

## Development of reference materials: Synergy with other work

Synergy with other work (for example, by iFAAM, EFSA, ILSI, MoniQA, JRC, and AOAC) has benefited this project and the consortium was well placed to engage with these stakeholders. The University of Manchester Food Allergy Network, MFAN, which meets alternately at University of Manchester and LGC was also well placed to engage with ELISA kit manufacturers, retailers, analytical service laboratories, regulators and food manufacturers. LGC is actively engaged with Public Analysts, providing the Association of Public Analysts Training Officer role. Particular synergy was established with the EFSA-funded project ThrALL, which aims inter alia to improve the detection and quantification of food allergens. A joint project meeting of ThrALL [34], [35] and FS 101206 project members was held on 22 March 2018. During this meeting it was evident that the activity would not overlap with the outputs of the ILSI working group in which the JRC was involved. Moreover, complementary work ensuing from ThrALL included a study on two highly processed matrices, chocolate bars and broth powder. These were incurred with six allergenic ingredients (egg, milk, peanut, soy, hazelnut and almond) at 2, 4, 10 and 40 mg total allergenic protein/kg food matrix using a pilot-scale food manufacturing plant. All the allergens tested were found to be stable in the incurred matrices for at least 30 months demonstrating they are suitable for method development [35].

The first (and so far the only) reference food allergen method traceable to the SI to enable the comparability of food allergen measurement results expressed in a decision-relevant manner was published in 2020 [36] based on work by Nitride et al. 2019 [37]. A workflow and stoichiometric calculations were demonstrated. Challenges included optimal extraction of marker proteins, complete digestion and equimolar release of peptides and the use of conversion factors to translate the amount of measured proteins into allergenic food. Importantly, the combined uncertainty of the final result was reported. This was followed by an interlaboratory comparison that indicated further harmonisation is required quantitatively to determine potentially allergenic constituents in food. [38] The assignment of the reference value for the interlaboratory comparison has been described noting that the proper application of isotope dilution mass spectrometry (IDMS) provides the shortest traceability to SI units and reference values with the lowest uncertainties. [39] A certified reference material for milk protein is expected from this work.