Development of reference materials: Discussion

8.1 Background

Food allergy is an increasing problem for those affected, their families or carers, the food industry and for regulators [40], [41]. The food supply chain is vulnerable to fraud involving food allergens and in the catering sector instances of poor compliance with basic allergen management have occurred with high media salience [44], [45], [46], [47]. There is the risk of fatalities and severe reputational damage to the food industry. Precautionary allergen labelling is widely regarded as suboptimal and in need of standardisation [48].

Many facets are being pursued to ameliorate the difficulties including better food labelling and the concept of thresholds of elicitation of allergy symptoms as risk management tools [46]. These efforts depend to a high degree on the ability reliably to detect and quantify food allergens; yet all current analytical approaches exhibit deficiencies that jeopardise accurate results being produced particularly in terms of the risks of false positive and false negative reporting. Three distinct but interrelated areas of analytical work have been recommended [22] to address the substantial gaps identified:

1. a coordinated international programme for the production of properly characterised clinically relevant reference materials and calibrants for food allergen analysis
2. an international programme to widen the scope of proteomics and genomics bioinformatics for the genera containing the major allergens to address problems in ELISA, MS and DNA methods.
3. the initiation of a coordinated international programme leading to reference methods for allergen proteins that provide results traceable to the SI.

Recommendation 1 has attracted much support [47], [48]; the work reported herein is a step in addressing recommendation 1 and in part recommendation 2. Confidence in analytical results is related to the availability of reference materials (RMs), which play an essential role in method calibration and validation, measurement uncertainty assessment, proficiency testing and quality assurance in order to guarantee accuracy and reliability of the analytical data, as well as comparability of results from different laboratories. Unlike reference materials for single analyte molecule or well-defined classes of compounds, the preparation and certification of RMs for food allergens are very challenging [43].

The consortium that carried out this study has previous experience [49], [50], in the preparation and utilisation of incurred materials that have proved useful in food allergen analysis. However given the constraints of the study it was important to consult the analytical community most concerned with food allergen analysis prior to planning and executing the preparation of the RM described herein, described in detail in section 7.3.

The consultation confirmed the utility of the medium difficulty industrially relevant Europrevall cold swelling chocolate flavoured vehicle as a relevant matrix in which to incur priority allergens. It also confirmed the priority allergens as hens' egg white powder, skimmed cows' milk powder, and the nuts almond, hazelnut and walnut. All of these are contained in the food allergens subject to specific labelling requirements in European law [51]. Equally importantly it emerged that as well

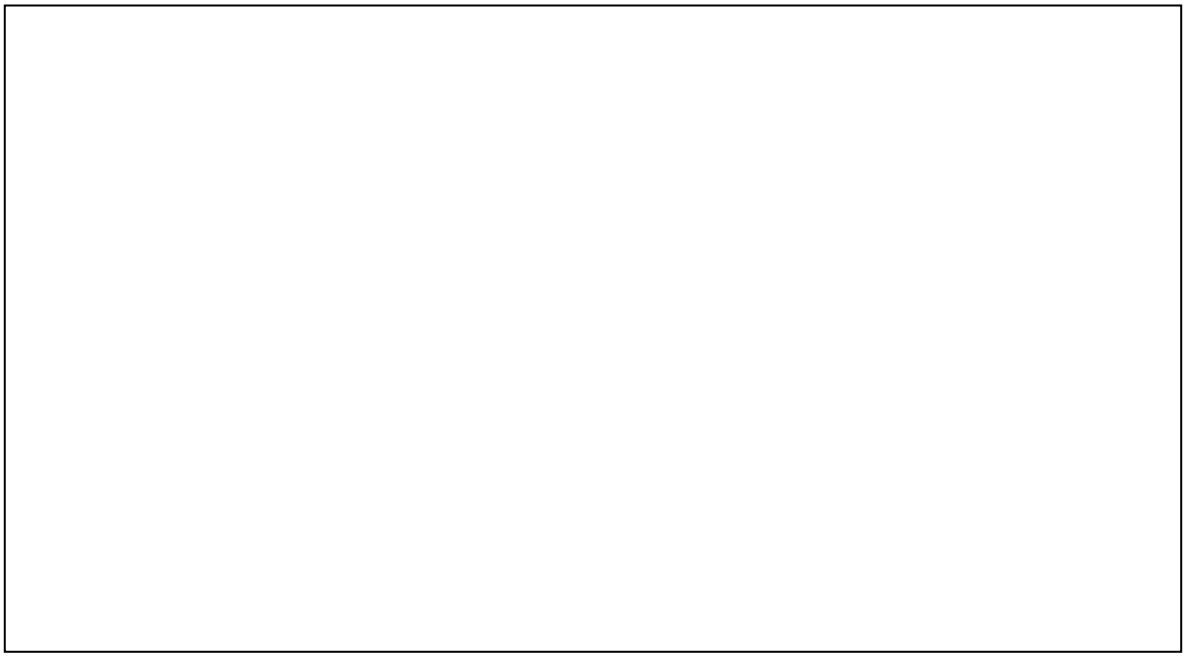
as an incurred matrix, a blank matrix and the food allergen commodities, as they appear in the food supply chain, were needed by the analytical community. The latter address a need for spike recovery experiments in matrices such as catering sector meals for which it is unlikely that dedicated RMs will appear owing to the diversity of the potential matrices.

Thus the RM has been prepared as a kit containing allergens chosen after consulting stakeholders, aimed to be of medium analytical difficulty and to be complementary to other RMs available (for example, from MoniQA).

8.2 Raw materials

The raw materials have been carefully sourced and characterised by proteomics (UoM, led by Professor Clare Mills), and at LGC by PCR and for Dumas N and Karl Fisher water, Figure 4.

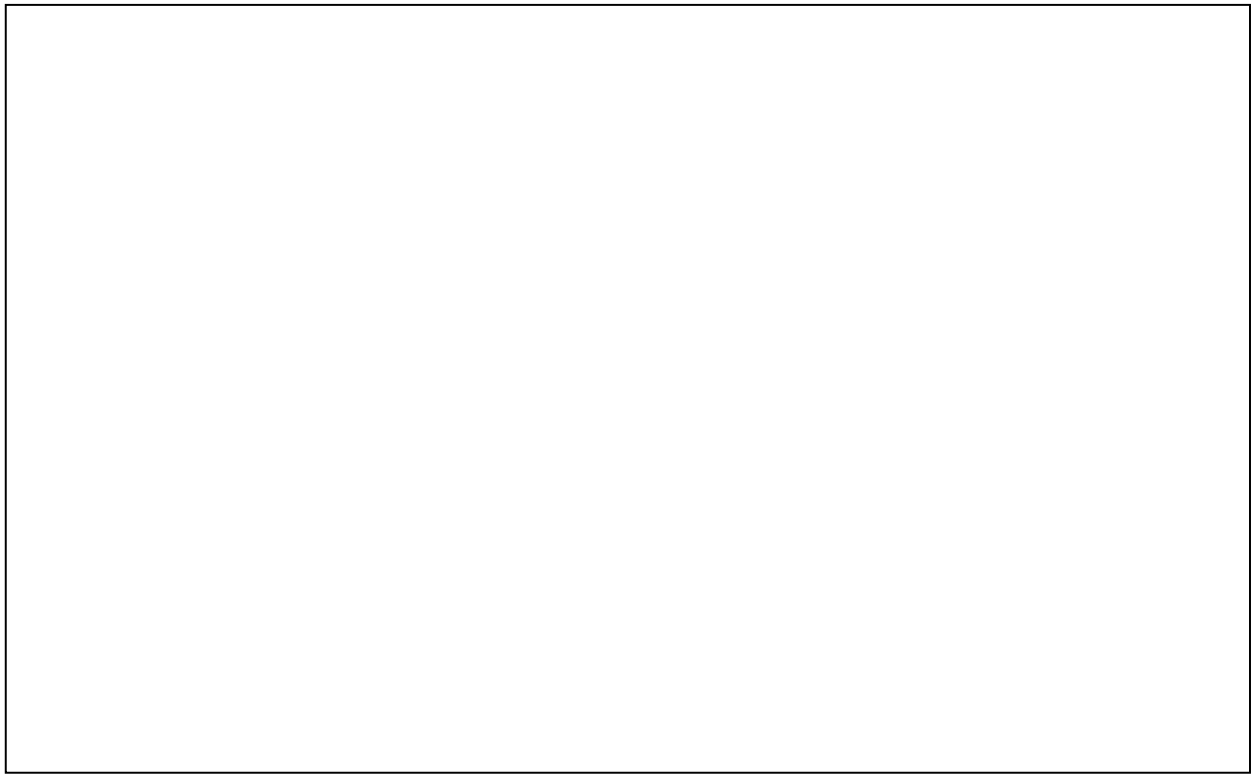
Figure 4: Characterisation of the raw material allergens



8.3 The matrix

The matrix is based on a food vehicle designed to blind the participants in oral food challenges to increasing doses of allergens to establish dose-distribution curves of allergic reactions. In general, the ingredients of the RM and the incurred allergens are from the same or similar sources as the foods used in the EuroPrevall studies that gave rise to reference doses for several allergens, hence the clinical relevance of the material. The approximate composition of the paste is shown in Figure 5. It was checked for workability - it is solid at room temperature and a viscous liquid at 37 ?C, the temperature at which mixing of the incurred allergens occurs and at which it is dispensed. It has been shown to be stable owing to its low water activity and devoid of the allergens intended to be incurred into it. There is no need to rehydrate this RM and it can be used directly from the bottle.

Figure 5 Overview of the RM kit



8.4 The incurred matrix

The allergen concentrations in the incurred material were prepared by weighing out appropriate amounts of each of the raw materials based on their total protein content. A high-level concentration was prepared to a checked written protocol and the final incurred concentration was arrived at by gravimetric serial dilution of the original preparation. Homogeneity and stability studies were carried out to the principles of ISO 17034 2016 (General requirements for the competence of reference material producers). From these, and separate ELISA assays within LGC, information can be given in the statement of measurement on the likely concentrations expected to be arrived at by laboratories using ELISAs from Romer and R-Biopharm.

8.5 Homogeneity

The raw materials and the blank matrix were examined for homogeneity and found to be fit for purpose in this respect. The incurred matrix was similarly studied and found to be sufficiently homogenous for the intended purpose with regard to its incurred hens' egg white protein and skimmed-cows'-milk protein. For hazelnut protein the homogeneity of the incurred matrix was challenging however a statistical model, with 95%/95% tolerance intervals calculated assuming (a) normality and (b) lognormality was developed that addresses the needs of potential users of the RM. Although funding was not available to assess the homogeneity of almond and walnut protein it is reasonable to assume a similar approach will be required.

In part the challenges to homogeneity studies arise from the available precision of current methods of allergen analysis. However, high lipid content materials such as nuts also present challenges in milling to a required low enough particle size to ensure adequate mixing. This is a topic for further work.

8.6 Stability

Not unexpectedly stability of the incurred material began to falter at 60?C however the stability studies have resulted in clear guidance for users of storage of the RM kit.

8.7 What is the kit intended for?

There are several possible applications for the food allergen RM kit. These include validation and verification studies of relevant (for example, relevant to the food allergens in the RM kit) analytical methods (ELISA, PCR, LC-MS/MS, LFD), and ELISA and PCR kits. Its applicability extends to new methods or detection kits, on-going competency assessments of existing detection kits and methods, and the competency of staff performing allergen analyses and of analytical service providers working in the food supply chain. The food allergen RM kit may be used directly to quality assure relevant non-routine analytical runs or to validate and verify in-house quality control materials for use in routine analytical runs.

Examples of usage include:

The raw materials can be used to

- generate kit calibrator extract solutions
- generate external check calibrator extract solutions
- spike various other matrices either by way of an extract but preferably by addition of the raw material itself to assess recovery in real life situations. It will be impossible to generate RMs for the required wide variety of matrices (for example, take-away curry type products) which are analysed by laboratories however the raw material may be spiked into such samples to provide matrix-specific recoveries.

The blank matrix can be used as

- a ‘no-template’ control to provide assurance of absence of in-lab allergen cross contamination (either environmentally, from personnel or in reagents)
- a material to assist method LoD calculation (as 3.3 times the standard deviation of a ‘blank’ dataset)

The incurred matric can be used to

- optimise analytical recovery from a chocolate-type matrix;
- inform risk assessors of the possible ‘true’ estimate of allergen in a questioned product.
- generate conversion factors from PCR copy number/copy number data to m/m data, and from peptides concentrations to protein and hence food concentrations (with appropriate definitions).