# Development of reference materials: Reference materials, quality control and harmonisation of analysis

## 5.1 Background

What can be done to address the limitations of all the available approaches to food allergen analysis? One solution is the availability of well characterised calibrants and reference materials (RM) to allow comparability and harmonisation of measurements obtained using diverse analytical methods and across different laboratories. More importantly, the production of reference materials which represent a real food product would help analysts to develop methods capable of providing food business operators with meaningful information that can be easily used for implementing risk assessment plans.

Work undertaken by the Joint Research Centre (JRC) has attempted to develop a candidate RM in relation to ELISA measurements of gluten. Gluten represents a challenging analyte as it is not clearly defined and there is no agreed common marker or conversion factor. The results of the work were mixed. On the one hand, the use of the gluten reference material as a calibrant helped to reduce within-kits and between-assay variability somewhat. On the other hand, when different kits target diverse fractions of the gluten molecule, this naturally leads to different antibody selectivity which was not improved by the use of a common calibrant [33]. Such data emphasises the need for development of internationally recognised sets of analytical targets and allergen reference materials to improve the reliability of allergen analysis.

Reference material (RM) and Certified Reference Material (CRM) are well defined terms within an associated international infrastructure. Food allergen RMs should comply with this infrastructure. Reference materials produced by National Measurement Institutes such as LGC exhibit the highest standards. Taylor et al [34]. were probably among the first to describe with examples the preparation of naturally incurred standards as allergenic food residues incorporated into various representative food matrices and then processed in a manner similar to ‘real-world’ food processing. This approach has stood the test of time in this and other contexts. Thus it is routine to produce very useful RMs of food in food (meat species for example) and LGC are long accustomed to analysing for food in food to appraise QUID (Quantitative Ingredients Declaration) – required by labelling law for certain foods.

## 5.2 Commercially available materials

### 5.2.1 Reference materials

The consortium investigated the reference materials already available on the market. They take two different forms: raw ingredients and incurred processed food matrices. The analysis included the major developers and producers of reference materials with Joint Research Centre (JRC), National Institute of Standards and Technology (NIST), LGC, and the National Metrology Institute of Japan (NMIJ). It is noteworthy that most were not originally intended as allergen protein reference materials.

### 5.2.2 Raw ingredients

Several milk powder reference materials are certified for the content of trace elements, organic pollutants, veterinary residues, natural toxins, and vitamins. JRC and NIST produce milk reference material as skimmed milk powder (BCR – 685) and whole milk powder (NIST – SRM 1549a) that can be used for measuring the nutritional values of the food product. The materials are certified for the crude protein content determined by Kjeldahl analysis. A factor of 6.38 was used to convert nitrogen results to protein.

A spray dried whole egg for allergen detection, produced without additives or stabilizers, is available from NIST (NIST – SRM 8445). The approximate content of protein was measured using a Leco protein analyser (Dumas). NIST also produces a whole egg powder for measuring the nutritional values of the food product (NIST – SRM 1845a). Nitrogen was determined by collaborating laboratories using Kjeldahl, thermal conductivity, and combustion (LECO). A factor of 6.25 was used to convert nitrogen results to protein.

A dry whole egg powder reference material (EGGS-1) is also available from the National Research Council Canada (NRC), where the protein concentration was determined using a factor of 6.25 to convert nitrogen in protein.

The JRC made available a material for peanut analysis including 6 vials containing peanuts prepared at different roasting temperatures and times (IRMM – 481). The kit is not considered a reference material since it was not tested for homogeneity and stability.

NIST produced a peanut butter standard reference material certified for the content of proteins (NIST – SRM 2387). A factor of 5.46 was used to convert nitrogen results to protein.

LGC has made available a mechanically defatted light roasted peanut flour in 5 g vials under Argon. (LGCQC1020) which is from the same source and batch of peanut flour used for recent protein detection studies. Values are provided for nitrogen and water content and the material will prove useful for allergen protein measurement.

### 5.2.3 Incurred processed food matrices

At the beginning of 2017, the MoniQA association made available the first validated reference material for food allergen analysis. They manufactured milk powder cookies at two agreed concentrations, 3.5 (LOW-MQA 102016) and 35 (HIGH-MQA 082016) mg of milk protein per kg of cookie. The materials are sold alongside the dry skimmed milk powder (SMP-MQA 092014) and a blank cookie (BLANK-MQA 082015). In the future, the production of egg and soy-based reference materials is expected.

The FAPAS (Food Analysis Performance Assessment Scheme) produced a cake mix reference material containing gluten, egg, and milk (FCAL7-PRO10RM). Differently from the MoniQA material, egg and milk were added as a known amount of total ingredient to a commercial cake mix manufactured to be gluten-free, egg-free and milk-free. The amount of egg and milk proteins is not traceable. Moreover, the effect of processing on the detection of the allergens is not taken into consideration. The material is currently not available. FAPAS produces two quality control materials: cooked biscuits containing hazelnut and peanut (T27171QC) and chocolate containing hazelnut (FCAL8-CON2QC). For all the products described, the protein concentration assignment was done by consensus assessment based on ELISA analysis.

LGC have made available a quality control set based on chocolate dessert. Each set contains a blank unit and a unit with 10 mg kg-1 gravimetrically incurred peanut protein (LGCQC101-KT). Within the iFAAM project, the material was used as a ring test sample for ELISA and Mass spectrometry analysis.