# Systematic review protocol to identify clinically relevant allergens

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## Abstract

In silico methods are an integral part of the allergenicity risk assessment process, both for novel foods and newly expressed proteins in Genetically Modified Organisms (GMOs) for food use. They help to identify novel proteins that have the potential to cause cross-reactive food allergies in the existing allergic population. Many of the tools developed rely on databases of curated allergen sequences but the curation process does not necessarily consider whether an allergen is clinically relevant and plays a role in triggering an IgE-mediated reaction. In order to fill this gap in our knowledge, a systematic review of allergens identified in a range of foods is planned. The foods include those for which food allergen labelling is mandated in the European Union and the UK, other foods such as fruit, which are known to cause IgE-mediated food allergies in at least 0.5% of the European population and foods of low allergenicity such as rice. The approach taken includes a Population-Outcome (PO) approach in order to address the primary research question “What scientific knowledge (evidence) is there that clinical manifestation(s) of IgE-mediated allergic reaction(s) are caused by ingestion of a food?”. A modified Population-Exposure-Comparator-Outcome (PECO) approach will be taken to address the secondary research question “Which food protein molecules are recognised by serum-IgE from individuals allergic to foods (identified by addressing the primary question) and are responsible for causing an IgE-mediated adverse reaction to those foods?” A protocol for executing the systematic review has been developed together with grading criteria and risk of bias analysis. The research process will be fully documented to allow the search to be assessed and reproduced as per PRISMA guidelines.

## 1. Background

Immune-mediated adverse reactions to food are almost entirely caused by exposure to protein components in foods. A classification of adverse reactions to foods undertaken by FAO-WHO recently identified the T-cell mediated gluten intolerance syndrome known as coeliac disease and IgE-mediated food allergies, as two major conditions of public health concern (FAO-WHO).

Foods and molecular triggers of coeliac disease are well characterised as the seed storage prolamins of cereals containing gluten (wheat, rye, barley, oats). A suite of peptide sequences, known as coeliac toxic motifs, have been defined (Sollid et al., 2020) that are able to bind to the human leukocyte antigen (HLA) DQ receptor (HLA-DQ) on antigen presenting cells in susceptible individuals. They present the peptide sequence to gluten reactive CD4+ T cells, triggering pro-inflammatory cytokine release and causing symptoms associated with CD (Sollid et al., 2020).

IgE-mediated food allergies are almost entirely triggered by proteins, known as allergens. Many different proteins have been recognised as allergens, the majority of which were identified - some years ago - as belonging to a relatively restricted number of protein families (Jenkins et al., 2005, Jenkins et al., 2007). However, experimental data demonstrating that proteins have the capacity to bind IgE, is of highly variable quality. For example, the 60S ribosomal protein from almond (Prunus dulcis) has been characterised as binding serum IgE from almond allergic subjects based on an immune-dot blot of protein produced using a cDNA expression library from pooled sera from a poorly described patient population (Abolhassani and Roux, 2009). Similarly, the profilin allergen from peanut, Ara h 5, was also identified by screening a cDNA expression library using serum IgE from peanut allergic patients. However, it is not found in peanut seed using proteomics approaches and, hence, not as such a relevant food allergen (Johnson et al., 2016). This contrasts with the level of detail and data quality available for the peanut allergens Ara h 2 and Ara h 6, where the importance of post-translational modification of hydroxy-proline for IgE-binding is acknowledged (Bernard et al., 2015). Furthermore, the clinical significance of Ara h 2 and Ara h 6 has been established, specific IgE to these proteins are markers of clinical allergy to peanut in many patient populations, in contrast to the birch pollen homologue, Ara h 8, which is more frequently associated with tolerance (Nicolaou et al., 2011, Ballmer-Weber et al., 2015, Asarnoj et al., 2012).

Such observations indicate there is a pressing need to identify clinically relevant allergens to support effective risk assessment of novel foods and genetically modified organisms (GMOs) regarding both IgE- and non-IgE-mediated adverse reactions (EFSA Panel on Genetically Modified Organisms et al., 2017). In silico methods have proven useful in the risk assessment process, helping to identify novel proteins that have the potential to cause cross-reactive food allergies. Such cross-reactive allergies have been well established for tree nuts, the concordance of walnut and pecan nut allergies, like that of pistachio and cashew, being very high. This reflects the close phylogenetic relationships between these tree nut species and underlying extensive sequence similarity and shared IgE-epitopes of the allergen molecules (Brough et al., 2020, Nesbit et al., 2020). Similar cross-reactive allergies exist between pollens and foods, sensitisation to the major birch pollen allergen, Bet v 1, being associated with development of IgE-mediated food allergies to a variety of fresh foods, notably fruits from the Rosacae family. These cross-reactive allergies again result from the sequence similarity and shared IgE-epitopes between Bet v 1 and its homologues in foods. Thus, the application of bioinformatic methods using multiple sequence alignments to characterise the levels of homology between novel proteins and known food allergens provides a well-founded approach to assessing the likelihood that a novel protein could act as a cross-reactive allergen and hence pose a risk to the existing allergic population (Poulsen, 2004). However, the

risk assessment process is much less certain in predicting which food proteins are likely to give rise to new food allergies, often termed de novo sensitisation. In part, this is because there is a lack of effective predictive animal models, and those that are available have widely acknowledged limitations. Such shortcomings are compounded by incomplete understanding of mechanisms whereby individuals become allergic.

Deployment of in silico comparisons of novel proteins and allergens has led to the compilation of several allergen databases although these face ongoing issues of data curation and updating as well as financing (Radauer and Breiteneder, 2019). Whilst some databases, such as the WHO/IUIS Allergen Nomenclature database (www.allergen.org), have clear and strict rules on data quality/ evidence required to designate a protein as an allergen (Sudharson et al., 2021), this is not the case for many others (Radauer and Breiteneder, 2019). There has also been an emphasis on identification of allergen molecules, whilst little attention has been given to characterisation of food proteins that could be identified as potential hypoallergens. Identifying allergenic comparators has the potential to provide a much-needed benchmark against which allergenic potential of novel proteins can be evaluated. Despite the importance of identifying clinically relevant allergen sequence sets to support assessment of in silico and experimental approaches for allergenicity risk assessment, even highly curated allergen sequence databases, such as WHO/IUIS and allergen-online (www.allergenonline.org), do not specify which allergens are the most clinically relevant. Thus, currently, the lack of a curated database of allergens with differing allergenic potentials is hampering development of improved methods in silico and in vitro for allergenicity risk assessment.

We propose to undertake a systematic review of the literature to assess the strength of evidence supporting identification of clinically relevant food allergens to support development of improved in silico and in vitro methods for allergenicity risk assessment. Systematic review of food hazards, such as allergens, is relatively novel. However, it is recognised that protocols used in medicine need to be adapted to support evidence-based toxicology (Stephens et al., 2016). A protocol has been developed based on a previously published method for identification of clinically relevant tree nut allergens (Javed et al., 2017) which was based on approaches to identify allergenic foods of public health importance (Bjorksten et al., 2008, Houben et al., 2016, van Bilsen et al., 2011), systematic review guidance provided by the European Food Safety Authority (EFSA, 2010), drawing on approaches established in healthcare (Higgins and Green, 2011, CRD, 2009) and PRISMA (Moher et al., 2009). This new protocol has been broadened to include both the priority food allergens which must be labelled on food products within the European Union and the UK together with other allergenic foods and potentially emerging food allergens.

## 2. Approach

The systematic review will address the following questions:

> **PRIMARY QUESTION**: "What scientific knowledge (evidence) is there that clinical manifestation(s) of IgE-mediated allergic reaction(s) are caused by ingestion of a food ?"
>
> **SECONDARY QUESTION**: "Which food protein molecules are recognised by serum-IgE from individuals allergic to foods (identified by addressing the primary question) and are responsible for causing an IgE-mediated adverse reaction to those foods?"

These questions will be addressed using a Population-Outcome (PO) with a modified Population-Exposure-Comparator-Outcome (PECO) approach (Figure 1; Table 1) as originally developed by Javed and co-workers (Javed et al., 2017). The PO approach (Figure 1) will be used to answer the primary question where P represents the population evaluated for an IgE-mediated allergy to food and the outcome (O) or condition of interest, in this case whether an individual has an IgE-mediated allergy to one of the selected foods (Table 1). As described in Javed et al. (2017), the population will be drawn from prospective cohort studies, longitudinal cohorts, or cross-sectional studies and case series. The outcome will be graded for quality of diagnosis (test accuracy), based on principals described in EAACI Food Allergy Guidelines (Muraro et al., 2014, Soares-Weiser et al., 2014) and criteria proposed by Bjorksten et al. (Bjorksten et al., 2008). The quality assessment builds on the following clinical definition of an individual having an IgE-mediated food allergy (Grabenhenrich et al., 2017, FAO-WHO, 2022) where they must have:

1. Symptoms including any of the following:
    a. Skin: Itching (pruritus) or tingling (paresthesia) in the mouth, lips, ears or throat; Swelling of the eyes, lips, or mouth; Nettle sting like rash or itchy skin, or red rash (urticarial rash, flush, erythema); angioedema
    b. Alimentary tract: blisters of the oral mucosa; dysphagia; hoarseness or swelling of throat; diarrhoea (other than food poisoning); vomiting (other than food poisoning); stomach cramps; nausea; bloating
    c. Respiratory tract: a runny, stuffy nose, or sneezing; red, sore, or running eyes; cough, wheeze, chest tightness, or breathlessness (dyspnea); laryngeal oedema; dysphonia; reduced peak expiratory flow/drop in FEV1; silence (in lung auscultation); cough
    d. Cardiovascular/neurological: Headache; anxiety; tiredness; fainting or dizziness; hypotension/drop of blood pressure; change in consciousness; seizures; change in heart rate/tachycardia; uterine cramps
2. Symptom onset occurring within 2h of consuming an offending food
3. Evidence of sensitisation to food established through skin prick testing and/or serum specific IgE testing.

This approach will allow the evidence that patient populations experience adverse reactions to food that are caused by an IgE-mediated mechanism to be assessed (i.e., diagnostic "test" accuracy) and allow patients to be classified into four groups as follows:

1. Non-IgE-mediated adverse reaction
2. Possible IgE-mediated adverse reaction (symptoms and time of onset only but no evidence of sensitisation)
3. Probable IgE-mediated adverse reaction (symptoms and time of onset and evidence of sensitisation to the same food)
4. Confirmed IgE-mediated adverse reaction (symptoms and time of onset and evidence of sensitisation to the same food confirmed by an oral food challenge)

The outcome is a population [P] with either a probable or a confirmed IgE-mediated food. allergy.

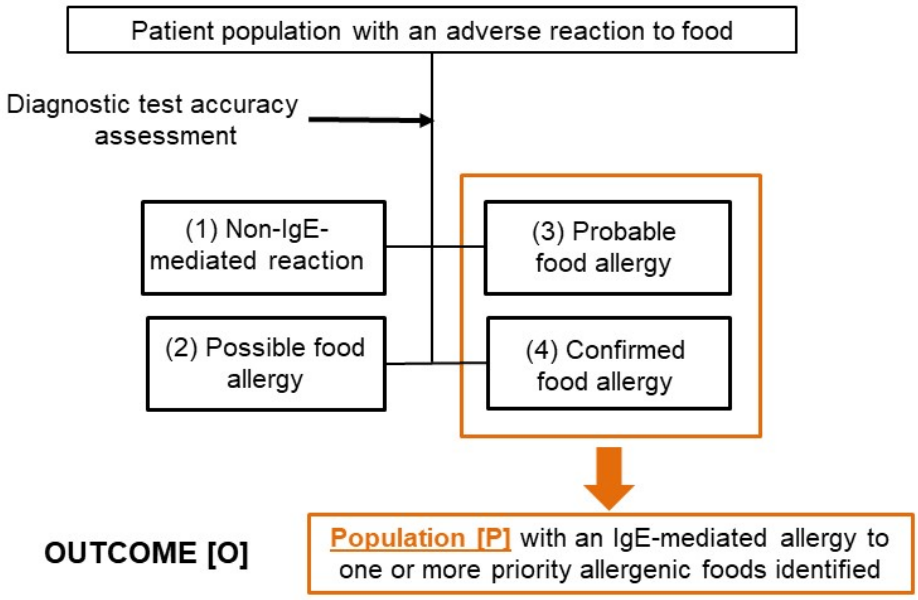


***FIGURE 1: Framework for addressing the primary research question using a Population-Outcome (PO) approach.***

Subsequently, a modified PECO approach (Figure 2, Table 1) will be used to address the secondary question. In this approach, the population (P) is that identified by addressing the primary question i.e., individuals classified as having either a probable or confirmed food allergy. The exposure [E] is taken as the contact/consumption of allergenic food protein molecules as determined by detection of food-specific serum IgE. The capacity of a given protein molecule (or derived fragments and peptides, as used in epitope mapping studies) to bind IgE can be determined by different methods such as immunoassay (e.g. enzyme-linked immunosorbent assay (ELISA)) or immunoblotting (where proteins are transferred from a gel after electrophoretic separation onto a membrane prior to detection using an immunological method) (Towbin and Gordon, 1984). In addition, cell-based assays, can be used to determine the capacity of a molecule to stimulate an effector cell (such as a basophil or a mast cell) sensitised with serum IgE from food allergic subjects, to release inflammatory mediators. In vivo assessments of IgE binding capacity can be performed

using skin prick testing with purified allergenic food proteins, although such studies are rare because of the regulatory requirements for the allergen preparations used in such analysis.

As described by Javed et al. (2017), in the PECO analysis test accuracy will be assessed in terms of the quality of IgE binding studies together with that of the allergen. Comparison (C) is made with regards prevalence of sensitisation to the different food proteins in the population (P). Where data of sufficient quality are available, allergens will also be compared for potency. One example potency parameter is the half maximal inhibitory concentration (IC50) a molecule that can inhibit IgE in a competitive immunoassay.

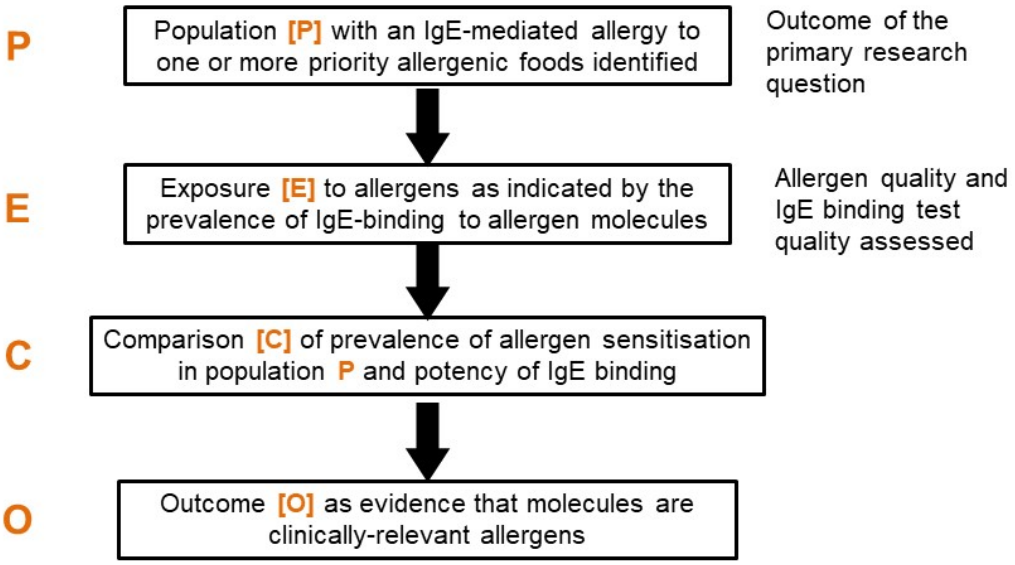


***FIGURE 2: Framework for addressing the secondary research question using a modified Population-Exposure-Comparator-Outcome (PECO) approach (based on Javed et al., 2017).***

The outcome in the PECO analysis then relates to evidence indicating that a food protein is responsible for eliciting IgE-mediated allergic reactions in the population. If data are of sufficient quality, some indication of potency might also be assessed through a meta-analysis of measures of IgE binding such as skin prick test wheal diameter or serum concentration of allergen specific IgE.

## 3. Search strategy

Initially, a list of search terms was compiled for allergenic foods listed in Annex II of Regulation (EU) No 1169/2011 FIC and which must be labelled irrespective of their level of inclusion in a recipe. This was then supplemented with foods identified as causing probable food allergies in at least 0.5% (ie. mixed and high prevalence) of a European population (adults, children, or infants) (Table 2). This list is based on the prevalence of probable IgE mediated food allergy in an unselected study population across Europe (Lyons et al., 2020, Lyons et al., 2019, Grabenhenrich et al., 2020, Nwaru et al., 2014a, Nwaru et al., 2014b) building on the classification of prevalence of immune mediated adverse reactions developed by the FAO-WHO expert consultation (FAO-WHO, 2022). Additional foods considered to be of lower allergenicity such as rice were included, together with novel foods, such as insects.

Using these selected "priority" foods a set of robust food names was compiled in English including common names and synonyms based on SNOMED altLabels, and common names in French, and Spanish. A list in Japanese was also developed using both characters and romaji (Table 3). These food-related search terms will be applied together with the wildcard allerg* as described by Javed et al. (2017) using validated study designed filters for retrieving any other relevant systematic reviews (Wilczynski & Haynes, 2007) and sound diagnostic studies (Wilczynski & Haynes, 2005) to search MEDLINE (OVID), ISI Web of Science, and Scopus (Falagas et al., 2008) (Table 4) together with derived wildcards (Table 5).

Searches will be executed using MEDLINE (OVID), ISI Web of Science, and Scopus (Falagas et al., 2008). Searches will be performed without language restrictions using the above search terms in English, Spanish, French and Japanese. Ultimately, references will be uploaded into EndNote. Duplicate copies will be removed using automated (DistillerSR) or manual screening, as necessary.

If an abstract of a non-English article is identified as being relevant, it will be translated into English by a native speaker or, if one is not available, using for example, Google translate or DeepL.

Study titles and abstracts will be reviewed independently by two reviewers using selection criteria and categorised as included, excluded, or unsure for further (full text) review using the inclusion and exclusion criteria listed in Table 6. Discrepancies remaining after full text review will be resolved by panel discussion by the study team and at least one representative from an external expert panel. The research process will be fully documented to allow the search to be assessed and reproduced as per PRISMA guidelines (Page et al., 2021).

# 4. Assessing quality of evidence in the PO analysis

## 4.1 Population (P)

Studies must include evidence of sensitisation as determined by serological analysis or skin prick testing. Other aspects to be taken into consideration are whether study participants are drawn from an unselected population (e.g., birth cohort, community survey) or biased population (e.g., outpatient clinic, case series) and if the study is multi- or single centre. These impact the quality of the population, which will be ranked building on the approach developed by the FAO-WHO expert consultation on Risk Assessment of Food Allergens (FAO-WHO, 2022). Thus, selected studies will be graded as follows:

1. Unselected study population or nested case control studies in single study centres
2. Surveys of out-patient clinic patients across multiple study centres
3. Surveys of out-patient clinic patients in a single study centre
4. Case reports

This grading reflects the validity of different study designs to deliver unbiased data with which the primary question can be addressed with grade 1 being the highest quality population to address the primary question. A geographic centre is defined as any location within a 50 mile/80 Km radius of another.

Risk of bias arises from how closely the study population represents the (food allergic) population. Sources of bias for outpatient clinics result from bias in on-demand healthcare referral systems that disadvantage low socio-economic (SES) groups, those from black and minority ethnic groups or indigenous peoples, and sex and gender biases, where more women than men seek healthcare support, but symptoms are more likely to be negated. These biases are reduced in unselected study populations, although these too are subject to biases arising from response rates. Similarly, bias from missing data might arise from lack of funding for high quality studies in an unselected study population or for developing outpatient clinic studies with higher numbers or spanning geographic centres. The approach described below to estimating risk of bias is based on the study design used in the EuroPrevall cohorts (Kummeling et al., 2009, Keil et al., 2010, Fernandez-Rivas et al., 2015). Thus, risk of bias estimates for the population are:

**VERY HIGH risk of bias**: Case reports and outpatient clinic studies describing <10 patients (single or multicentre).

**MEDIUM risk of bias**: outpatient clinic studies with at least 100 patients from a single geographic centre

**MEDIUM-LOW risk of bias**: outpatient clinic studies with at least 100 patients from multiple geographic centres

**LOW risk of bias**: Unselected study populations e.g., birth cohorts and nested case-control studies appropriately powered.

### 4.2 Outcome (O)

Studies will be graded for according to the following diagnostic outcome based on as the approach of Bjorksten et al. (2008) and (Lyons et al., 2019, Lyons et al., 2020). Grading reflects the quality of diagnosis (test accuracy), i.e., robustness of the outcome for addressing the primary research question and will be as follows:

1. Challenge confirmed food allergy: gold standard diagnosis of IgE-mediated food allergy where a clinician confirmed food allergy has been further confirmed by oral food challenge (double blind placebo controlled [DBPCFC] or open).
2. Clinician confirmed food allergy: a clinician has diagnosed a patient based on reported symptoms associated with consumption of a particular food which are typical of an IgE-mediated food allergy, symptom onset within 2 hours of contact with food and evidence of sensitization to the same food (either a positive skin prick test (a mean wheal diameter ≥3mm compared to the negative control) or a positive serum specific IgE (≥0.35kU/L) to the same food)
3. Probable food allergy: where self-reported food allergy is combined with evidence of sensitization to the same food in the form of a positive skin prick test (a mean wheal diameter ≥3mm compared to the negative control) or positive serum specific IgE (≥0.35kU/L) to the same food. Individuals with evidence of sensitisation to selected foods and a convincing history of a reactions to those same foods within two hours of consumption.
4. Possible food allergy: self-reported food allergy with symptoms consistent with an IgE-mediated food allergy occurring within 2h of consuming the problem food.

Studies of populations with confirmed food allergy will be ranked higher than those with probable food allergy, the lowest ranking given to those with possible food allergy.

In this aspect, risks of bias arise from:

**HIGH risk of bias**: where clinical history and evidence of sensitisation are not linked

**MEDIUM risk of bias**: linking clinical history to sensitisation (probable food allergy) but there is still a risk of bias since clinical history relies on patient recall and access to healthcare.

**LOW risk of bias**: evidence of past anaphylaxis or a positive oral food challenge (open, single or a double-blind placebo-controlled food challenge).

Biases from missing data might arise from lack of funding for high quality studies employing oral food challenges, lack of clinical staff and facilities for undertaking oral food challenges or reluctance of patients to undergo a food challenge.

### 4.3 Primary question outcomes

Aggregated scores based on the grading for population and outcome will allow the quality of evidence that a specific food can cause IgE-mediated food allergies.

If the quality of evidence and available resources allow the prevalence, potency (e.g., severity of reactions using numerical scoring systems developed in iFAAM, Fernández-Rivas et al., 2022) and sensitivity (using threshold dose distributions, Bjorksten et al., 2008) will be integrated with prevalence to classify food proteins as major, minor, or emerging allergenic risk.

# 5. Assessing quality of evidence in the PECO analysis

## 5.1 Exposure assessment

Prior to assessing exposure [E], two tests of accuracy will be applied, one related to the quality of allergen preparations (5.1.1) and the other methodology used to assess IgE binding (5.1.2).

### 5.1.1 Quality assessment of the allergen (food protein) preparation and quality characteristics

Food protein preparations can be crude allergen extracts, native purified proteins, or recombinant proteins from food as consumed. The grading reflects the quality of allergenic food proteins used for analysis including their relationship with the food source with the highest quality rank being 1 .

1. Well-characterised purified native allergen (sequence confirmation including N-terminal sequence and mass data) from the food as consumed.
2. Recombinant allergen with confirmed sequence, folding and aggregation information, and protein-level evidence of expression in foods as consumed.
3. Native allergen with no sequence information.
4. Recombinant allergen without folding and/or aggregation confirmation, or peptides corresponding to segments of the allergen sequence, and protein-level evidence of expression in foods as consumed.
5. Partial purified allergen from foods as consumed.
6. Crude extract from foods as consumed.
7. Purified protein, recombinant protein or extracts, but no protein-level evidence of expression or presence in the food as consumed.

**HIGH risk of bias**: lack of data demonstrating allergens are expressed or present in the food as consumed (e.g., present in root but not in leaves that are typically eaten).

**MEDIUM-HIGH risk of bias**: allergens have not been authenticated with respect to sequence or folding.

**MEDIUM-LOW risk of bias**: Purified native allergens or recombinant allergens for which at least molecular masses have been determined by, for example, SDS-PAGE; synthetic peptides used that, whilst retaining parts of the primary sequences, lack post-translational modification or tertiary structures attributes of intact native proteins.

**LOW risk of bias**: native proteins with a confirmed structural information

Biases from missing data might arise from lack of funding for high quality studies employing well characterised allergens. Clinical studies of IgE reactivity often lack details on biochemical characterisation of allergen molecules used for analysis and vice versa.

### 5.1.2 Quality assessment of the test used to determine whether a food protein can bind IgE and cause an allergic reaction

Different types of (diagnostic) tests can be used to define whether a particular protein is an allergen that can induce IgE-mediated reaction(s), with in vivo assessments graded higher (1 or 2) than in vitro tests using biological samples from patients with a relevant food allergy (graded 3-6). Specifically:

1. In vivo challenge test in a confirmed food allergic individual.
2. Skin prick test in a confirmed food allergic individual.
3. Effector cell activation (e.g., basophil histamine release) using either cells or serum from confirmed food allergic individual.
4. IgE-immunoassay using serum samples from confirmed food allergic individual.
5. IgE-dot blotting with a purified protein or immunoblotting following separation of allergen from a confirmed food allergic individual.
6. Dot blotting using allergen extracts and serum samples from confirmed food allergic individual.

It is known that sensitisation to certain types of allergen molecule varies across Europe with the prevalence of sensitisation to Bet v 1 homologues being higher in northern Europe where birch tree are fond, whilst sensitisation to lipid transfer proteins (LTPs) is more common in the Mediterranean area (Fernandez-Rivas et al., 2006, Datema et al., 2015, Lyons et al., 2021, Vereda et al., 2011). Consequently the risk of bias in serological analysis is dependent on the both the number of study subjects and their geographic location, with a minimum number of patient sera based on that used for IUIS allergen designation (Pomés et al., 2018) [n=5].

**HIGH risk of bias**: any of poor technical replication or low sample numbers (≤5 subjects), or serum pools used, lack of quantitative data, lack of control sera from healthy non-atopic or atopic controls* .

**MEDIUM-HIGH risk of bias**: good technical replication but sera from a small study population in only one or multiple centres (≥5-10) used and may lack of control sera from healthy non-atopic subjects or atopic controls.

**MEDIUM risk of bias**: good technical replication, control sera (atopic and non-atopic control sera) used and sera from a small study population (≥10<20) from either a single or multiple centres.

**MEDIUM-LOW risk of bias**: good levels of technical replication, control sera (atopic and non-atopic control sera), sera from individuals from single centre (n= ≥20).

**LOW risk of bias**: good levels of technical replication, control sera (atopic and non-atopic control sera), sera from individuals used, large numbers from multiple centres (n=≥20).

Biases may also result from differences in test methodology. Therefore, the risk of bias will always be lower in studies where multiple test methods are applied. Biases from missing data might arise from lack of funding for high quality studies using proper sampling for biological and technical replicates, control sera, and complementary test methods.

Numerical outcomes of the analysis will be combined to provide an accuracy score.

### 5.1.3 Exposure assessment

Where sufficient data are available the exposure to an allergen, as indicated by the extent of sensitisation to an allergen in the population will be assessed. The option of integrating the test accuracy scores will be explored to provide a scale of exposure and an indication as to its accuracy.

## 5.2 Secondary question outcomes

Comparison (C) will assess prevalence of sensitisations to the different allergenic food proteins in the population (P). If data are of sufficient quality and quantity, these will be ranked for capacity to induce an IgE-mediated allergic reaction in a sensitised individual.

If the quality of evidence and available resources allow allergenic food proteins will be compared with regards their potency, as indicated by capacity to bind IgE or trigger mediator release in an effector cell assay, such as the stripped-basophil histamine release assay, and classified as major, minor, or emerging allergenic risk.

Outcome relates to evidence indicating that an allergenic food protein is responsible for eliciting IgE-mediated adverse reactions to foods and, therefore, is clinically relevant. Thus, ranking and quality assessment will allow clinically relevant allergens to be identified and form a basis for assessing the risk novel food proteins present in terms of clinically relevance.

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## 7. Disclaimer

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

## 8. Conflicts of interest statement

The authors declare no conflict of interest in relation to the published work. All interests declared by the authors have been scrutinised by the European Food Safety Authority as part of the tender process to assesses whether a declared interest constitutes a conflict.

## 9. CRediT author statement

CM was involved in conceptualisation of the systematic review methodology and together with SBA and PMF was involved in Funding acquisition. CM and SA drafted the protocol, which was reviewed and edited by PMF, FO, CN and HW.

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## 11. Tables

| | |
|---|---|
| POPULATION (P) | What is the EVIDENCE is there that the POPULATION had an IgE-mediated allergy to a food?<br>[Identified by addressing the primary question using a PO approach] |
| EXPOSURE (E) | How many individuals in the POPULATION with a food allergy have serum-IgE that binds a specific allergen molecule(s)? |
| COMPARATOR (C) | How do different allergen molecules compare with regards to:<br>Extent of sensitisation in the food allergic POPULATION<br>Levels of specific IgE, measures of IgE binding capacity and/or activation effector cells involved in driving allergic reactions (POTENCY) |
| OUTCOMES (O) | What is the quality of EVIDENCE that specific FOOD PROTEIN MOLECULES can cause IgE-mediated reactions in the POPULATION? |

***TABLE 1: Modified population, exposure, comparator, and outcome (PECO) approach***

| Country | Age group | % Prevalence of probable food allergy (96%CI) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Apple | Peach | Kiwi | Banana | Melon | Carrot | Tomato | Lentil | Sunflower seed |
| Greece | Children | - | - | - | 0.56 (0.00-2.51) | - | - | - | 0.56 (0.00-2.51) | - |
| The Netherlands | Adults | 0.91 (0.34-1.77) | 0.60 (0.17-1.33) | 0.57 (0.15-1.29) | - | - | - | - | - | - |
| | Children | 0.84 (0.18-2.05) | 0.53 (0.06-1.55) | 0.63 (0.09-1.72) | - | - | - | - | - | - |
| Lithuania | Children | 0.89 (0.01-3.17) | - | - | - | - | 0.89 (0.01-3.17) | - | - | - |
| Poland | Adults | 0.75 (0.17-1.83) | - | - | - | - | - | - | - | - |
| | Children | 1.1 (0.3-2.4) | - | - | 0.95 (0.25-2.18) | - | - | 0.63 (0.10-1.68) | - | - |
| Spain | Adults | 0.57 (0.08-1.65) | 1.6 (0.6-3.2) | 0.64 (0.11-1.77) | - | 0.95 (0.25-2.24) | 0.81 (0.18-2.03) | - | - | - |
| | Children | - | 1.1 (0.2-2.7) | 1.06 (0.19-2.74) | - | - | - | - | 0.53 (0.02-1.85) | 0.53 (0.02-1.85) |
| Switzerland | Adults | 1.9% (1.0-3.1) | 2.6% (1.5-4.0, | 1.3% (0.6-2.4) | _ | _ | 1.0 (0.4-2.0 95) | - | - | - |
| | Children | 0.54 (0.02-1.80) | - | - | - | - | 0.81 (0.10-2.27) | - | - | - |

***Table 2: Foods not included in Annex II FIC Regulation No. 1169/2001 with prevalence of probably food allergy > 0.5% in EU countries (Lyons et al., 2019, 2020)***

| English common | English synonyms | Spanish | French | Japanese |
|---|---|---|---|---|
| **Cow's milk** (Bos taurus) | | Leche | Lait | ミルク \| Miruku |
| **Hen's egg** (Gallus domestica) | | Huevos | Oeufs | 卵 \| Tamago |
| **Fish** | | Pez | poisson | 魚 \| Sakana |
| Salmon (Salmo salar) | Atlantic salmon, Oncorhynchus (Pacific Salmon; Chinook salmon [O. tshawytscha], Chum [O. keta], Choho [O. kisutch], Masu [O. masou], Pink [O. gorbuscha], Sockeye [O. nerka] | Salmón | Saumon | 鮭 \| Sake |
| Trout | Rainbow (Oncorhynchus mykiss), Brown (Salmo trutta) | Trucha | Truite | マス \| Masu |
| Cod | Atlantic cod (Gadus morhua), Pacific cod (Gadus macrocephalus) | Bacalao | Morue | タラ \| Tara |
| Mackerel | Atlantic mackerel (Scomber scombrus), Short mackerel (Rastrelliger brachysoma), Island mackerel (R. faughni), Indian mackerel (R. kanagurta), Blue mackerel (Scomber australasicus), Atlantic chub mackerel (S. colias), Chub mackerel (S. japonicus); Spanish Mackerel (genus Scomberomorus, Grammatorcynus and Acanthocybium); other mackerel (families Carangidae, Hexagrammidae and Gempylidae) | Caballa | Maquereau | サバ \| Saba |
| Pollock (Pollachius pollachius) | Pollack, Coalfish (Pollachius virens) | Abadejo | Goberge | ポロック \| Po rokku |
| Tuna | Thunnus (bluefin group), Thunnus neothunnus) (yellowfin group) | Atún | Thon | シーチキン \| shīchikin |
| Pike (Esox lucius) | Northern pike | Lucio | Brochet | パイク \| Paiku |
| Carp (Cyprinus carpio) | Common carp, Asian carp [Catla (Gibelion catla), rohu (Labeo rohita), mrigal (Cirrhinus cirrhosus); black carp (Mylopharyngodon piceus), Grass carp (Ctenopharyngodon Idella), Silver carp (Hypophthalmichthys molitrix), Bighead carp (Hypophthalmichthys nobilis) | Carpa | Carpe | 鯉 \| Koi |
| Talapia (Oreochromis mossambicus) | Mozambique tilapia, Sarotherodon galilaeus, Sarotherodon melanotheron | tilapia | Tilapia | ティラピア \| Tirapia |
| Pangasius (Pangasianodon hypophthalmus) | Striped catfish | (pez) panga | (poisson) pangasius | パンガシウスの魚 \| Pangashiusu no sakana |

| | | | | |
|---|---|---|---|---|
| **Crustacean shellfish** | | Marisco crustáceo | Coquillages et crustacés | 甲殻類の貝 \| Kōkaku-rui no kai |
| Crab (Charybdis feriatus) | true crabs, short-tailed crabs | Cangrejo | Crabe | カニ \| Kani |
| Blue swimmer crab (Portunus pelagicus) | Blue crab, flower crab, blue manna crab, sand crab, Rajungan, Alimasag | Cangrejo nadador azul | Crabe bleu | ワタリガニ \| Watarigani |
| Chinese mitten crab (Eriocheir sinensis) | | Cangrejo de Shanghai | Crabe chinois | モクズガニ \| Mokuzugani |
| Mud crab (Scylla paramamosain) | Mangrove crab | | Crabe nageur | 泥ガニ \| Doro-gani |
| Warrior swimming brown crab (Callinectes bellicosus) | | | Crabe de boue | ワタリガニを泳ぐ戦士 \| Watarigani o oyogu senshi |
| Lobster (Homarus spp., Panulirus spp. Nephrops and Metanephrops spp.) | Scampi (Dublin Bay or Norway lobsters; Bay prawn; Lobsterette; Baby lobster; Deep sea lobster) | Langosta | Homard | ロブスター \| Robusutā |
| American Lobster (Homarus americanus) | | Bogavante americano | Homard d'Amérique | アメリカンロブスター \| Amerikanrobusutā |
| Spiny lobster (Panulirus stimpsoni) | | | Langouste rouge | 伊勢海老 \| Ise ebi |
| Shrimp (Caridea spp.) | Crangonidae; penaeidae; prawn; palaemondidae; caridea | Gamba, camarón | Gambas ou crevette | エビ \| Ebi |
| Black tiger shrimp (Penaeus monodon) | tiger shrimp; black tiger prawn | Camarón tigre negro | Crevette tigrée noire | ブラックタイガーシュリンプ \| Burakkutaigāshurinpu |
| Brine shrimp (Artemia franciscana) | | | Crevette des salines | ブラインシュリンプ \| Burainshurinpu |
| Brown shrimp (Farfantepenaeus aztecus; Penaeus aztecus) | | Camarón marrón | Crevette grise | ブラウンシュリンプ \| Buraunshurinpu |
| Greasyback shrimp (Metapenaeus ensis) | | | Crevette glissante | 脂性エビ \| Aburashō ebi |
| Neptune rose shrimp (Parapenaeus fissurus) | Penaeus longirostris; Parapenaeus paradoxus; Neopenaeopsis paradoxus; Penaeus cocco; Parapenaeus longirostris; Penaeopsis paradoxus; deepwater rose shrimp; gamba; penaeus bocagei | | Crevette neptune | ネプチューンローズシュリンプ \| Nepuchūnrōzushurinpu |
| North Sea shrimp (Crangon crangon) | Crangon vulgaris; brown shrimp | Quisquilla del Mar del Note | Crevette de la mer du Nord | 北海エビ \| Hokkai ebi |
| Northern shrimp (Pandalus borealis) | Coldwater prawn; cold-water prawn; deepwater prawn | Camarón boreal | Crevette nordique | 北海老 \| Kitaebi |
| White shrimp (Litopenaeus setiferus; Litopenaeus vannamei) | Penaeus setiferus; pacific white shrimp; whiteleg shrimp | Camarón blanco | Crevette à patte blanche | 白エビ \| Shiraebi |
| Crawfish/ Crayfish (Astacoidea and Parastacoidea spp.) | crawfish, craydids, crawdaddies, crawdads, freshwater lobsters, mountain lobsters, rock lobsters, mudbugs, baybugs or yabbies Procambarus spp.; cambarus spp.; Cambaridaa | Cangrejo de río | Ecrevisses | ザリガニ \| Zarigani |
| Crayfish (Archaeopotamus sibiriens) | | | | |

| | | | | |
|---|---|---|---|---|
| Narrow-clawed crayfish (Pontastacus leptodactylus) | Danube crayfish, Galician crayfish, Turkish crayfish | | Écrevisses à pattes grêles | 狭い爪のザリガニ \| Semai tsume no zarigani |
| Red swamp crayfish (Procambarus clarkii) | red swamp crayfish, Louisiana crawfish; mudbug | Cangrejo americano | Écrevisses à pattes rouges | レッド・スワンプ・ザリガニ \| Reddo suwanpu zarigani |
| Prawns (Dendrobranchiata spp.) | Shrimp | Langostinos | Langoustine | 車海老 \| Kurumaebi |
| Giant freshwater prawn (Macrobrachium rosenbergii) | giant river prawn or giant freshwater prawn | Camarón gigante de agua dulce | Crevette géante d'eau douce | 巨大淡水エビ \| Kyodai tansui ebi |
| Indian prawn (Fenneropenaeus indicus; Penaeus indicus) | | | Crevette des Indes | インド海老 \| Indo ebi |
| King prawn (Melicertus latisulcatus) | | Langostino | Gambas | キングエビ \| Kinguebi |
| Silk moth (Bombyx mori) | | | Bombyx du mûrier | カイコガ \| Kaikoga |
| **Molluscan shellfish** | | Moluscos | Mollusque | 軟体動物 \| Nantaidōbutsu |
| Abalone (Haliotis midae) | South African abalone; perlemoen abalone | Abulón | Ormeau | アワビ \| Awabi |
| Jade tiger abalone (Haliotis laevigata x Haliotis rubra) | blacklip abalone; greenlip ablone | | | |
| Snail (Helix aspersa; Cornu aspersum) | Common garden snail; European brown snail | Caracola | Escargot | カタツムリ \| Katatsumuri |
| Portuguese oyster (Crassostrea angulata) | | | Huître portugaise | ポルトガルのカキ \| Porutogaru no kaki |
| Pacific oyster (Crassostrea gigas) | Japanese oyster; Miyagi oyster (Magallana gigas) | Ostra del pacífico | Huître creuse japonaise | パシフィック・オイスター \| Pashifikku oisutā |
| Sydney rock oyster (Saccostrea glomerata) | New Zealand rock oyster; Auckland oyster | | Huître creuse d'Australie | シドニーロックオイスター \| Shidonīrokkuoisutā |
| Veined rapa whelk (Rapana venosa) | Asian rapa whelk | | Rapana veiné | ツブツブ \| Tsubutsubu |
| Japanese flying squid (Todarodes pacificus) | Japanese common squid or Pacific flying squid | Calamar volador | Encornet japonais | スルメイカ \| Surumeika |
| **Wheat** (Triticum aestivum) | | Trigo | Blé | 小麦 \| Komugi |
| **Peanut** (Arachis hypogea) | | Cacahuete | Cacahuète | 落花生 \| Rakkasei |
| **Soybean** (Glycine max) | Bean sprout; sprout | Soja | Soja | 大豆 \| Daizu |
| **Sesame** (Sesamum indicum) | | Sésamo | Sésame | ごま \| Goma |
| **Mustard** (Brassica nigra, Brassica juncea) | Black mustard; brown mustard; | Mostaza | Moutarde | マスタード \| Masutādo |
| **Buckwheat** (< 0.5%) (Fagopyrum esculentum) | | Trigo sarraceno, alforfón | Sarrasin | そば \| Soba |
| **Hazelnut** (Corylus avellana) | Cobnut; Filberts | Avellana | Noisette | ヘーゼルナッツ \| Hēzerunattsu |
| **Pistachio** (Pistacia vera) | | Pistacho | Pistache | ピスタチオ \| Pisutachio |
| **Cashew** (Anacardium occidentalis) | | Anacardo | Noix de cajou | カシュー \| Kashū |
| **Almond** (Prunus amygdalus) | | Almendra | Amande | アーモンド \| Āmondo |
| **Brazil nut** (Bertholletia excelsa) | | Nuez de Brasil, nueces pecanas | Noix du Brésil | ブラジルナッツ \| Burajirunattsu |

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|---|---|---|---|---|
| **Walnut** (Juglans regia, Juglans nigra) | | Nuez | Noisette<br>Noyer noir<br>Noix de pecan | クルミ \| Kurumi<br>ペカン \| Pekan |
| **Celery** (Apium graveolum),<br>**Celeriac** (Apium graveolens var. rapaceum) | | Apio | Céleri | セロリ \| Serori<br>セルリアック \| Seruriakku |
| **Peach** (Prunus persica)<br>**Nectarine** (Prunus persica) | | Melocotón<br>Nectarina | Pêcher<br>Nectarine | 桃 \| Momo<br>ネクタリン \| Nekutarin |
| **Apple** (Mallus domestica) | | Manzana | Pomme | りんご \| Ringo |
| **Kiwi fruit** (Actinidia deliciosa) | | Kiwi | Kiwi | キウイ \| Kiui |
| **Banana** (Musa acuminata, Musa balbisiana) | Dessert banana; dwarf banana; sweet banana; plantain; Balbis banana; starchy banana | Plátano | Banane | バナナ \| Banana |
| **Carrot** (Daucus carota subsp. sativus) | | Zanahoria | Carotte | にんじん \| Ninjin |
| **Lentil** (< 0.5%) (Lens culinaris) | | Lenteja | Lentilles | レンズ豆 \| Renzu mame |
| **Melon** (Cucumis spp.) | Cucumber (Cucumis sativus), Muskmelons (Cucumis melo including cantaloupe and honeydew), horned melon (Cucumis metuliferus), West Indian gherkin (Cucumis anguria) | Melón | Melon | メロン \| Meron |
| **Sunflower seeds** (< 0.5%) (Helianthus annuus) | | Pipas de girasol | Graine de tournesol | ヒマワリの種 \| Himawari no tane |
| **Tomato** (Solanum lycopersicum) | Lycopersicon esculentum; Lycopersicon esculentum var. esculentum; Solanum esculentum; Solanum lycopersicum var. humboldtii | Tomate | Tomate | トマト \| Tomato |

***Table 3: Food search terms including English common names, English synonyms, Spanish, French and Japanese***

| Common name (EN) | Latin name | Search terms (EN) |
|---|---|---|
| Cow's milk | Bos taurus; Bos indicus | milk* AND allerg* |
| Buffalo milk | Bubalus bubalus | |
| Ewe's milk | Ovis aries | |
| Goat's milk | Capra hircus | |
| Hen's egg | Gallus domestica | egg* AND allerg* |
| **Fish** | | fish AND allerg* |
| Salmon | Atlantic salmon (Salmo salar), Oncorhynchus (Pacific Salmon; Chinook salmon [O. tshawytscha], Chum [O. keta], Choho [O. kisutch], Masu [O. masou], Pink [O. gorbuscha], Sockeye [O. nerka] | |
| Trout | Rainbow (Oncorhynchus mykiss), Brown (Salmo trutta) | |
| Cod | Atlantic cod (Gadus morhua), Pacific cod (Gadus macrocephalus) | |
| Mackerel | Atlantic mackerel (Scomber scombrus), Short mackerel (Rastrelliger brachysoma), Island mackerel (R. faughni), Indian mackerel (R. kanagurta), Blue mackerel (Scomber australasicus), Atlantic chub mackerel (S. colias), Chub mackerel (S. japonicus); Spanish Mackerel (genus Scomberomorus, Grammatorcynus and Acanthocybium); other mackerel (families Carangidae, Hexagrammidae and Gempylidae) | |
| Pollock | Pollachius pollachius | |
| Tuna | Thunnus spp. | |
| Pike | Esox lucius | |
| Carp | Cyprinus carpio | |
| Talapia | Oreochromis mossambicus | |
| Pangasius | Pangasianodon hypophthalmus | |
| Crustacean shellfish | | shellfish* OR crustac* AND allerg* |
| Tiger prawn | Penaeus monodon | |
| White leg prawn | Litopenaeus vannamei | |

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|---|---|---|
| | (Penaeus vannamei) | |
| North Atlantic prawn | Pandalus borealis | |
| Brown shrimp | Crangon crangon | |
| Northern brown shrimp | Penaeus aztecus | |
| Scampi/ Dublin Bay Prawn/ Norway Lobster/ langoustine | Nephrops norvegicus | |
| Lobster | Homarus gammarus | |
| | Homarus americanus | |
| Blue swimming crab | Portunus Pelagicus | |
| Brown Crab | Cancer Pagurus | |
| Molluscan shellfish | By species | mollus* AND allerg* |
| Wheat | Triticum aestivum | wheat* AND allerg* |
| Peanut | Arachis hypogea | peanut* AND allerg* |
| Soybean | Glycine max | soy* OR sprout* AND allerg* |
| Sesame | Sesamum indicum | sesame* AND allerg* |
| Mustard | Brassica nigra, Brassica juncea | mustard* AND (nigra or juncea) AND allerg* |
| Buckwheat | *Fagopyrum esculentum* | buckwheat* OR Fagopyrum AND allerg* |
| Hazelnut | *Corylus avellana* | hazelnut* OR cobnut* OR filbert* AND allerg* |
| Pistachio | *Pistacia vera* | pistachio* AND allerg* |
| Cashew | *Anacardium occidentalis* | cashew* AND allerg* |
| Almond | *Prunus amygdalus* | almond* AND allerg* |
| Brazil nut | *Bertholletia excelsa* | brazil AND (nut or nuts) AND allerg* |
| Walnut | *Juglans regia, Juglans nigra* | walnut* AND (regia or nigra) AND allerg* |
| Celery<br>Celeriac | Apium graveolum<br>Apium graveolens var. rapaceum | celer* AND allerg* |
| Peach<br>Nectarine | Prunus persica | peach* OR nectarine* AND allerg* |
| Apple | Mallus domestica | apple* AND allerg* |
| Kiwi fruit | Actinidia deliciosa | kiwi* AND allerg* |
| Banana | Musa acuminata, Musa balbisiana | banana OR plantain AND allerg* |
| Carrot | Daucus carota subsp. sativus | carrot AND allerg* |

| | | |
|---|---|---|
| Lentil | Lens culinaris | lentil AND allerg* |
| Melon | Cucumis spp. | melon AND allerg* |
| Sunflower seeds | Helianthus annuus | sunflower AND allerg* |
| Tomato | Solanum lycopersicum | tomato AND allerg* |

***Table 4: Examples of search terms, Boolean operators, and truncation***

| Search term | Wildcards and tuncations |
|---|---|
| Allergy (FR) Allergie<br><br>Allergies (FR) Allergies<br>Allergen (FR) Allergéne<br>Allergens (FR) Allergénes<br>Allergenicity (FR) Allergénicité<br>Allergenicities (FR) Allergénicités | → search term allerg* (e.g., lait* AND allerg*) |
| Allergy (ES) Alergia<br>Allergies (ES) Alergias | → search term alerg* (e.g., leche* AND alerg*) |
| Allergen (ES) Alérgeno<br><br>Allergens (ES) Alérgenos<br>Allergenicity (ES) Alergenicidad<br>Allergenicities (ES) Alergenicidades | → search term alérg* (e.g., leche* AND alérg*) |
| Allergy (JA) アレルギー \| Arerugī<br><br>Allergies (JA) アレルギー \| Arerugī<br><br>Allergen (JA) アレルゲン \| Arerugen<br>Allergens (JA) アレルゲン \| Arerugen<br>Allergenicity (JA) アレルギー誘発性 \| Arerugī yūhatsu-sei<br>Allergenicities (JA) アレルギー誘発性 \| Arerugī yūhatsu-sei | → search term アレル* \| arerug*<br>(e.g., ミルク OR miruku AND arerug*) |

***Table 5: Examples of search term wildcards and truncation***

| Inclusion criteria | Exclusion criteria |
|---|---|
| • Peer-reviewed articles<br>• Articles directly related to the defined research questions<br>• Articles not closely related to the topic of the research question (IgE binding molecules) but provide information about patients from whom serum samples were obtained to characterise the allergen (e.g., clinical manifestation) and those providing information about the physicochemical characteristics and biological activity of allergens.<br>• Case studies or case reports that are peer reviewed and related to IgE- mediated food allergy where an IgE-binding molecule is described.<br>• Articles which are published in languages other than English, if relevant to the defined research question. | • Full text is unavailable<br>• Studies that do not describe the IgE-binding molecules or are unrelated to the question being addressed<br>• Abstract and summary of the following will not be included in the study: book chapters, non-peer reviewed case reports or case studies, editorial materials which are expressing the opinion of the editor or publisher, meetings, conferences, seminars, workshops, congress, symposiums, patents and proceeding papers<br>• Review articles<br>• Animal model studies |

***Table 6: Inclusion and exclusion criteria used for screening***