Validation of a targeted mass spectrometry method for confirmation of peanut allergens in serum
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Background: The iFAAM project is investigating whether the passage of allergen into the circulation may contribute to determining the severity of an allergic reaction by comparing uptake in healthy individuals and individuals undergoing oral food challenge. A mass spectrometry based method to detect the presence of peanut allergens in serum samples is being developed which will allow confirmation of peanut allergens in serum identified by other methods such as immunoassay and mediator release.

Methods: A set of heavy labelled tryptic peptide targets for detection of peanut allergens Ara h 1, Ara h 3 and Ara h 2,6 and 7 have been used to develop a targeted mass spectrometry (MS) method using multiple reaction monitoring, for detection of peanut in serum. Using serial isotopic dilution (SID) series and blank serum spiked with a peanut protein extract, initial validation studies have been undertaken exploring the use of different depletion methods to remove the most abundant serum proteins. MS analysis has made use of different TOF and triple quadrupole platforms.

Results: Undepleted serum samples had a marked matrix effect on SIDs, likely due to the presence of other serum peptides causing suppression effects on peanut peptide ionisation. These effects were less marked in depleted serum samples. Analysis of peanut spiked into blank serum and analysed after depletion showed differential matrix effects, with some peanut peptides having been lost during the depletion process. The general limit of detection of peanut peptide targets was achieved in the femtomolar range, on column.

Conclusion: Further method development is required to optimise serum depletion, trypsin digestion and chromatographic separation steps to allow detection of peanut at levels likely to be found in human subjects after ingestion of peanut. This mass spectrometry method shows promise as a complimentary tool to immunoassay methods to detect the presence of peanut in serum.