

## IDENTIFICATION OF HAEMOGLOBIN VARIANTS USING MASS SPECTROMETRY BASED EXPERIMENTAL STRATEGIES

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In order to obtain meaningful mass spectrometric data for haemoglobin variants or their isoforms, it is essential that the information linkage is maintained during the entire analytical procedure for a particular haemoglobin variant with the associated structural changes due to the specific sequence variation or post-translational modification. We present novel mass spectrometry based experimental strategies that overcome these obstacles, with the methodology consisting of an on-target proteolysis after either “separation free” sample preparation or after capillary liquid chromatographic separation of intact haemoglobins and micro-fraction collection, spotting and subsequent MALDI TOF MS analysis. Experimental workflows are presented with examples from procedures developed to characterise haemoglobin variants from human EDTA-treated whole blood via rapid (< 3 min) mass spectrometry-compatible surfactant-aided on-target enzymatic proteolytic digestions and subsequent MALDI TOF MS analyses. These methods allow minimal sample volumes to be used (less than 1 micro litre) whilst maintaining the capacity to rapidly identify haemoglobin variants. These new techniques are expected to significantly contribute to fast and efficient biomedical diagnostic method developments in which protein variant identification forms a crucial component for disease identification.