Randomised Controlled Multicentre Trial on the Clinical Impact of the Basophil Activation Test and the Mast Cell Activation Test as Food Allergy Biomarkers in Children and Young People

BAT IMPACT STUDY

PROTOCOL VERSION 3.0, 06MAY2024

CO-SPONSORS:
KING'S COLLEGE LONDON
GUY'S AND ST THOMAS' NHS FOUNDATION TRUST

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Table 1. Key study team members and their contact details.

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LIST OF ABBREVIATIONS

BAT	Basophil activation test
BMI-C	Body Mindset Inventory- Child Version
BTMS	Bodily Threat Monitoring Scale
DBPCFC	Double-blind-placebo-controlled-food-challenge
FA	Food allergy
FAQLQ	Food allergy quality of life questionnaire
FARE	Food allergy research and education
FFQ	Food frequency questionnaire
HADS	Hospital anxiety and depression scale
MAT	Mast Cell Activation Test
OFC	Oral food challenges
PBMC	Peripheral blood mononuclear cell
POC	Patient organisations committee
QoL	Quality of life
RCADS	Revised Children's Anxiety and Depression Scale
RCT	Randomised control trial
sIgE	Specific IgE
SPT	Skin prick test
STAI-AD	State trait anxiety inventory for adults
STAI-CH	State trait anxiety inventory for children

TRIAL SUMMARY

Trial Title	Randomised Controlled Multicentre Trial on the Clinical Impact of the Basophil Activation Test and the Mast Cell Activation Test as Food Allergy Biomarkers in Children	
Internal ref. no. (or short title)	BAT Impact Study	
Trial Design	Biomarker-led multicentre randomised controlled trial	
Trial Participants	Children aged from 6 months to 15 years with suspected allergy to peanut, cow's milk, egg, sesame or cashew nut and requiring an oral food challenge to confirm the diagnosis.	
Planned Sample Size	398 participants	
Intervention	Basophil activation test ± mast cell activation test (the biomarker)	
Follow up duration	2 months after the oral food challenge	
Planned Trial Period	3 years	
Outcomes	Objectives	Outcome Measures
Primary	To determine the impact in the use of the basophil activation test in the number of allergic reactions experienced as part of the oral food challenge (OFC)	Proportion of positive oral food challenge in the biomarker arm (BAT) compared to the standard-of-care arm
	To determine the impact in the use of the basophil activation test tt in the number of OFC	Number of OFC in the biomarker arm (BAT) compared to the standard-of-care arm
Secondary	To assess the quality of life of children and parents at the start and at the end of the diagnostic work-up for food allergy	QoL score as measured by standardised and validated questionnaire
	To assess the impact of the tests on anxiety levels of parents and children	Food allergy-specific anxiety questionnaires
	To assess the cost-effectiveness of incorporating BAT/MAT in the diagnostic assessment	NHS and societal costs using bespoke form
	To determine the diagnostic accuracy of BAT, MAT, SPT, sIgE to allergen extracts, sIgE to individual allergens and the proposed diagnostic algorithm	Sensitivity and specificity of BAT, MAT, SPT, sIgE to allergen extracts, sIgE to individual allergens and the proposed diagnostic algorithm

Table 2 Study synopsis

FUNDING AND SUPPORT IN KIND

FUNDER(S) (Names and contact details of ALL organisations providing funding and/or support in kind for this trial)	**FINANCIAL AND NON FINANCIALSUPPORT GIVEN**
Food Allergy Research and Education (FARE)	Grant to Dr Alexandra Santos at King's College London

Table 3 Study Funders and Supporters

ROLE OF TRIAL SPONSOR AND FUNDER

The lead sponsor, King's College London, will take primary responsibility for ensuring that the design of the study meets appropriate standards and that arrangements are in place to ensure appropriate conduct and reporting. King's College London also provides cover under its No Fault Compensation Insurance, which provides for payment of damages or compensation in respect of any claim made by a research subject for bodily injury arising out of participation in a clinical trial or healthy volunteer study (with certain restrictions). The co-sponsor, Guy's & St Thomas' NHS Foundation Trust, take responsibility for arranging the initiation and management of this research, and will take responsibility for ensuring that appropriate standards, conduct and reporting are adhered to regarding its facilities and staff involved with the project.

ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITEES

Data Monitoring Committee

The Data Monitoring Committee is formed by two independent experts and the Chief Investigator (**Table 1**). This Committee will meet at a minimum twice per year via video conference. A report on the progress of the study and any issues will be sent in advance of the meeting. The Committee will also be asked to advise on any issues that may affect data integrity or safe progression of the study.

Trial Management Group

The Trial Management Group is formed by the Chief Investigator, the Study Coordinator, the Statistician, the Lead Laboratory Technician and one Investigator from each of the Study Sites (**Table 1**). The Trial Management Group will meet at a minimum of 4 times per year and will produce a report on progress and plan for the next reporting period of 3 months.

Protocol contributors

The protocol was defined by the Chief Investigator with contribution from the Statistician and the Study Coordinator. All study co-investigators reviewed the protocol prior to its submission.
Expert patients and patient organisation representatives such as Allergy UK, the EAACI POC, and Natasha Allergy Research Foundation also provided input on the protocol.

KEY WORDS:
Food allergy; Oral food challenge; Diagnosis; Basophil activation test; Mast cell activation test; Specific IgE

TRIAL FLOW CHART

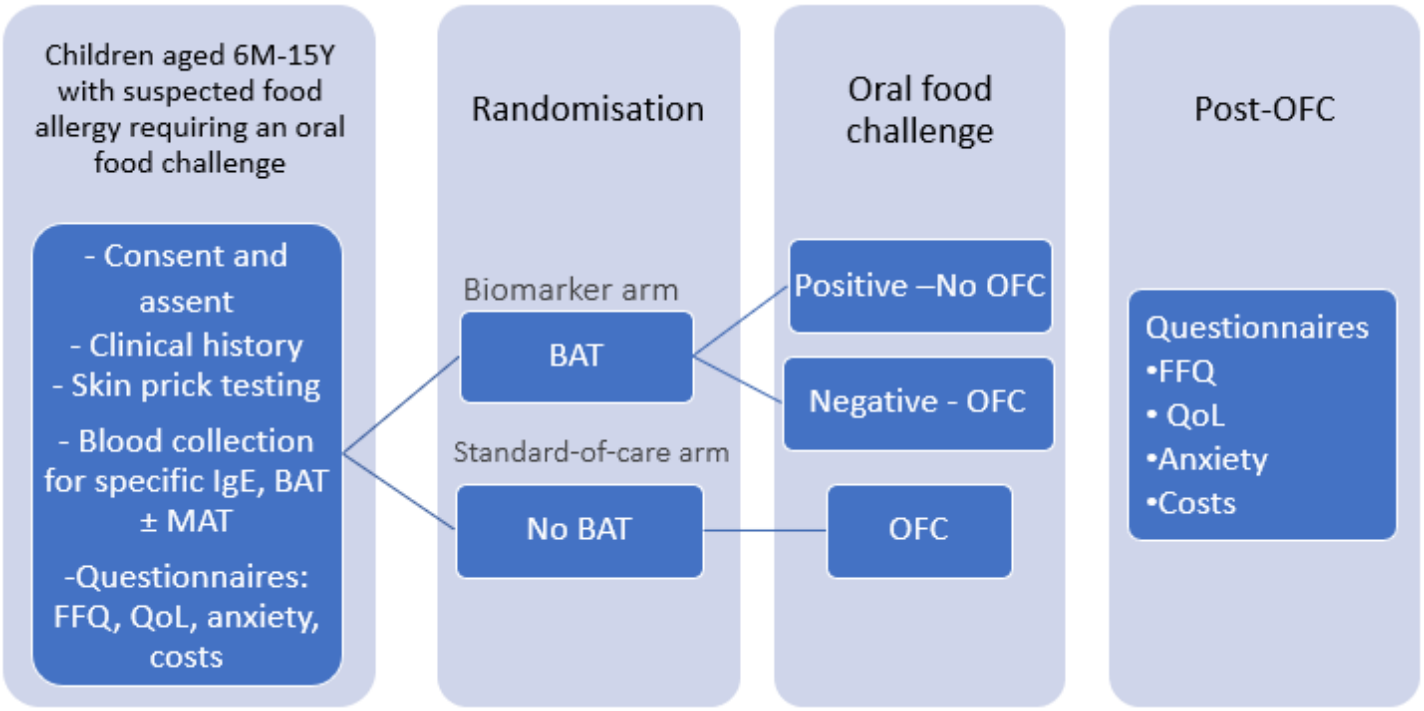


Figure 1. Trial flow chart. OFC, oral food challenge; BAT, basophil activation test; FFQ, food-frequency questionnaire; QoL, quality of life questionnaire.

1. BACKGROUND

1.1. Impact of food allergy on the lives of allergic children and their families

Food allergy (FA) affects 8% of children in the US and UK[1, 2]. There is currently no curative treatment for FA and the mainstay of management is allergen avoidance and carrying emergency medication to treat allergic reactions that may result from accidental allergen exposure, which are, unfortunately, common and potentially severe[3]. Allergen avoidance and the fear of accidental reactions lead to, not only dietary restrictions with possible nutritional consequences, but also social restrictions and an unmeasured psychological burden from stress and anxiety[4-10]. All these factors have a **negative impact** on the quality of life of allergic children and their families and underscore the importance of an accurate diagnosis of FA.

The tests currently used to support the diagnosis of FA, such as skin prick test (SPT) and specific IgE (sIgE), have a high number of false-positive results, i.e. they are positive in many children who do not have FA[11, 12]. This can lead to **over-diagnosis of FA**, if SPT and sIgE are considered in isolation. In order to reach an accurate diagnosis of FA, children need to undergo OFC, which consists of consuming the food suspected of causing an allergic reaction in a medically supervised environment, starting with small amounts and progressively increasing the dose at regular intervals up to a cumulative dose corresponding to an age-appropriate portion of the food. As OFC involve exposure to the suspected food, they cause a degree of **anxiety for patients and families**, and can lead to **allergic reactions of unpredictable severity**, which may add to the negative experience of children and families of FA and enhance the recall response related to anxiety of encountering the food or create food aversion[13].

1.2. Current state-of-the-art of the diagnosis of food allergy

OFC are the most accurate test and are considered the gold-standard to diagnose FA[14, 15]. However, OFC carry the risk of acute allergic reactions of unpredictable severity and thus require a highly skilled multidisciplinary clinical team, sophisticated medical equipment needed to treat allergic reactions, including anaphylaxis, with access to intensive care support if required. Given the increasing prevalence and awareness about FA, there are long waiting lists for OFC, specialised services have difficulty in responding to demand and children often avoid foods for longer than necessary, which has, not only dietary implications, but also implications in terms of their social and family life and overall well-being. OFCs are also the gold-standard to confirm eligibility for new treatments and to monitor response to that treatment in children already known to be allergic.

In the majority of cases of suspected FA, to try and reduce the number of OFC, the diagnosis of FA is based on a combination of a history of allergic reactions and the documentation of allergen-specific IgE, using **SPT and/or specific IgE**[11, 16]. In the presence of a clear history of an immediate reactions to a specific food with symptoms that are typical of IgE-mediated FA, evidence of allergen-sIgE is enough to confirm the diagnosis. However, in the absence of a clear history of allergic reaction or of uneventful consumption of the suspected food, the results of SPT or sIgE may be difficult to interpret.

Specific IgE to individual allergen components can increase the specificity of sIgE testing compared to sIgE to allergen extracts. For instance, sIgE to Ara h 2 in peanut has higher diagnostic accuracy than peanut-sIgE. In a previous study, we showed that Ara h 2-sIgE had 91% accuracy compared to 82% for peanut-sIgE[17]. However, sIgE to components are only helpful for some foods (e.g. Ara h 2 in peanut and Cor a 9/Cor a 14 in hazelnut). Even for these foods, component-sIgE are not always conclusive – e.g. in the Healthnuts study, 18% of peanut allergic infants had undetectable sIgE to Ara h 2[18].

In cases in which SPT, sIgE to extracts and sIgE to allergen-components do not allow us to reach a conclusive diagnosis, children need to undergo OFC. Despite being the reference standard of choice, the OFC outcome is not absolute and involves 3% of false-positives, 3% false-negatives and 2-9% inconclusive results[19-21]. For all these reasons, the discovery and development of **new and better tests to support the diagnosis of FA** has been pursued over the years.

2. RATIONALE

2.1. Principles and science behind the novel biomarkers: BAT and MAT

Basophils and mast cells are the key drivers of food allergic reactions and anaphylaxis to foods. We have developed new tests that measure the reaction of mast cells and basophils by flow cytometry following stimulation with allergen, the BAT[22, 23] and the MAT[24, 25]:

- **BAT** uses fresh whole blood from patients added to allergen and antibodies in a test tube (**Figure 2**). The tube containing the allergic cells are then analysed one by one to estimate how many and how much express activation markers on their surface, CD63 and CD203c.
- **MAT** uses a human mast cell line (LAD2 cells) which are human mast cells grown in the laboratory to which patients' plasma is added in order to mimic the patients' own mast cells. Sensitised LAD2 cells are then stimulated with allergen or controls and analysed by flow cytometry to assess the expression of the activation marker CD63 on the cell surface, similar to what happens in the BAT.

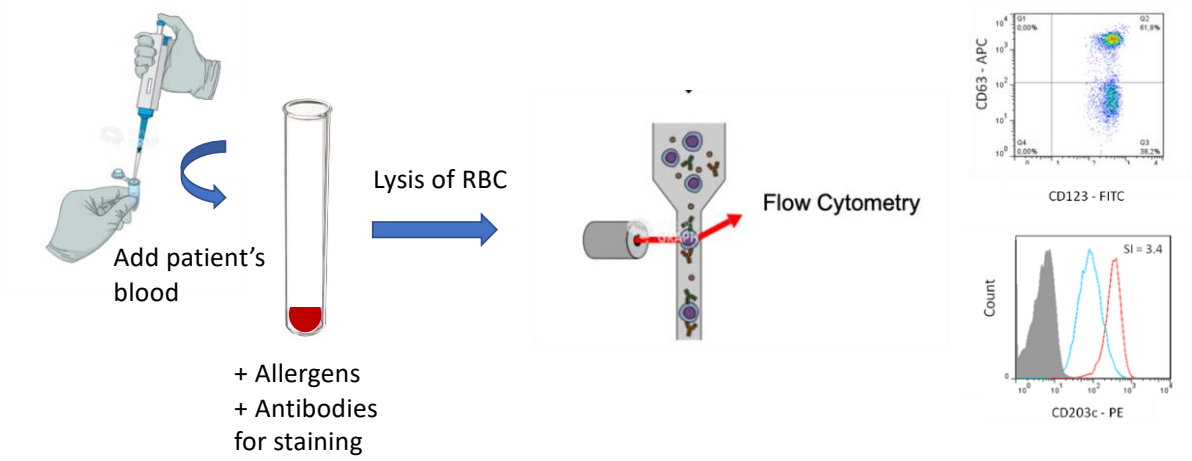


Figure 2 Schematic representation of the procedure for the basophil activation test (BAT). Representative results for a peanut allergic are shown for activation markers CD63 and CD203c on the surface of basophils, following stimulation with 10ng/ml of peanut extract (top figure and red line on bottom figure). Grey shade and blue line on bottom figure represent the isotype and negative controls for CD203c.

2.2. Clinical utility of BAT and MAT

BAT and MAT have shown to **discriminate very well between food allergic and non-allergic children** who test IgE+ (**Figure 3**). In a peanut study, BAT showed 98% sensitivity and 96% specificity to diagnose peanut allergy[17]. On an external validation cohort, the previously defined cut-offs showed 100% specificity and 83% sensitivity[17]. We have applied the BAT to peanut to the LEAP and related cohorts, which included 981 BATs, performed with the exact same methodology as previously. In the LEAP and associated cohorts, BAT had 98% specificity and 75% sensitivity to diagnose peanut allergy[26]. MAT has also shown a very high specificity (98%) to diagnose peanut allergy but lower

sensitivity (73%)[25]. When comparing the results of BAT and MAT, they showed a strong correlation (Rs=0.808).

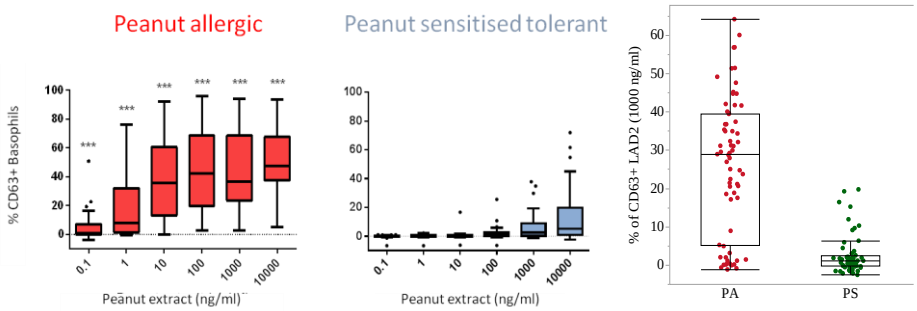


Figure 3. Basophil and mast cell activation (expressed as %CD63+ basophils and %CD63+ LAD2 cells, respectively) to peanut in peanut allergic children (PA) and children who were peanut sensitised (i.e. IgE+) but not allergic (PS)1, 2.

A positive BAT or MAT confirms FA and could dispense patients from OFC, who would otherwise react[22, 23]. The reduction in OFC would therefore be mainly a reduction in positive OFC, making the diagnostic work-up for FA accurate, safer and more comfortable for patients. Given that it is easier to perform SPT or sIgE, we propose that BAT and MAT are reserved for the cases with equivocal SPT and sIgE and that would otherwise be undergoing OFC. In a previous peanut study, used as a second step in the diagnostic work-up, BAT led to a 60% reduction in the number of positive OFC[17], which were OFC that resulted in allergic reactions. In the Pronuts study (NCT01744990), applying a similar two-step approach, **BAT allowed a reduction in the number of positive OFC that ranged between 50 and 75%**; specifically, 75% for cashew nut, 50% for sesame seed and 60% for peanut.

2.3. Proposed study

We propose to do a **prospective multicentre study in the UK using a biomarker-led study design** to compare the incidence of adverse events (defined as allergic reactions during OFC) in a **randomized-controlled trial** in which patients either follow the standard-of-care (i.e. OFC in case of equivocal SPT/sIgE) or follow a BAT based strategy, i.e. patients with a positive BAT are dispensed of OFC and patients with a negative BAT undergo an OFC (**Figure 1**)[27, 28].

Hypothesis: The incidence of allergic reactions during OFC is lower in the biomarker-based strategy arm compared to that of the standard-of-care arm.

Biomarkers: BAT and MAT have been developed and validated in our laboratory to diagnose peanut allergy, as part of research projects funded by the MRC, and we have been using them continuously since 2010. We studied the clinical utility of the BAT and MAT to peanut, as reflected in recent publications, and are currently doing the same for BAT and MAT to other common food allergens, such as cow's milk, egg, cashew and sesame (as part of the BAT 2 study, NCT03309488; https://bat2study.uk), which, together with peanut, constitute more than 90% of children's food allergies.. BAT results will be considered to decide whether a patient needs a challenge. If BAT is positive, patient won't undergo challenge, if BAT is negative or if the patient has non-responding basophils, a challenge is indicated.. MAT testing will not be used in real time as part of routine diagnostics and as part of the decision making to identify if a challenge is required. MAT will be performed in all subjects at a later date as part of batch testing so that we can meet the secondary outcome regarding the diagnostic accuracy of MAT in this cohort.

Food allergens: We plan to include patients being tested to peanut, cow's milk, egg, sesame and cashew nut, which are the foods to which we have defined optimal diagnostic cut-offs that can be applied following randomisation, for the cases assigned to the biomarker-based strategy arm of the study.

2.4. Assessment and management of risk

The OFC involves the risk of allergic reactions of unpredictable severity and can cause significant anxiety in patients and families. Skin prick test can potentially induce allergic reactions. These procedures will be undertaken in clinical facilities with the resources to treat allergic reactions of any severity, including anaphylaxis. The study procedures will be conducted as per routine clinical practice in the various centres. The risk is comparable with normal standard practice.

The study intervention, which is additional to current routine clinical practice, is to take the results of BAT into consideration to decide on the clinical management of the patient and whether or not the patient will undergo OFC. As all patients with a negative BAT will undergo OFC, the risk of misdiagnosis and accidental unexpected allergic reactions in the community is negligible. The potential risk is to over diagnose patients with a positive BAT, who will be dispensed from OFC and recommended allergen avoidance. However, this false positive rate is low (ca. 2%) and it does not expose the patient to risk of allergic reactions.

i.e. This study will not pose a higher than the risk of standard medical care.

3. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

This study is a biomarker-led UK multicentre randomised controlled trial assessing the clinical impact that incorporating BAT ± MAT in the diagnostic work-up for food allergy in children and young people.

3.1 Primary objective

- **Research question:** What is the change in the number of allergic reactions to the suspected food during OFC with the incorporation of the BAT (if BAT is inconclusive that we go ahead with a challenge) in the diagnostic work-up for food allergy in children and young people?
- **Hypothesis:** Including BAT in the diagnostic work-up for food allergy in children and young people reduces the number of positive OFC and therefore the number of allergic reactions to the suspected food, making the process as accurate, safer and more comfortable for patients.
- **Primary objective:** To determine the number of allergic reactions to the suspected food with the incorporation of the BAT in the diagnostic work-up for food allergy in children and young people compared to standard-of-care.

3.2 Secondary objectives

We will assess the number of OFCs to the suspected food with the incorporation of the BAT in the diagnostic work-up for food allergy in children and young people compared to standard-of-care.

We will assess the quality of life (QoL) of children/young people and their parents at the start and at the end of the diagnostic work-up and compare this between study arms.

We will also compare the cost-effectiveness of the approaches considered in each study arm, which will largely depend on the number of OFC in each group, in addition to the costs related to the performance of the biomarker.

Using the population of patients who underwent OFC in both arms of the study, we will calculate the sensitivity, specificity, PPV and NPV of the given cut-offs for BAT and MAT to each food, namely cow's milk, egg, peanut, sesame and cashew nut, in this new independent and prospectively recruited real-

life cohort of children with suspected food allergies. We will also validate the diagnostic performance of the other tests (i.e. SPT, sIgE to allergen extracts and components) and of the proposed diagnostic algorithm represented in **Figure 4**.

3.3 Outcome measures

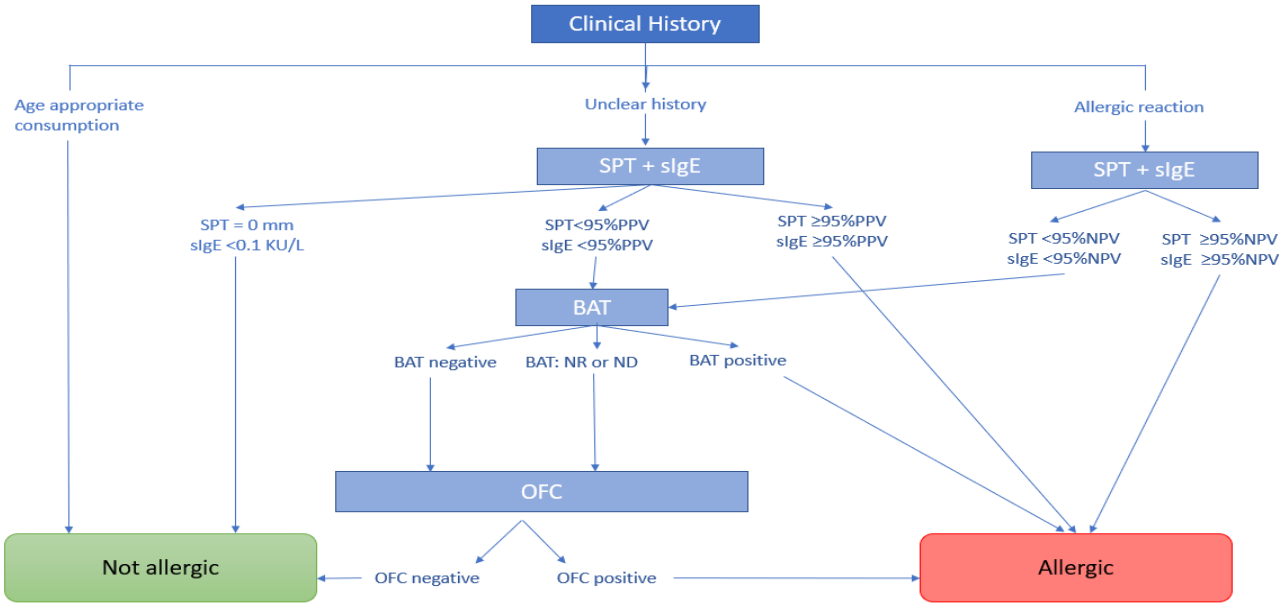


Figure 4. Proposed algorithm to diagnose food allergy including clinical history, skin prick test (SPT), specific IgE (sIgE), basophil activation test (BAT), mast cell activation test (MAT) and oral food challenge (OFC). 95% PPV refers to the cut-off with 95% positive predictive value and 95% NPV refers to the cut-off with 95% negative predictive value (for instance, for peanut allergy, the 95% PPV cut-off is 8mm for SPT and 15 KU/L for specific IgE and the 95% NPV cut-off is 3 mm for SPT and 0.10 KU/L for specific IgE). NR, non-responders; ND, not done.

The outcome endpoints are summarised in **Table 4**.

- **Primary endpoint:** number of positive OFC in the biomarker arm (in which the decision of whether to perform OFC is made based on the outcome of the BAT, or MAT if BAT inconclusive) compared with the standard-of-care arm (in which all subjects undergo OFC).

- **Secondary endpoints:**
  - QoL score for children and QoL score for parents at start and end of diagnostic work-up
  - Cost-effectiveness of the diagnostic work-up taking into account the performance of BAT/MAT and OFC.
  - Sensitivity and specificity of individual tests: BAT, MAT, SPT, sIgE to allergen extracts, sIgE to individual allergens in comparison with the gold-standard OFC
  - Sensitivity and specificity of the proposed diagnostic algorithm represented in figure 4 in comparison with the gold-standard OFC.

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure
Primary Objective	Proportion of positive OFC in the biomarker arm (BAT ± MAT) compared to the standard-of-care arm	Assessment during OFC to suspected food
Secondary Objectives	Number of OFC in the biomarker arm (BAT) compared to the standard-of-care arm	During diagnostic work-up
	QoL score as measured by standardised and validated questionnaire	Before and after diagnostic work-up
	Food allergy-specific anxiety questionnaires	Before and after diagnostic work-up
	Cost-effectiveness using NHS and societal costs using bespoke form	After diagnostic work-up
	Sensitivity and specificity of individual tests: BAT, MAT, SPT, sIgE to allergen extracts, sIgE to individual allergens compared with the gold-standard OFC	At the time of diagnostic work-up done in the study
	Sensitivity and specificity of the proposed diagnostic algorithm represented in figure 4 compared with the gold-standard OFC	At the time of diagnostic work-up done in the study

Table 4. Summary of outcome measures.

4. TRIAL DESIGN

This is a randomised controlled trial designed to assess the impact of including BAT in the diagnostic work-up for food allergy on the number of children experiencing allergic reactions as part of OFCs.

We have opted for a parallel group design, in which each group of participants is assessed for food allergy, including or not including the new biomarkers BAT ± MAT.

The primary outcome is the proportion of positive OFC in the biomarker arm compared with the standard-of-care arm.

5. TRIAL SETTING

This is a multicentre trial, in which 10 different specialised Allergy services across the UK will act as recruiting centres (**Table 5**). All samples will be shipped to the Santos Lab at King's College London, where specific IgE and BAT will be performed. Although MAT will be carried out in batch testing at a a later stage of the study, only BAT will be used as part of routine diagnostics and will be critical in the decision making of needing an oral food challenge.

Location	Centres	Partners
Birmingham	Sandwell and West Birmingham Hospital	Dr Nick Makwana
Cambridge	Addenbrookes Hospital	Dr Zaraquiza Zolkipli
Leicester	Leicester Royal Infirmary	Dr Gary Stiefel
London A	Evelina London Children's Hospital	Dr Alexandra Santos
London B	University College London Hospital	Dr Penny Salt
Manchester	Royal Manchester Children's Hospital	Dr Vibha Sharma
Newcastle	Great North Children's Hospital	Dr Louise Michaelis
Sheffield	Sheffield Children's Hospital	Dr Eleanor Minshall
Southampton	University Hospital Southampton	Prof Graham Roberts
Glasgow	Royal Hospital for Children	Dr George Raptis

Table 5 Recruiting centres in the study and lead clinician at each centre

6. PARTICIPANT ELIGIBILITY CRITERIA

The eligibility of participants will be determined according to the criteria listed below.

6.1 Inclusion criteria

- Children and young people aged 6 months to 15 years
- Suspected allergy to one of the study foods (peanut, cow's milk, egg, cashew, sesame) – defined as:
    - history of clinical reaction **or**

- evidence of IgE sensitisation (SPT>0mm and/or specific IgE>=0.10 KU/L) to the respective food **or**
- reassessment for possible resolution of allergy to the specific food following previous diagnosis of food allergy

- Need for an OFC to the study food
- OFC to reach amount of food protein in a typical portion size for child's age (
- **Table** 6**)**
- Consent from adults with parental responsibility and assent from children and young people in an age-appropriate form.

6.2 Exclusion criteria

- Clinically significant chronic illness other than atopic diseases;
- Previous history of severe life-threatening reaction to the suspected food with documented decrease in oxygen saturation (<90%), hypotension (≥20% reduction in systolic blood pressure) and/or admission to intensive care;
- Unwillingness to comply with study procedures, namely to undergo a diagnostic food challenge;
- Contra-indication for diagnostic food challenge, namely:
  - Uncontrolled atopic diseases (e.g. eczema, asthma, rhinitis);
  - Chronic medical conditions that pose significant risk in the event of anaphylaxis or treatment of anaphylaxis (e.g. cardiac disease, severe lung disease, pregnancy, mastocytosis);
  - Inability to discontinue medications that might interfere with assessment or safety (e.g. antihistamines, β-agonists, β-blockers, NSAIDs, ACE inhibitor, antacids);
  - Recent (within 7-14 days) treatment with systemic steroids or prolonged high-dose systemic steroids or immunosuppressants;
- Undergoing treatment or currently taking part in studies with omalizumab, ligelizumab, dupilumab, food or inhalant allergen immunotherapy or other systemic immunomodulatory treatment;
- Inability to stop anti-histamines prior to SPT or OFC.

Food	Approximate protein content of minimum cumulative dose (g)	Approximate quantity of food to meet minimum cumulative dose
Fresh cow's milk	3.5g	100ml
Cooked egg	7.0g	1 egg (+/- 50g)
Peanut	2.0g	8g (7 whole) peanuts or 8g (1 teaspoon) of peanut butter*
Cashew	2.0g	11g (11 whole) cashew nuts or 10g (1.3 teaspoons) of cashew nut butter**

Sesame	2.0g	8g (1.3 teaspoons) of tahini paste***

Table 6 The minimum cumulative dose, in protein content and quantity of food, to be used as reference in the oral food challenge. Oral food challenge will be performed as per local NHS procedures.
**Assuming whole unroasted peanuts are 26g protein/100g and peanut butter is 25g protein/100g and 1 teaspoon of peanut butter is 8g*
***Assuming whole cashew nuts are 18.2g protein/100g and cashew nut butter is 20g protein/100g and 1 teaspoon of cashew nut butter is 8g*
****Assuming 26g protein/100g in tahini paste and one teaspoon of tahini paste is 6g*

7 TRIAL PROCEDURES

For a detailed schedule of procedures, see **Appendix 2**.

7.1 Recruitment

Children and young people aged 6 months to 15 years with suspected allergy to cow's milk, egg, peanut, cashew nut or sesame, requiring an OFC will be recruited from 10 different specialised Allergy services across the UK. Participation requires signed consent from adults with parental responsibility and assent from children and teenagers in an age appropriate form.

Potential participants will be identified by the NHS clinical team from the outpatients' clinic lists at the recruiting centres. The clinician assessing the patient in clinic will confirm the eligibility.

Information on participants who are eligible and invited to participate in the study but not randomised will be collected on a screening log, including:

- food they were being assessed for,
- the reason not eligible for trial participation, or if they are eligible but declined.
- recruiting centre

7.2 The randomisation scheme

Patients will be randomised using a 4:5 allocation between standard-of-care/biomarker arms. Stratified randomisation will be adopted according to recruiting centre, using a web-based allocation system.

7.3 Blinding

The laboratory research assistant performing BAT and MAT will be blinded to the identifiable information and the clinical features of the study participants.

The clinical team will be blinded as to the results of the BAT. They will only be informed as to whether an OFC is or is not required.

The OFC will be performed as per standard clinical care with a minimum cumulative dose of food protein detailed in **Table 6**. Only if in the case of subjective symptoms, psychological issues and equivocal outcome of OFC as a result, DBPCFC will be considered, as per standard clinical care.

7.4 Data collection

All participants will undergo clinical assessment, skin prick testing, blood testing (specific IgE BAT. and MAT). Participants in the standard-of-care arm, and those in the Biomarker arm with a negative BAT result, will undergo an OFC. In addition, all participants will fill in a FFQ, a QoL questionnaire and anxiety questionnaires at the start and end of the diagnostic work-up. A cost-effectiveness questionnaire will also be filled in per study participant.

At the baseline visit, the clinical data, including history and SPT results, will be collected electronically using online forms.

7.5 Trial assessments

Clinical History	A food allergy-focused history will be collected using a previously validated questionnaire using an online platform. The clinicians will be able to fill in the form in real-time, while seeing the patients in clinic. The results of SPT and any previous specific IgE will also be recorded in this form.
FFQ1	An electronic food-frequency questionnaire to the respective food will be collected to document the consumption of the food prior to the diagnostic assessment.

QoL1	Parents of children younger than 12 years will complete the FAQLQ-parent, and children aged 12 and above will fill in the FAQLQ-teen.
Anxiety 1	We will assess general as well as procedure-related parental and patient anxiety ahead of diagnostic procedures using the following questionnaires: STAI-CH in children, STAI-AD in parents, RCADS in children, and HADS in parents, BMI-C in children, and BTMS in children**.** Both BTMS and BMI-C questionnaires should NOT be completed by the parents and are ONLY to be completed by patients aged 12-15 years.
Costs 1	NHS and societal costs of food allergy testing will be considered and resource use data for each test will be considered using a bespoke form. All parents and clinicians will complete relevant forms prior to the diagnostic assessment.
Resource Use	NHS resource use for each child's food allergy will be captured using a bespoke form. All physicians will complete this prior to the diagnostic assessment.
SPT	SPT will be performed to the culprit food as well as positive control (histamine) and negative control (saline) in each clinic where the patient is seen as per standard of care and the result will be recorded on the same electronic form.
Specific IgE	The results of specific IgE done in clinic and used to make the decision on whether to do an OFC will also be recorded in the same electronic form.
Blood collection for Biomarkers BAT and MAT	**Blood sample** will be collected before the OFC (as close to the OFC as possible, maximum of 12 months) and sent to the Santos Lab at KCL for BAT the suspected food and for specific IgE testing. Although the time window between blood collection and the OFC is a maximum of 12 months, OFC should ideally be done as close to the 6 months' as possible. **Specific IgE testing** to the suspected food will be performed by ImmunoCAP (Thermofisher). MAT will be done using stored samples at a later stage of the study. **BAT and MAT** will be performed according to the methodology established in the Santos Lab and used in previous studies[17, 25, 26]. These results shall NOT be communicated to the clinical team (blinding).
Procedure in the Biomarker arm	Following blood collection, each participant will be randomly allocated to the biomarker arm, or the standard-of-care arm.

	If the study participant is allocated to the Biomarker arm, BAT will be used to decide about whether the participant will undergo an OFC: - positive BAT will dispense from OFC; - participants with negative or inconclusive BAT will undergo OFC.
Procedure in the Standard-of-care arm	All study participants allocated to the Standard-of-care arm will undergo OFC.
OFC	OFC will be performed as per standard clinical care and needs to reach amount of food protein in a typical portion size for child's age. The outcomes of OFC will be recorded online by the clinical teams. OFC should be no longer than 1 year after the blood collection.
FFQ2	An electronic food-frequency questionnaire to the respective food document allergic reactions or continued consumption of the food, depending on the outcome of diagnostic work-up. The FFQ2 should be completed ideally 2 months after OFC/BAT, and no later than 6 months.
QoL2	Electronic QoL questionnaires will be filled in by parents and young people similar to the one that was filled in at the start of the diagnostic work-up. Parents of children younger than 12 years will complete the FAQLQ-parent, and children aged 12 and above will fill in the FAQLQ-teen. This should be completed ideally 2 months after OFC / BAT, and no later than 6 months.
Anxiety 2	We will reassess general as well as procedure related parental and patient anxiety after the diagnostic procedures using the following questionnaires: STAI-CH in children, STAI-AD in parents, RCADS in children, and HADS in parents**,** BMI-C in children, and BTMS in children**.** Both BTMS and BMI-C questionnaires should NOT be completed by the parents and are ONLY to be completed by patients aged 12-15 years. This should be completed ideally 2 months after OFC / BAT, and no later than 6 months. ..
Costs 2	NHS and societal costs of food allergy testing will be considered and resource use data for each test will be considered using a bespoke form. All parents will complete this ideally 2 months after OFC / BAT, and no later than 6 months.

Table 7. Description of the assessments planned as part of the study.

7.6 Follow-up assessments

There are no planned follow-up assessments beyond the questionnaires indicated in **Table 7** above.

7.7 Withdrawal criteria

Study participants may withdraw at any time without giving reasons. If the study visits have been completed, the data can be used after withdrawal in the case that specific consent has been provided.

If the study visits have not been completed (namely BAT/and OFC, if applicable) at the time of withdrawal, the participant needs to be replaced.

7.8 Collection, Transportation, Storage and Analysis of clinical samples

Blood will be collected to the following tubes:

- Gold top Vacutainer tube containing clot activator and gel for serum separation for specific IgE;
- Green top Vacutainer tube containing lithium heparin for BAT;
- Blue top Vacutainer tube containing citrate for peripheral blood mononuclear cell (PBMC) and plasma separation and storage. Plasma will be used for MAT.

Venous blood collection methods will be as per local NHS procedures.

Maximum volumes of blood collected per age group are indicated in **Table 8**

Tube	**Volume of blood per age group (ml)**			
	6M-2Y	2-5Y	5-10Y	10-15Y
Serology tube	4	4	4	4
Lithium heparin tube	4	4	4	4
Citrate tube	4	12	22	42
Total volume (ml)	12	20	30	50

Table 8 Volume of blood collected per age group

Samples will be collected at each individual site and transferred to the KCL Santos Lab based at Guy's campus within 24 hours using a private courier service.

Samples will be stored at the KCL Santos Lab based at Guy's campus during the study and beyond, according to the use consented by study participants. All patients are to be tested on specific IgE, BAT and MAT. Additionally, individual participants will be given the option to consent for other future laboratory assays to explore the immune response to food allergens in allergic and tolerant children.

BAT will be performed within 24 hours of blood collection. Serum and plasma will be stored at -80C and PBMC will be stored in liquid nitrogen until use.

All samples will be appropriately labelled in accordance with the trial procedures to comply with the 2018 Data Protection Act. Biological samples collected from participants as part of this trial will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act and the 2006 Human Tissue (Scotland) Act.

8 STATISTICS AND DATA ANALYSIS

8.1 Sample size calculation

We expect about 20% positive OFC in the standard-of-care arm and about 7% positive OFC in the biomarker-based arm. Group sample sizes of 159 in the non-biomarker group and 159 in the BAT negative group achieve 90% power to detect a difference between the group proportions of 0.14. We expect 20% of the participants in the biomarker-based arm to be BAT positive, 40 participants will therefore be added to the total sample size.

The test statistic used is the two-sided Fisher's Exact Test with a significance level of 5%. Considering the possibility of 10% drop-outs, the total number of participants is 398.

The study design is independent of the foods the patients are being tested to, because it focuses on the impact in terms of number of positive OFC, and thus the power calculation was done taking into account all the 5 foods together.

8.2 Statistical analysis plan

Qualitative variables will be compared between the 2 arms of the study using the Fisher Exact test or Chi-Square tests, and continuous variables will be compared using the Mann-Whitney U test, the Kruskal-Wallis test or a t-test, as appropriate.

The primary outcome of the study, i.e. the proportion of positive OFC between the standard-of-care arm and the participants with negative BAT in the biomarker arm, will be determined using proportion Test (Chi2 or Fischer exact test if rates <5%).

The proportion of positive OFC will be calculated using the number of participants who developed an allergic reaction in each study arm as numerator and the number of participants who underwent OFC in each study arm as denominator.

	Biomaker-based (e.g. biomarker negative group)	Standard care	Total
OFC positive			
OFC negative			
Total			

The proportion of OFC will be calculated using the number of participants who underwent OFC as in each study arm as numerator and the total number of participants in that study arm as denominator.

The diagnostic accuracy of BAT and MAT, SPT, sIgE to allergen extracts and sIgE to allergen components, and of the proposed algorithm will be determined in comparison with OFC and sensitivity, specificity, predictive values, likelihood ratios, and accuracy will be calculated.

A secondary analysis to test for non-inferiority of the biomarker-based arm will be performed using a proportion test. For which the biomarker-based arm will include both the biomarker positive and biomarker negative participants. Assuming all biomarker positive participants are also OFC positive.

Statistical analyses will be performed with SPSS 20.0 and STATA 12.1 for Windows. Significance will be determined using a 2-sided a level of 0.05.

8.3 Procedures to account for missing or spurious data

Only participants with non-missing OFC test results will be included in the primary analysis. Participant characteristics will be presented by arm in a table and checked for balance. No imputation methods will be performed.

9 DATA MANAGEMENT

9.1 Data collection tools and source document identification

Clinical data about the history, SPT and the outcome of OFC will be filled in in real-time as the patient is assessed by the various clinical teams at the recruiting centres using online forms. The data will be automatically exported to the REDCap database licensed to KCL Division of Health and Social Care Research.

Laboratory data including the results of specific IgE, BAT and MAT will not include any patient identifiable information but only the study subject ID and will be stored at KCL throughout the study.

The clinical and laboratory databases will be merged at the end of the study prior to data analyses.

9.2. Access to Data

The Chief Investigator and the KCL Study Team will have access to the study data. Access will be granted to the clinical teams, health economist and statistician, as appropriate. Research data will be pseudo anonymised outside of the direct care team.

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections- in line with participant consent. This may include access to medical records as required and participants will be informed of this in the participant information sheet.

Each site will archive their consent forms and identifiable data locally. If archiving costs are applicable, this will be funded by the FARE grant.

10 ETHICAL AND REGULATORY CONSIDERATIONS

10.1 Research Ethics Committee (REC) review& reports

This study will receive ethical review and approval. Before the start of the study, approval will be sought from a REC for the trial protocol, informed consent forms and other relevant documents.

Substantial amendments that require review by REC will not be implemented until the REC grants a favourable opinion and will also be reviewed and accepted by the R&D department and HRA before they can be implemented in practice at the different sites.

All correspondence with the REC will be retained in the Trial Master File/Investigator Site File.

An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended.

The Chief Investigator will notify the REC of the end of the trial. If the trial is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination. Within one year after the end of the trial, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC.

10.2 Peer review

The study was reviewed by two external experts commissioned by the funder FARE, prior to grant award. The study protocol was reviewed by additional experts and the Trial Management group.

10.3 Public and Patient Involvement

The study was reviewed by nine parents of children of varying ages who have food allergies. The Study Coordinator had a thirty minute discussion with each parent, to obtain their views and experiences of OFCs and blood tests. Parents were shown a simplified version of the Trial Flow Chart to understand any concerns or questions that potential participants might have about the study. Parents were overall very positive about the design of the study and the research objectives, and could see the value of conducting the research. Specific elements of the design, such as the recruitment process and the completion of questionnaires, were based on parents' preferences. Parents' feedback was also taken into consideration when creating the parent information sheets and consent forms.

10.4 Protocol compliance

Protocol non-compliances are departures from the approved protocol.

Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations on Clinical Trials and must not be used.

Accidental protocol deviations must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately.

Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

10.5 Notification of Serious Breaches to GCP and/or the protocol

A "serious breach" is a breach which is likely to effect to a significant degree the safety or physical or mental integrity of the participants of the trial; or the scientific value of the trial.

The sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase. The sponsor of a clinical trial will notify the licensing authority in writing of any serious breach of the conditions and principles of GCP in connection with that trial; or the protocol relating to that trial, as amended from time to time, within 7 days of becoming aware of that breach.

10.6 Data protection and patient confidentiality

Patient confidentiality will be maintained throughout the study.

All investigators and trial site staff must comply with the requirements of the Data Protection Act 2018 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

The pseudo-anonymisation spreadsheet relating to patient IDs and personal information will remain locally at each site, and will only be accessible by the local NHS study team. KCL will not have access to this nor will KCL have access to any identifiable information. Each site is to create and maintain the spreadsheet which is to be password protected and saved in a shared drive only accessible by the paediatric allergy research team.

Personal information is collected using REDCap database licensed to KCL Division of Health and Social Care Research and stored in the respective cloud provider. All participants' identifying information is replaced by an unrelated sequence of characters in all forms and sample tubes in the laboratory. The linking code will be kept in separate locations from the samples using encrypted digital files within password protected folders and storage media. Access will be granted to the core study

team and representatives of the co-Sponsors at King's College London and Guy's and St Thomas' NHS Foundation trust team as necessary for quality control, audit, and analysis and participants will be informed of this in the participant information sheet..

The confidentiality of data will be preserved when the data are transmitted to sponsors and co-investigators.

The data will be stored for 25 years after the end of the study. The Chief Investigator is the data custodian.

End of study is defined as the date of the last visit of the last participant – this will include completion of the 2-month post-diagnostic questionnaires.

10.7 Future use of samples

With permission from the participant's parent(s), stored samples may be used in future allergy research studies and genetic testing. This may involve sending samples to either a national or international commercial company/laboratory based within the UK or outside of the UK. These samples will only be used for research that has been approved following scientific and ethical review. The storage of samples for additional research is optional. Permission will be obtained in the consent form. However, parent(s) can withdraw their consent at any time, and the samples will be destroyed. Residual blood samples will be stored for 25 years.

10. 8 Financial and other competing interests

The conflicts of interest for the Chief Investigator and the study committees will be collected and archived in the Study Folder.

10.9 Insurance and Indemnity

The study is co-sponsored by King's College London (KCL) and Guys and St Thomas' NHS Foundation Trust (GSTT). The sponsors will, at all times, maintain adequate insurance in relation to the study. KCL through its' own professional indemnity (Clinical Trials) and no fault compensation and the GSTT having a duty of care to patients via NHS indemnity cover, in respect of any claims arising as a result of negligence by its employees, brought by or on behalf of a study participant.

11 DISSEMINATION POLICY

The results of the trial will be published in peer-reviewed journals. Summary of the results will be disseminated to study participants in a study newsletter.

The Chief Investigator and the co-Sponsor's King's College London and Guy's and St Thomas' NHS Foundation Trust own the data arising from the trial. On completion of the trial, the data will be analysed and tabulated and a Final Trial Report prepared. Any publication of the trial data will need to involve and be reviewed at least one month prior to submission and approved by the Chief Investigator. The participating investigators will not have the right to publish parts of the study data separately without prior approval of the Chief Investigator.

The funder will be acknowledged in all publications but will not have review and publication rights of the data from the trial.

Guidelines on authorship will follow the authorship criteria of The International Committee of Medical Journal Editors for manuscripts submitted for publication. No professional medical writers will be hired.

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13. APPENDICES

13.1 Appendix 1 - Trial management / responsibilities

13.1.1 Patient registration/randomisation procedure

Patients will be registered and randomised using a web-based registration and randomisation tool monitored by the data management team.

13.1.2 Data management

The KCL Trial Coordinator will be responsible for management of clinical data, namely checking CRF and export to the study database and addressing any queries from the study site teams. The KCL Lead Lab Technician will be responsible for management of the laboratory data, namely making multiple copies of the data, ensuring appropriate record keeping and maintenance of the lab study database.

13.1.3 Amendments and annual reports

The KCL Trial Coordinator will be responsible for preparing and submitting amendments and Annual Safety Report to the REC.

13.2 Appendix 2 – Schedule of Procedures

Study procedures	**Visit 1**	**Visit 2**	**Visit 3**	**-**
	Clinic	**Bloods**	**Challenge**	**2 month Post-challenge**
Informed consent	X			
Demographics	X			
Medical history	X			
Physical examination	X			
Skin prick testing	X			
FFQ	X			X
QoL questionnaires	X			X
Anxiety questionnaires	X			X
NHS and societal cost questionnaires	X			X
Resource use questionnaire	X			
Blood collection	X*	X*		
Oral food challenge			X	

**Blood collection will be done on Visit 1 or Visit 2 depending on availability of phlebotomy services at site or patient's convenience.*