Mass spectrometry is a method used to weigh molecules. A program, using electrophoresis and chromatography techniques, the clinically significant hemoglobin variants are routinely detected so that early treatment can be given. Symptoms but a small proportion are life-threatening and require treatment.

Chain produce structural hemoglobin variants. Most cause minimal symptoms but a small proportion are life-threatening and require rapid detection so that early treatment can be given.

Mass spectrometry (MS) is a method used to weigh molecules. A mass spectrometer is able to generate ions from a sample and then separate these ions based on their mass-to-charge ratio. As well as providing mass information, mass spectrometric data of peptides and proteins can be used to infer their sequence and shape.

Advantages
- Definitive characterisation of hemoglobinopathy using expert-based approach for interpretation and diagnosis
- Variant identification and quantitation of glycation and delta-chain in one experiment
- Rapid identification
- Capable of 24/7 operation
- High initial cost but little consumables required
- MS use in clinical setting already established for metabolite screening

Method
1.) 10 µl of blood sample diluted in 490 µl of H2O to make stock solution. Stock solution diluted 10-fold in 50% acetonitrile 0.2% formic acid and analysed by mass spectrometry.

2.) 100 µl of stock solution digested with the enzyme trypsin. After 30 minutes digestion diluted 10-fold in 50% acetonitrile 0.2% formic acid and analysed by mass spectrometry.

3.) The identified variant peptide is analysed by tandem mass spectrometry. The peptide of interest is selected and fragmented by collisions with a gas. The resulting fragments are analysed to determine its amino acid sequence and thus the hemoglobinopathy present.

Hemoglobin screening
The clinically significant hemoglobin variants are routinely screened for, under the NHS Sickle Cell and Thalassaemia program, using electrophoresis and chromatography techniques. These techniques rely on a ‘trace-matching’ approach and therefore do not provide definitive diagnosis. If a variant is detected within a blood sample that cannot be identified by this approach the sample is then sent for mass spectrometry or DNA analysis for conclusive characterisation.

Mass spectrometry experiment
Schematic representation of a typical mass spectrometry experiment

The tetrameric adult human hemoglobin complex
- 2 α-chains
- 2 β-chains
- 4 heme groups

Mutations in the amino acid composition of the α-chain or the β-chain produce structural hemoglobin variants. Most cause minimal symptoms but a small proportion are life-threatening and require rapid detection so that early treatment can be given.

Any variant peptides present in the tryptic digest are detected by mass spectrometry.