Development of reference materials: Conclusions and recommendations for further work

Allergen reference materials have been produced: hens' egg white powder, skimmed cows' milk powder, almond powder (full fat), hazelnut powder (partially defatted), and walnut powder (partially defatted) which are available as the appropriately characterised individual foods and added to a chocolate dessert paste to make a material at the clinically and industrially relevant incurred concentration of 10 mg kg-1 as the total protein. The homogeneity and stability of the materials were investigated and found to be fit for purpose.

The process of preparation and characterisation of the reference materials has been described. Traceability to the SI was achieved gravimetrically. The RM kit, which is on the market, has been confirmed within the scope of ISO 17034 accreditation. Statement of measurement have been published and assigned values compared with independently obtained data from two ELISA platforms.

The successful conclusion of this project does not, of course, solve all the problems in food allergen analysis and we make recommendations for further work to build on the firm foundations reported herein. These include:

- comparison of data from the reference materials on ELISA platforms other than the two exhibited in the project and two allergens (almond and walnut) were not extensively characterised in the project and the reference materials would benefit from further study, including in multiple laboratories.
- although the proteomics of some allergens in the kit have been reported there is scope for further work on the allergen profiles and on value assignment by liquid chromatography –tandem mass spectrometry (LC-MS/MS).
- value assignment for the nut ingredients by Polymerase Chain Reaction (PCR) DNA methods would add to extant data and achieve copy number to mass fraction conversion factors.
- homogeneity data in the reference materials are satisfactory but for hazelnut the data are more dispersed and it is not possible to distinguish the inherent variability of the ELISA from effects perhaps caused by the raw material particle size.The application of digital PCR, (dPCR) which offers much lower variance than ELISA methods and absolute single molecule quantification of DNA species without an external calibration curve, would give more precise information on the homogeneity of the reference materials.
- if dPCR homogeneity data retain the same dispersion driven by the particle size and mixing into the matrix exploration of cryogenic (-80?C) milling would be useful. Assessment of the impact on protein structure of these low temperatures would also be interesting.