A comparison of IEF and MSMS for Clinical Hemoglobinopathy Screening in 40,000 Newborns

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INTRODUCTION

Currently, newborn hemoglobinopathy screening is carried out using HPLC or isoelectric focusing (IEF). We have previously described a rapid and specific electrospray mass spectrometry – mass spectrometry (MSMS) technique, using multiple reaction monitoring (MRM) based peptide analysis, for simultaneous detection of the clinically significant hemoglobinpathies; hemoglobin (Hb)S, HbC, HbE, HbD^{Punjab} and HbO^{Arab}[1] and subsequently for the detection of Hb Lepore and HbA_2 quantitation[2]. Although a significant improvement on previous approaches, in the context of newborn screening the sample preparation was technically demanding, requiring multiple pipetting steps and therefore not ideal. The original surviving ion MRM transitions for HbC, HbE, HbD^{Punjab} and HbO^{Arab} were also reviewed and potential specific MRM's investigated to improve sensitivity and specificity, of particular importance at the low level beta chain expression of newborns. Here we report the results of a comparison of 40,000 newborn blood spots screened by both IEF and the improved MSMS strategy.

AIM

To develop a simple and robust method for detecting the clinically significant hemoglobinopathies in a newborn screening programme.

METHOD

For both IEF and MSMS analysis, blood spots (3.2mm) were punched into separate 96 well plates. IEF was performed using the Resolve haemoglobin test kit (PerkinElmer Life Sciences, Waltham, USA) and Isoscan imaging system. For MSMS analysis, the blood spots were digested for 30min at 37°C with a trypsin reagent, and diluted in mobile phase (acetonitrile: water, 50:50, with 0.025% formic acid. Sample, 2µl, was injected directly into the mobile phase (flow rate 80µl/min) and analysed, in positive ion mode, using a Sciex API4000 (Applied Biosystems, Warrington, UK). Specific MRM transitions for HbS, HbC, HbE, HbD^{Punjab}, HbO^{Arab}, normal beta, alpha, gamma and delta chains were acquired; total acquisition time per sample was 60 sec. This enabled identification of sickling disorders and thalassaemia major, as well as assessment of transfusion state and potential identification of HbLepore and HbBarts.

Table 1. MRM Target peptide and ion masses

Tryptic peptide	Target Peptide Ion (Da)	Target fragment ion (Da)
Wild Type (Beta) T1	476.9	y4 502.3
HbS	461.9	y4 472.4
HbC	694.5	b4 451.3
Wild Type T13	689.9	b3 378.1
HbD^{Punjab}	689.4	b3 377.1
Wild Type T13	689.9	y4 1001.4
HbO^{Arab}	625.3	y3 1001.4
Wild Type T3	657.9	y5 887.5
HbE	458.7	y5 489.3
Delta chain T2	480.3	y4 688.4
Delta chain T13	721.4	y4 1064.3
Wild Type T2	466.8	y4 675.4
Gamma Chain T2	488.6	y4 691.6
Alpha Chain T1	365.2	y4 430.4
Alpha Chain T13	626.9	y3 992.5

Fig 1. Flow injection Profile: HbFAS

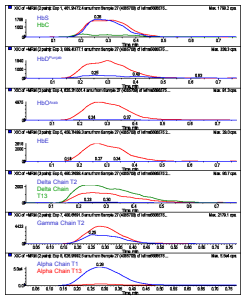


Fig 2. Flow injection Profile: HbFAE

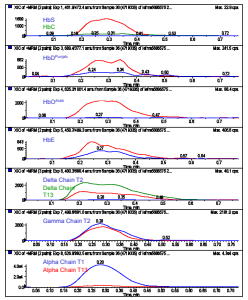


Fig 3. Ratio Plot of Sickle/WT peptide signal

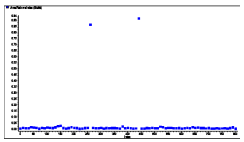
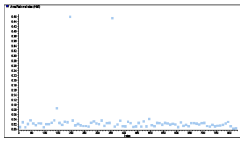


Fig 4. Ratio Plot of HbE/WT peptide signal



RESULTS

40,000 blood spot samples for routine newborn hemoglobinopathy screening were analysed in parallel. MSMS data was analysed by inspection of the flow injection profile, and of plots of the abundance ratio of variant peptide to corresponding wild type peptide (Table 1, Figures 1 to 4). HbS was detected in 199 samples; 8 were HbS/HbF only and 3 HbSC. HbC was detected in 39 samples, HbD^{Punjab} in 52, HbE in 48. No HbO^{Arab} or HbLepore mutations were detected by either method. There have been no discrepancies between the analytical techniques. Using MSMS, mutation positive samples can be re-run in product ion scan mode to provide peptide sequence and hence unequivocal confirmation of the hemoglobin variant. In addition, 5,000 samples were analysed on a Sciex API4000 Q trap; using the information dependent acquisition facility provided "real time" peptide sequencing thus removing the requirement for re-injection (Figures 5 & 6).

Fig 5. Qtrap IDA spectrum of HbS with library match

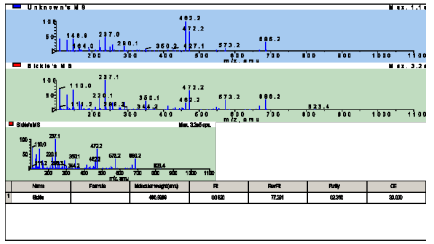
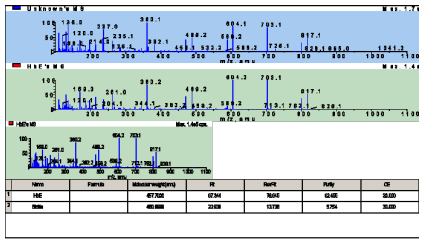


Fig 6. Qtrap IDA spectrum of HbE with library match



DISCUSSION

Boemer et al[3] have recently published an MSMS method, based on our original method[1,2], for newborn hemoglobinopathy screening. We have significantly reduced the processing steps required for conventional tryptic digestion of proteins and, together with the 30min digestion[1,2], modified the sample preparation for MSMS to be no more onerous than for IEF. The consumable costs associated with the MSMS technique are <10% of those for IEF and the capital cost of MSMS can be offset by high throughput and/or integration with current newborn inherited metabolic disease screening by MSMS. The specificity of the MRM analysis implies that hemoglobinopathy detection can be limited to specified conditions, based on agreed screening policy, and can eliminate the need for costly and time consuming second line testing. Furthermore, subsequent product ion scanning or rapid "real-time" product ion scanning on a linear ion trap instrument provide unequivocal sequence data.

CONCLUSIONS

This study demonstrates that newborn hemoglobinopathy screening can be carried out rapidly, easily, and cost effectively using MSMS technology. It enables rationalisation of technology platforms in newborn screening by consolidating screening for hemoglobinopathies and inherited metabolic diseases (

REFERENCES

YA Daniel *et al*. Br J Haem 130 (2005) 635
YA Daniel *et al*. Clin Chem 53,8 (2007) 144
F Boemer *et al*. Clin Chem 54,12 (2008)

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