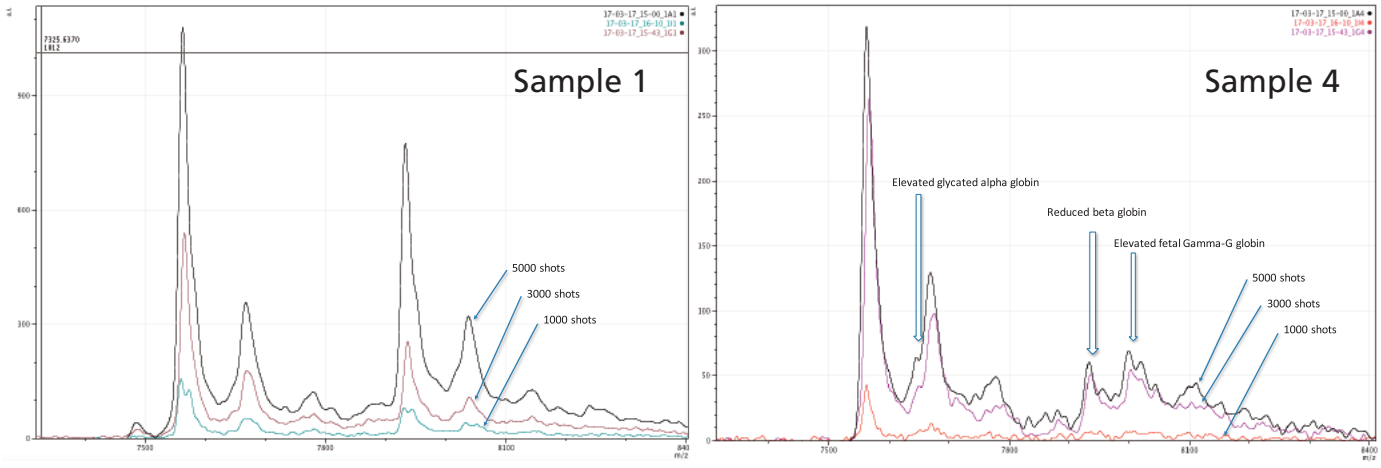

Figure 1. Illustration of the effect on peak recognition of accumulating and averaging 1000s of MALDI spectral shots. Although peak heights remain largely in ratio (left panel - sample 1), consistent reproducibility is critical for diagnosis of complex conditions, such as concomitant diabetes in a beta-thalassemia patient (right panel - sample 4).

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Table 3. Average reproducibility of spectra for all 8 samples under the conditions tested

	Simple Raster pattern (10 spectra per position)			Blast Raster (10 shot burn pattern then 10 spectra per position)		
Total number of shots	Mean replicate CV of alpha peak a.i.	Beta globin peak detection[1]	Mean replicate CV of beta/alpha globin peak ratio[2]	Mean replicate CV of alpha peak a.i.	Beta globin peak detection[1]	Mean replicate CV of beta/alpha globin peak ratio[2]
1000	0.56	5/8	0.23	0.47	7/8	0.17
3000	0.43	6/8	0.19	0.50	8/8	0.13
5000	0.43	6/8	0.13	0.46	8/8	0.05

[1] Beta globin peaks or sickle beta globin greater than 3 x baseline in all three replicates

[2] Mean CV calculated in samples in beta globin or sickle beta peak globin was detected

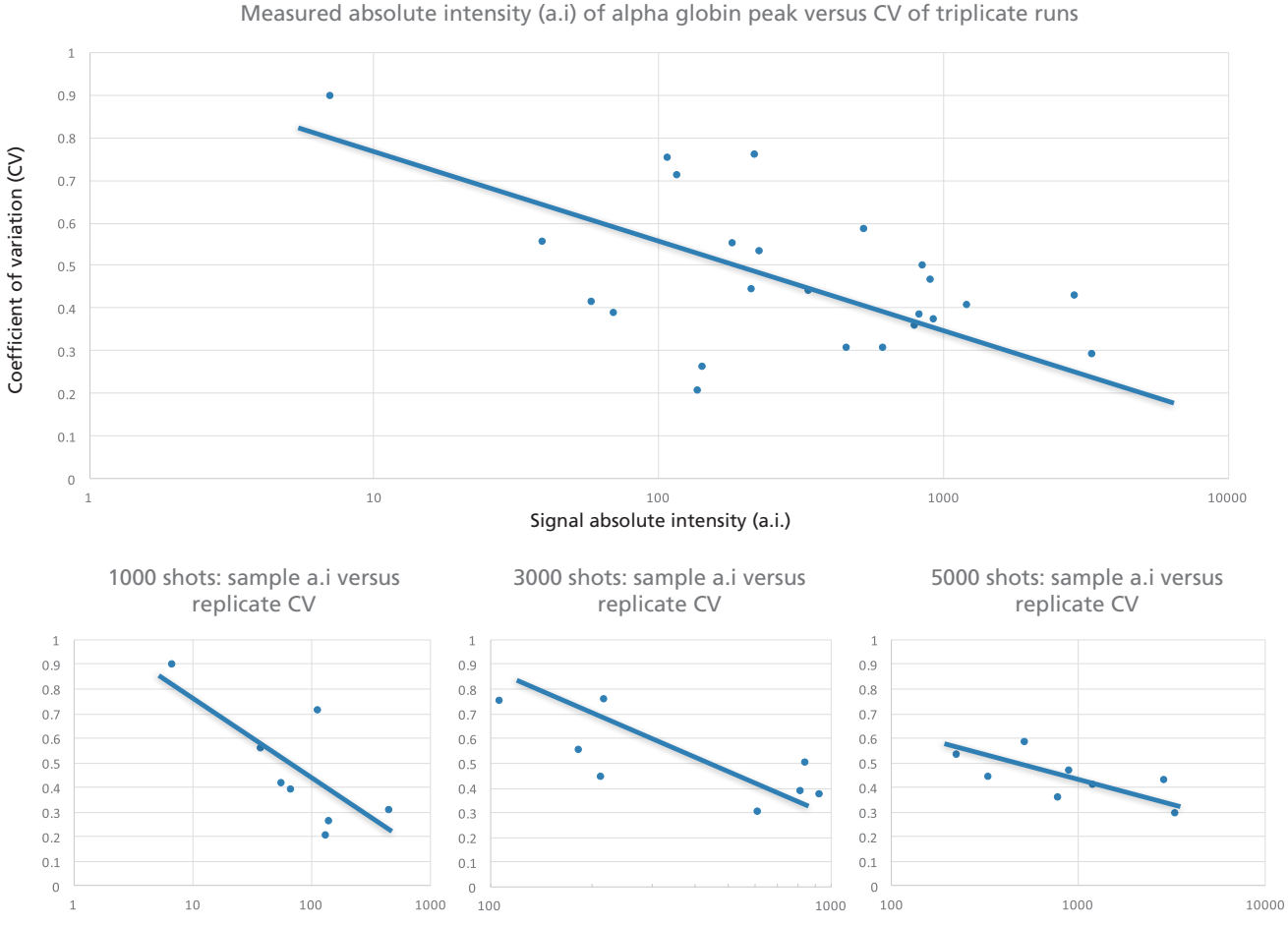

Figure 2. Illustration that absolute intensity correlates with reproducibility (top panel). Increasing the number of accumulated spectral shots averaged per sample decreases variability; however at 5000 shots CVs remain high (lower panels).

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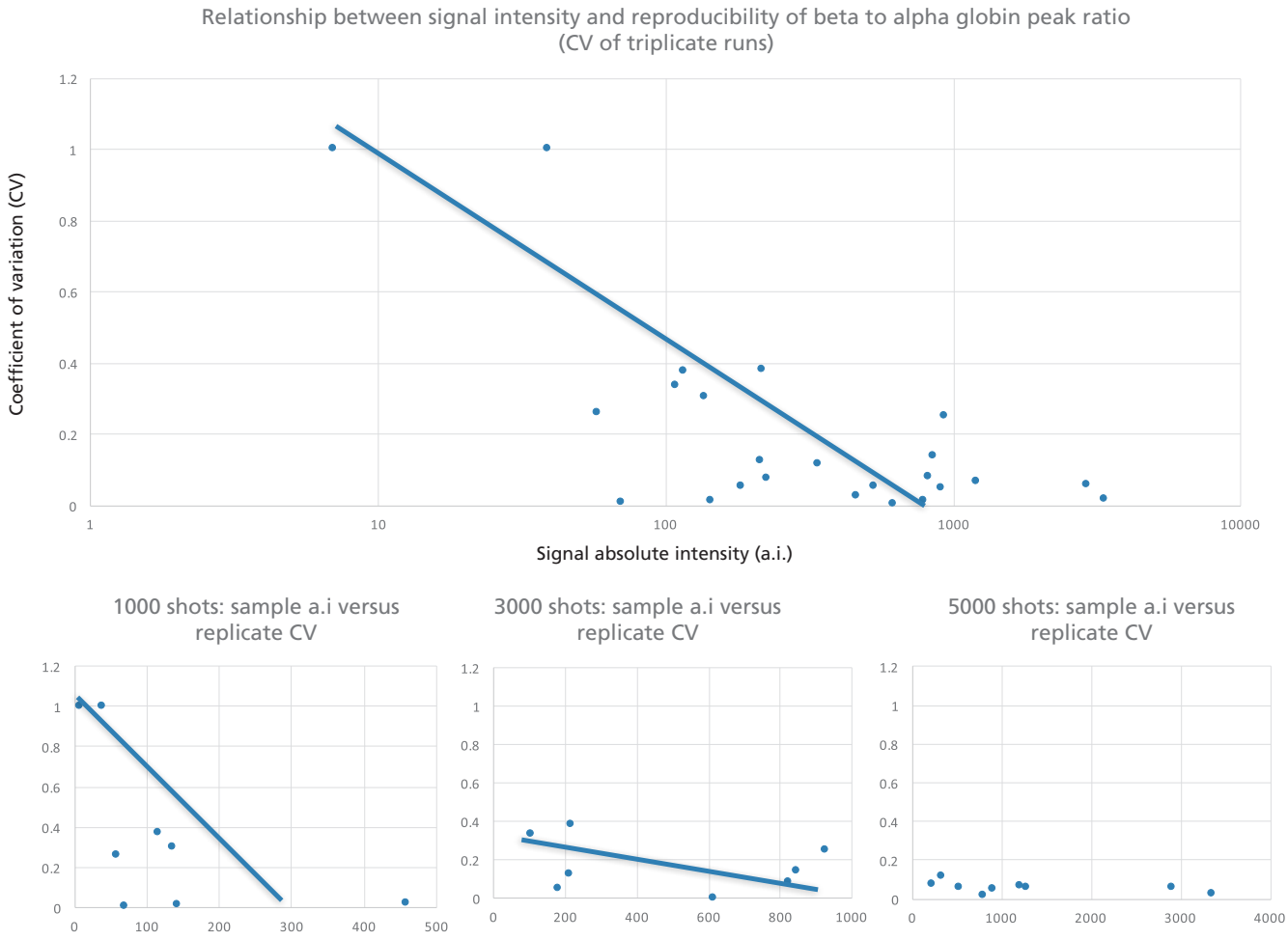

Figure 3. Illustration that quantification based on relative peak height of endogenous globins is highly reproducible and at 5000 shots per sample extremely low CV are achieved.

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Discussion

Adding 1ul of pin prick blood to distilled water causes red cells lysis and haemoglobin dissociation, including release of haem from the protein globins. These globins are the most abundant protein in the lysed sample. Simple dilution to the order of 103 effectively reduces signals from other component proteins such that only the blood globins are seen in mass spectral analysis. By accumulating 5000 spectra, clinically significant and highly reproducible data can diagnose sickle cell and fetal globin expression disorders and conditions such as unstable alpha globin carrier (sample 8, Table 1). Quantification is necessary for further diagnosis of alpha and beta thalassemia, and post translational disease modification such as glycation, by this technique. To achieve quantification as reflected in reproducible absolute intensity (a.i.), requires averaging 100,000s of spectra from the sample. However, relative signal intensity of blood globin proteins is maintained such that relative quantification of the various globins to alpha globin can be made with just 5000 data shots. This also allows quantitative determination of relative glycation of globin proteins, even in those with a haemoglobinopathy.

Haemoglobinopathies are the largest group of inherited disorders, affecting 7% of the world's population. Diabetes is a major contributor to the morbidity and mortality indices of national health organisations. Governments and regional health programmes are implemented to screen for these disorders before they become complicated and less manageable. From a single pin prick of blood, Haemoglobinopathy and diabetes can be simultaneously determined in minutes by MALDI-ToF MS, whereas conventional laboratory medicine takes days and weeks of expensive testing.

References

1) **METHOD FOR DETECTING ABNORMALITIES IN HEMOGLOBIN** RK Iles, JK Iles, T Abban, SM Docherty, M Naase. WO Patent App. PCT/GB2015/052,491
2) **RAPID SCREENING AND EVALUATION OF DIABETES AND PREDIABETES BY GLYCATED HEMOGLOBIN MASS SPECTROMETRY.** RK Iles WO Patent App. PCT/GB2015/052,487
3) **Analysis and Quantitation of Glycated Hemoglobin by Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry.** Hattan, S.J., Parker, K.C., Vestal, M.L. et al. J. Am. Soc. Mass Spectrom. (2016) 27: 532. doi:10.1007/s13361-015-1316-6

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