

Development of reference materials: Appendices

Appendix 1: Project Participants

A1.1 LGC

LGC is a global leader in the Life Science Tools sector, which serves customers across a number of end markets, including human healthcare, agri-food & the environment. LGC provides a comprehensive range of measurement tools, proficiency testing schemes, supply chain assurance standards and specialty genomics reagents underpinned by leading analytical and measurement science capabilities. Its scientific tools and solutions form an essential part of its customers' quality assurance procedures and enable organisations to develop and commercialise new scientific products and advance research.

LGC's 3,200+ employees include internationally-recognised scientists who are experts in their field. Headquartered in London, it operates out of 22 countries worldwide and is extensively accredited to quality standards such as GMP, GLP, ISO 13485, ISO 17034, ISO 17043, ISO/IEC 17025 and ISO 9001.

LGC has been home to the UK Government Chemist for more than 100 years and is the UK National Measurement Laboratory and Designated Institute for chemical and bio measurement. LGC has been privately-owned since 1996 and has diversified through internal investment and acquisition to be an international leader in its chosen markets.

For more information, please visit www.lgcgroup.com

About the National Measurement Laboratory

The National Measurement Laboratory (NML), hosted at LGC, delivers underpinning chemical and bio-measurement science for the UK and forms part of the UK National Measurement System (NMS). The NML is the Designated Institute for chemical and bio-measurement and supports the work of the Government Chemist, a unique statutory function that provides expert opinion and advice to Government.

Our research areas span the sectors of advanced therapeutics, diagnostics and safety & security and are delivered through the four core streams of measurement research, calibration facilities, reference materials, and training and consultancy. Our measurement capabilities comprise state-of-the-art mass spectrometry, PCR and cell characterisation of products and processes, with many of our testing and calibration services accredited to ISO/IEC 17025. We play a leading role internationally to develop best practice and standardise measurements across the world, in turn providing further confidence in the UK's science and technology capabilities.

Through improved chemical and bio-measurements we support manufacture and trade, protect consumers, further skills development and enhance quality of life.

A1.2 University of Manchester Institute of Biotechnology

The strong interdisciplinary focus and mode of operation in the Manchester Institute of Biotechnology (MIB) enables our scientists and engineers to drive state-of-the-art biotechnology research through establishing new types of collaboration. The MIB's approach is pluralistic, integrative and non-exclusive functioning flexibly as a partner to researchers across a broad spectrum of disciplines which are fluid and constantly evolving. Using advanced quantitative methods to explore the relationship between the macro behaviour of biological systems and the properties of their nanoscale components we see this understanding as a basis for developing new biotechnologies that will find applications in areas such as human health, the energy economy, food security, industrial transformations and the environment. A defining feature of our mission is the incorporation of rigorous, cutting-edge principles and technologies derived from the physical sciences, engineering, mathematics and computation into novel approaches to elucidating the molecular mechanisms that underpin living processes and systems. An extensive programme of technology development lies at the heart of our research programmes, driven by expertise from the quantitative sciences and engineering, allowing us to accelerate progress through the implementation of state-of-the-art enabling technologies.

A1.3 Romer Labs Ltd

Founded in Washington, MO, in 1982, [Romer Labs](#) became over the years a leading provider of diagnostic solutions for the agricultural, food and feed industry.

Today, Romer Labs offers a broad range of innovative diagnostic solutions covering mycotoxins, food pathogens, food allergens, gluten, GMO, veterinary drug residues, and other food contaminants. The portfolio includes:

- ELISA test kits - AgraQuant®
- Lateral flow devices - AgraStrip® and RapidChek®
- Reference materials - Biopure™
- Cleanup Columns - MycoSep®, MultiSep®, MycoSpin®, StarLine™
- Sampling mills

Furthermore, Romer Labs operate 4 accredited, full-service laboratories in Austria, UK, US and Singapore. Using cutting-edge technology in the fields of chromatography and immunological analysis, our labs offer services for the analysis of mycotoxins, food allergens, meat speciation, VDR and GMO.

Romer Labs is at the forefront of diagnostic technology and we are constantly expanding our product and service portfolio to meet your continuously evolving demands.

Our key objective at Romer Labs is to provide scientifically sound, high-quality products and an exceptional service, in line with our mission – Making the World's Food Safer®.

Appendix 2: Refereed publications

The following refereed publications were prepared for publication during the project.

A2.1 Paper D1.1 - Pilolli *et al.*

Citation

Pilolli, R., Nitride, C., Gillard, N., Huet, A.C., van Poucke, C., de Loose, M., Tranquet, O., Larré, C., Adel-Patient, K., Bernard, H. and Mills, E.C., 2020. Critical review on proteotypic peptide marker tracing for six allergenic ingredients in incurred foods by mass spectrometry. *Food Research International*, 128, p.108747. DOI: 10.1016/j.foodres.2019.108747

Abstract

Peptide marker identification is one of the most important steps in the development of a mass spectrometry (MS) based method for allergen detection, since the robustness and sensitivity of the overall analytical method will strictly depend on the reliability of the proteotypic peptides tracing for each allergen. The European legislation in place issues the mandatory labelling of fourteen allergenic ingredients whenever used in different food formulations. Among these, six allergenic ingredients, namely milk, egg, peanut, soybean, hazelnut and almond, can be prioritized in light of their higher occurrence in food recalls for undeclared presence with serious risk decision. In this work, we described the results of a comprehensive evaluation of the current literature on MS-based allergen detection aiming at collecting all available information about proteins and peptide markers validated in independent studies for the six allergenic ingredients of interest. The main features of the targeted proteins were commented reviewing all details available about known isoforms and sequence homology particularly in plant derived allergens. Several critical aspects affecting peptide marker reliability were discussed and according to this evaluation a final short-list of candidate markers was compiled likely to be standardized and implemented in MS methods for allergen analysis.

A2.2 Paper D1.2 Adaba *et al.*

Citation

Rosemary Adaba, Chiara Nitride, Anuradha Balasundaram, Ernest Long, Ivona Baricevic-Jones, Daniel Schaeffer, Jason Robothom, Gill Holcombe, Michael J Walker, and E. N. Clare Mills, 20xx, Characterisation of hazelnut, walnut and almond ingredient for use as reference materials for allergen analysis, (in preparation).

Abstract

Quantification of allergens in foods using different test methods can give rise to conflicting results, an issue that can be addressed by access to good quality incurred allergen reference materials which provide a mean of harmonising test results. Powdered ingredients were sourced from foods that frequently cause severe allergic reactions and/or give rise to product recalls and are representative of foods as they are commonly consumed. These included roasted peanut (*Arachis hypogea*), hazelnut, (*Corylus avellana*), walnut (*Juglans regia*) and almond (*Prunus dulcis*). The extraction of ingredients was optimised, and the allergen profiles characterised using a combination of immunoblotting using specific antibodies and discovery mass spectrometry using data dependent acquisition. Homologous allergens from the major plant food allergen families were identified across the plant species and shown to be present in an immunologically active form. These data demonstrate the suitability of the ingredients for the preparation of allergen reference materials.

A2.3 Paper D4.2 Holcombe *et al.*

Citation

Gill Holcombe, Michael J Walker, Malvinder Singh, Kirstin Gray, Simon Cowen, Stephen L R Ellison, Adrian Rogers, Anuradha Balasundaram, Malcolm Burns and E. N. Clare Mills, 20XX, Clinically and industrially relevant incurred reference materials to improve analysis of food allergens, milk, egg, almond, hazelnut and walnut, (paper submitted for publication).

Abstract

Measurement of critical food allergen protein concentrations against concentrations derived from quantitative allergen risk assessment has the potential to improve allergen risk management and precautionary allergen labelling. However, such measurement suffers well known problems. The availability of well characterised reference materials (RMs), called for by many, would allow comparability and harmonisation of measurements between methods and across different laboratories and help analysts provide meaningful information for risk assessors. We describe the preparation and characterisation of the first consensus informed industrially and clinically relevant multi-allergen matrix RM kit for five priority allergens. It consists of a medium analytical difficulty polyphenol-containing processed food chocolate paste matrix (a) devoid of allergens, and (b) incurred with five allergenic materials 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), are also available as RMs. The preparation, gravimetric traceability to the SI, homogeneity, short term (transportation) stability and long-term stability of the RM have been demonstrated and are described. Further, 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 RM. The RM kit, which is on the market, has been confirmed within the scope of LGC's ISO 17034 accreditation. Assigned values are compared with independently obtained data from two ELISA platforms and a statement of measurement has been published.