# Development of reference materials: Executive summary

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## Abbreviations and glossary

| Abbreviation | Meaning of the abbreviation |
|---|---|
| AOAC | Association of Analytical Communities, (formerly Association of Official Analytical Chemists |
| APA | Association of Public Analysts |
| dPCR | Digital PCR |
| AQA | Analytical Quality Assurance |
| EFSA | European Food Safety Authority |
| EHO | Local Authority Environmental Health Officer |
| ELISA | Enzyme-linked Immunosorbent Assay (a type of immunoassay) |
| EuroPrevall | A European and international research programme on the prevalence, cost and basis of food allergy – headed by Prof Clare Mills |
| FSA | Food Standards Agency |
| iFAAM | Integrated approaches to food allergen and allergy management, an international research programme to develop evidence-based management of allergens in food and integrate knowledge derived from their application into food allergy management plans and dietary advice |
| ILSI | International Life Sciences Institute, a non-profit, worldwide organization whose mission is to provide science that improves human health and well-being and safeguards the environment |
| ISO | International Organization for Standardisation |
| JRC | Joint Research Centre (of the European Commission) |
| LGC | Formerly Laboratory of the Government Chemist, now a private company |
| NML | The UK's National Measurement Laboratory (NML) at LGC is the UK's designated National Measurement Institute for chemical and bio- measurement |
| MFAN | University of Manchester Food Allergy Network |
| MoniQA | International Association for Monitoring and Quality Assurance in the Total Food Supply Chain, an international and interdisciplinary network working in food research, regulatory bodies and trade. |
| MS | Mass Spectrometry |

| Abbreviation | Meaning of the abbreviation |
|---|---|
| OCL | Official Food Control Laboratory (see below Public Analyst) |
| PA | Public Analyst |
| PAL | Precautionary allergen labelling ('may contain') |
| PCR | Polymerase Chain Reaction, a technique used to amplify DNA sequences so that they can be identified |
| qPCR | Real time-PCR, which uses fluorescent markers on PCR primers to follow the PCR amplification process as it is occurring (for example, in real time) |
| RM | Reference material |
| TSO | Local Authority Trading Standards Officer |
| UKAS | United Kingdom Accreditation Service, licensed to accredit laboratories to the ISO/IEC 17025 standard |
| UoM | University of Manchester |

# Executive Summary

This research has now been published as a scientific paper in the Food Chemistry journal, which can be found here.

Reference: Gill Holcombe, Michael J. Walker, Malvinder Singh, Kirstin Gray, Simon Cowen, Stephen L.R. Ellison, Adrian Rogers, Anuradha Balasundaram, Malcolm Burns, E.N. Clare Mills, 2023, Clinically and industrially relevant incurred reference materials to improve analysis of food allergens, milk, egg, almond, hazelnut and walnut, Food Chemistry, 434, 2024, 137391

Food hypersensitivity is a serious and growing problem, of which food allergy is a significant facet with up to about 2 million people in the UK estimated to be affected. In the absence of a currently accepted cure, affected individuals must throughout their life avoid eating foods to which they are allergic. This can be difficult to achieve in practice. Allergens can sometimes find their way into foods unintentionally. Even very small quantities of foods (as little as a hundredth of a gram in the case of some nuts) can cause an unwanted reaction in a person with an allergy to the food.

Analysis of food and food ingredients for allergens is vital to secure safe food for people with allergies, protect the supply chain and support businesses and risk assessors. However, analysis is hampered by lack of reliable reference materials to check and harmonise the proper performance of laboratory tests. Food Standards Agency (FSA) project FS101206 set out to partially address this need. LGC led a consortium with the University of Manchester and Romer Laboratories Ltd which was awarded the project following an open competitive tender.

The consortium members brought unique skills. The UK's National Measurement Laboratory (NML) at LGC is the UK's designated National Measurement Institute for chemical and bio-measurement with extensive experience in the production of reference materials to ISO 17034:2016 'on the general requirements for the competence of reference material producers' (previously ISO Guide 34:2009). The University of Manchester (Professor ENC Mills) (UoM) applies molecular science to better understand, diagnose and treat food allergies with experience of running a series of high value EU Framework Programmes on food allergy including EuroPrevall, iFAAM and ThrALL and extensive knowledge of protein chemistry. Professor Mills also runs the Manchester Food Allergy Network (MFAN) bringing together stakeholders in allergen risk assessment and risk management. Romer Laboratories Ltd (Adrian Rogers) (Romer Labs) is a leading global supplier of diagnostic platforms for food safety including food allergens, with ISO/IEC 17025 accredited laboratories in Austria, UK, US and Singapore and a contributor to harmonisation on food allergen measurement.

The project successfully produced and characterised the first multi-allergen reference material kit containing five common allergens (milk, egg, almond, hazelnut, walnut), traceable to the SI (International System of Units). It will help scientists and industry by supporting method

development to determine ‘true’ allergen content, and assist laboratories in monitoring the performance of methods on a day-to day basis.  This is improving the safety of food products for people with food allergies by helping analysts provide meaningful information for risk assessors.

This report briefly describes the governance of the project, stakeholder engagement to optimise the format and contents of the reference material kit and its preparation and characterisation. More detailed information is available in three peer reviewed publications referenced in the report. We also touch on dissemination of information about the reference materials.

The reference materials have been prepared as a ‘kit’ consisting of

A medium analytical difficulty polyphenol-containing processed food chocolate paste matrix

- (a) devoid of the five allergens, and
- (b) incurred with each allergen at the clinically relevant concentration of 10 mg kg-1 expressed as protein.

The allergen raw materials,

- hens’ egg white powder,
- skimmed cows’ milk powder,
- almond powder (full fat),
- hazelnut powder (partially defatted), and
- walnut powder (partially defatted)

The raw materials are also individually available as well as being included in the kit.

The preparation, gravimetric traceability to the SI, homogeneity and short term (transportation) stability and long term stability of the RM have been demonstrated and are described. Long term stability continues to be monitored. These data together with species characterisation by Sanger sequencing and allergen profiling in the raw materials by a combination of immunoblotting and discovery mass spectrometry demonstrate the suitability of the reference materials. The reference materials, which have been available since early 2020, have been confirmed within the scope of LGC’s ISO 17034 accreditation. Assigned values are compared with independently obtained data from two ELISA platforms and statements of measurement have been published.

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. Two allergens (almond and walnut) were not funded to be 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.