



Biosensors for food allergy detection according to specific IgE levels in serum

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ABSTRACT

People respond differently to food components, which can sometimes cause dramatic and unpredictable effects, such as intolerance, toxicity and allergy, and are a major global problem today. Of all the different reactions induced by food allergens, the most frequent are hypersensitivity mediated by specific immunoglobulins E (IgE). The *in vitro* biosensing of specific IgE levels is an alternative approach to invasive risky *in vivo* tests for diagnosing food allergy in humans. This work reviews the current analytical approaches in food-specific IgE biosensing, including immunochemical and lateral flow assays, electrochemical biosensors, array technologies, nanomaterial-based techniques, label-free sensors and microfluidic systems. The aim is to draw a closer more stimulating scenario for innovation in the food allergies topic. Perspectives on the future of biosensor technology applied to *in vitro* IgE determinations are also offered from an analytical viewpoint.

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1. Food allergy

Food allergy, defined as an adverse immune response to a substance in food, is becoming an increasingly frequent disease that affects many people all over the world [1]. Effects of allergy can vastly differ and most cause minor issues, but some are responsible for serious health disorders and illnesses. Consequences are considerable morbidity, a negative impact on quality of life and high medical care costs [2,3].

Food allergies include a very wide spectrum of clinico-pathological disorders (i.e. hypersensitivities), but are very distinct from non-immune reactions to food components, such as

intolerance, toxic, pharmacologic or other disturbing mechanisms [4]. Although any food can trigger an allergic response, reactions to egg, milk, peanut, tree nuts, fish, shellfish, wheat, and soya account for most significant food allergies [5]. In fact, relatively few protein families explain the vast majority of allergic reactions. Potent food allergens are usually water-soluble glycoproteins with molecular weights of 10–60 kD that remain stable at a low pH. Nevertheless, genetic factors probably lie behind food allergy development [6]. In addition, marked changes in diet suggest that differences in macronutrient and micronutrient dietary contents might explain the increase in allergies [7]. The control and reduction of food allergens are carried out at different levels, from dietary change to determining the allergen content in raw or treated foods, and also by clinically establishing if someone really suffers food allergy or intolerance [8,9].

An atypical immune system response to specific allergens leads to an allergic reaction [10]. Typical food allergy symptoms include disturbances to skin, respiratory and gastrointestinal tracts, as well as cardiovascular aberrations like acute urticaria/angioedema, atopic dermatitis and eosinophilic gastrointestinal disease. In its most severe form, known as anaphylaxis, immediate hypersensitivity to food can involve several organ systems and induce life-threatening hypovolemic shock and respiratory compromise [5].

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Immune allergies are usually grouped into two general categories: IgE-mediated and non-IgE-mediated [11]. Typically, the first category is of rapid onset with clinical symptoms usually developing within minutes to a few hours after ingestion. The second category is characteristically chronic, such food protein-induced enterocolitis syndrome. Accordingly, the categorisation of allergic disorders to make classification more appropriate and usable was proposed by Demoly [12].

Food allergy prevalence has increased in recent decades and is now recognised as a substantial public health burden in developed countries. Food avoidance has been the time-honoured approach for both prevention and treatment, which requires making changes in dietary habits and social interactions [1]. However, recent findings indicate that early contact with food can induce tolerance and desensitisation [13,14]. The exact incidence of food allergy is yet to be established as major discrepancies between reported and diagnosed food allergy remain [15]. Up to one third of the population believes that they are allergic to food, but the reported prevalence is roughly 5–10% in children and 4–5% in adults. In westernised countries, data from oral food challenges are 3–8% and 1–3%, respectively. In conclusion, more people believe they have food allergies than those clinically diagnosed with allergies. In addition, physicians' awareness of clinical history limitations alone in the diagnostic accuracy for food allergies and the judicious use of testing will help to decrease false diagnoses.

2. IgE as an allergy biomarker

In food allergies, as in other types of hypersensitivity reactions mediated by IgE production, the biochemical mechanism involves an initial phase called 'sensitisation' in which a susceptible individual produces IgE antibodies against a specific allergen present in food. The second phase is 'elicitation', which is a robust allergic reaction in the sensitised individual upon subsequent re-exposure to the same allergen. Therefore, when the culprit food, protein or component penetrates the intestine lining or skin, IgE antibodies bind to it. Antigen food-specific IgE binds to the FcεRI receptors on mast cells, and basophils and the cross-linking of IgE and IgE receptors occur, when preformed mediators are released to circulation (Fig. 1) [16,17], such as histamine and proteases tryptase and chymase. At the same time, activation of the *de novo* synthesis of arachidonic acid metabolites takes place, which include leukotrienes, prostaglandins and platelet-activating factors.

These mediators promote vasodilation and increased vascular permeability, which contribute to bowel wall swelling. Smooth muscle contraction and mucus production also occur, and the influence of these factors results in vomiting, diarrhoea and, in some cases, anaphylaxis (a rapid systemic allergic reaction that can cause death).

As IgE plays a critical control role in the allergic mechanism, this molecule was chosen as a biomarker to support the diagnosis of allergy and treatment of patients made by physicians. Other recent studies reveal that serum IgE can be used for predicting allergen immunotherapy effectiveness [18]. These approaches are very interesting from an analytical point of view because serum IgE levels are very low. This requires researching and developing new methods that allow specific IgE to be detected in serum at very low concentrations (below 1.0 µg/L). Therefore, this review aims to provide an overview of the current developed biosensing technologies for the quantitation of food-specific IgE by highlighting advantages and weaknesses in order to obtain a general perspective of analytical performances in the food allergy diagnosis topic.

3. Clinical guidelines

There are several approaches to diagnose immediate allergy hypersensitivity reactions type I, which range from absolute avoidance of the culprit food to specialised methods looking for evidence by either the direct detection of specific IgEs (*in vitro* tests) or indirect assays (*in vivo* and *ex-vivo* tests).

The methods used for food allergy diagnosis are briefly described.

3.1. *In vivo* methods

The term *in vivo* methods includes all tests performed directly on the body. Skin tests and oral provocation tests are the most common *in vivo* methods and must be performed by experienced allergy professionals under strictly controlled conditions to avoid consequent risks. In an allergy skin test for a food, a small drop of a liquid food extract is placed on the skin. The total procedure takes 15–20 min on average. The test is safe but it should be avoided in the event of pathological dermal alteration and during acute allergic reactions. The allergy skin test has to be carried out individually on each patient, which makes it laborious and time-consuming. Sensitivity is not optimal, but ranges from 60% to 70% [19].

The oral provocation tests are currently the diagnostic method that establishes a cause–effect relationship between the ingestion of a certain food or food additive and the reported reaction [20]. An individual risk-benefit evaluation and protocol have to be performed before starting the test. Pregnant women, patients at high risk due to co-morbidity (e.g., acute infections) and those suffering severe life-threatening reactions should not be analysed. These tests might facilitate allergic sensitisation or induce adverse reactions, and they are not always available as in emergencies and primary care centres, nor reliable due to either the severity of medical conditions or effects of concomitant food.

3.2. *Ex vivo* tests

The basophil activation test (BAT) is the most widely used *ex vivo* test. BAT uses flow cytometry to measure the expression of activation markers on the surface of basophils that are up-regulated following the cross-linking of the IgE antibodies bound to the high-affinity IgE receptor (FcεRI) that result from allergen or anti-IgE stimulation [21]. It is a functional assay that measures IgE function as the ability to induce the activation of basophils in the presence of any allergen type by measuring basophil degranulation using histamine and other mediator releases. The sensitivity can be expressed as the concentration at which 50% of the maximal basophil response occurs. Currently, BAT and histamine release tests show challenges for the majority of relevant foods, such as poor sensitivity and lack of pure allergens. Despite the fact that both tests have existed for decades, their validation is still underway.

3.3. *In vitro* methods

As both *in vivo* and *ex vivo* tests are inconvenient and perform poorly, safer, cheaper and faster *in vitro* tests are required to analyse physiological fluids. The *in vitro* diagnosis of food allergies in blood is based on the immunoanalytical determination of the specific IgE concentration to a particular allergen or derivatives.

Food allergy has been categorised into classes according to the IgE concentration found in plasma or serum, which starts from class 0 (<0.1 kU/L; absent or not detectable) and continue to class VI

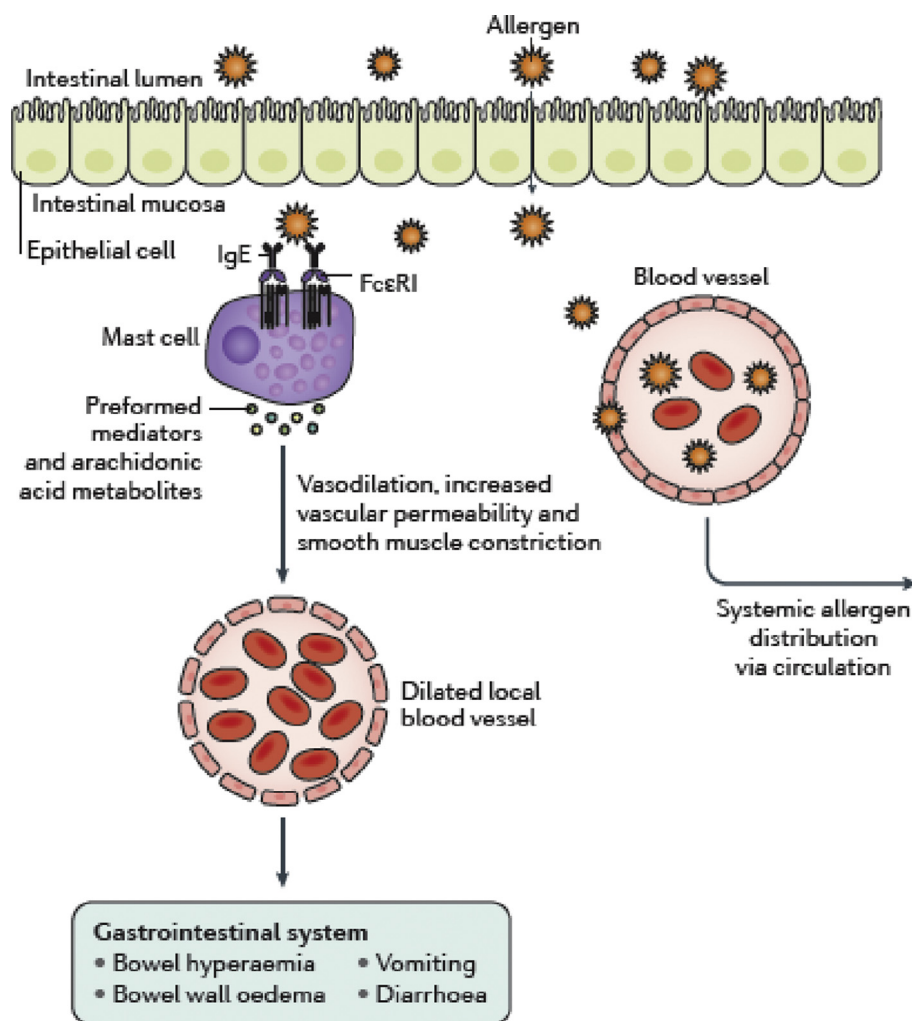


Fig. 1. Scheme of Ig-mediated reactions to food allergens [17].

(>100 kU/L, very high). Note that 1.0 kU/L is the equivalent to 2.4 µg IgE/L. These limits are only guidelines because each user laboratory establishes its own reference ranges.

The main currently used commercial *in vitro* IVD tests are ImmunoCAP (PHADIA, Thermofisher Scientific), the 3gAllergy Universal Kit (IMMULITE, Siemens) and that commercialised by Dr. Fooke-Achterrath Laboratorien. The three companies employ autoanalyzers for the fully automated determination of specific IgEs and total IgEs in times ranging from 60 to 180 min. Detection principles are absorptiometry, fluorescence and bioluminescence. Tests are always carried out in fully equipped laboratory settings and they all have a limit of detection (LOD) ≥ 0.35 kU/L. The price for instrumentation ranges from €15,000 to more than €100,000 depending on performance and working capacity. Today, the clinical sensitivity of *in vitro* blood allergy testing is lower than *in vivo* skin tests [22], which is the main reason why these tests are not often included in regular clinical protocols.

Compared to other types of blood biomarkers, the sensitive and specific determination of IgE is by far the most important issue in allergy diagnosis. There is certainly no *in vitro* test on the market capable of determining IgE concentrations below 0.1 kU/L. Besides, current allergy tests lack mainly sensitivity and selectivity. Hence, it is clearly necessary to overcome these limitations by different biosensing approaches that can determine specific IgE for food allergy diagnosis.

4. Biosensing technologies

Biosensors are cutting-edge analytical devices for the detection of a wide range of targets in numerous fields mediated by isolated enzymes, immunosystems, nucleic acids, tissues, organelles or whole cells. They are chemical sensors in which the recognition system utilises a biochemical mechanism. A typical device has three elements: an analyte bioreceptor immobilised on the analytical surface; a transducer for converting chemical or physical data into measurable signals; a signal processing system. The processor can store, display and analyse output signals to produce diagnostic data results. Biosensors can be categorised by transduction mode as electro-chemical, optical, magnetic, mass-sensing, electro-mechanical, etc. They provide selective semi-quantitative or quantitative analytical information. Several technologies have been employed to diagnose food allergy and are the object of this review (Fig. 2).

4.1. Immunoassays and lateral flow systems

Lateral flow technology was originally developed for pregnancy diagnoses in the 1980s. Currently, this technology is widely used for many other applications, e.g. to detect drugs of abuse, diagnose tuberculosis and HIV, determine cardiac markers, detect infectious diseases and test food. Today the gold standard assay

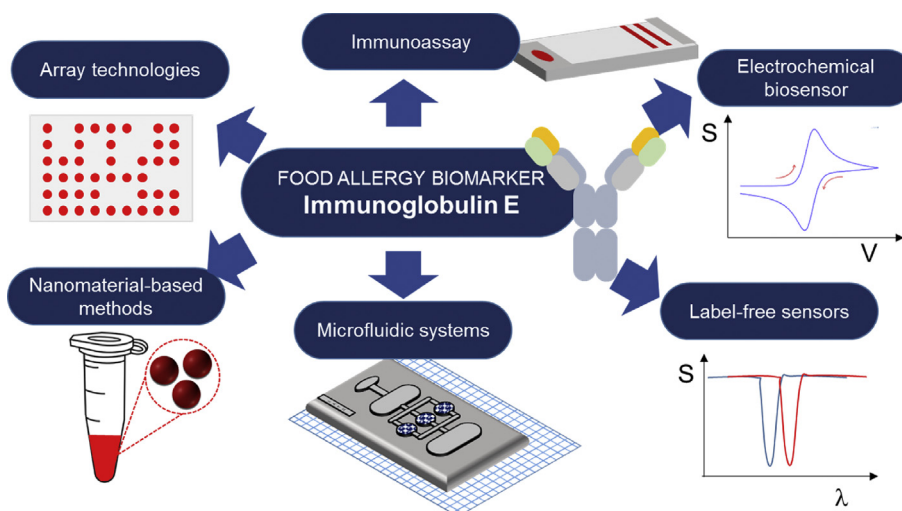


Fig. 2. Classification of biosensing technologies applied to determine specific IgEs associated with food allergy.

for determining specific IgE is the single-plexed ImmunoCAP system and the multiplexed array-based ImmunoCAP ISAC. Both require sophisticated instrumentation, which make lateral flow-based tests a promising alternative to occupy specialised market niches in the clinical field. In line with this, Diaz-Vazquez et al. described the benefits of using the ImmunoCAP rapid lateral flow system for point-of-care applications because it allows up to 10 specific IgE to be analysed in less than 20 min. The single-plexed ImmunoCAP system provides good correlation results above 1.0 kUA/L [23]. Chinnasamy et al. reported a scalable lateral flow paper microarray assay that employs anti-human IgE and HRP dual labelled gold-nanoparticles conjugates (Fig. 3) [24]. In this approach, different allergens, represented as coloured spots, are printed on a nitrocellulose membrane. IgE antibodies attach to specific allergen spots, followed by washing and exposed with dual labelled gold nanoparticles, substrate TMB is finally added to improve the signal intensity of the different allergen spots.

The assay is performed in 10 min and uses a flatbed scanner for detection and commercial software for data analyses. The assay was evaluated using 35 human serum samples to reach an agreement with the ImmunoCAP results for a threshold of 1 kUA/L. According to the conventionally accepted cut-off level of 0.35 kUA/L for a positive *in vitro* test of specific IgE, this system's clinical sensitivity was limited to discriminate positive allergic patients.

Another interesting reverse phase microarray approach is described as a paper-based Vertical Flow Microarray (VFM) assay (Fig. 4). This colorimetric test is able to simultaneously detect IgEs with a limit of detection of 1.9 $\mu\text{g/mL}$ (832 kUA/L) in whole serum in 8 min [25] using a flatbed scanner as a detector. The performance of this assay was established by screening 113 serum samples, and the results agreed with those provided by a reference method.

The results obtained with a fully automated analyzer for the multiple detection of allergen-specific immunoglobulin E are reported [26]. AdvanSure AlloStation Smart is a fully clinical laboratory analyzer based on a line blot enzyme immunoassay that determines multiple allergen specific IgE antibodies in approximately 4 h using 50 μL of human serum or plasma. The strip-processing carousel holds 60 strip housing reagents (Fig. 5A and B). The analyzer's performance was evaluated by analysing 341 serum samples tested for 23 allergens. The results demonstrated the potential for determining specific IgE for food allergens.

A paper-supported aptasensor for total IgE determination is reported [27]. The sensor is based on the luminescence resonance energy transfer with upconversion carbon nanoparticles. The sensor's performance was evaluated using 10-fold diluted human serum samples. A LOD of 0.5 ng/mL was achieved, which is the equivalent to 0.2 kU/L. The sensor was not used to determine specific IgE for food allergens but, according to sensitivity, it has the potential to run such an analysis type.

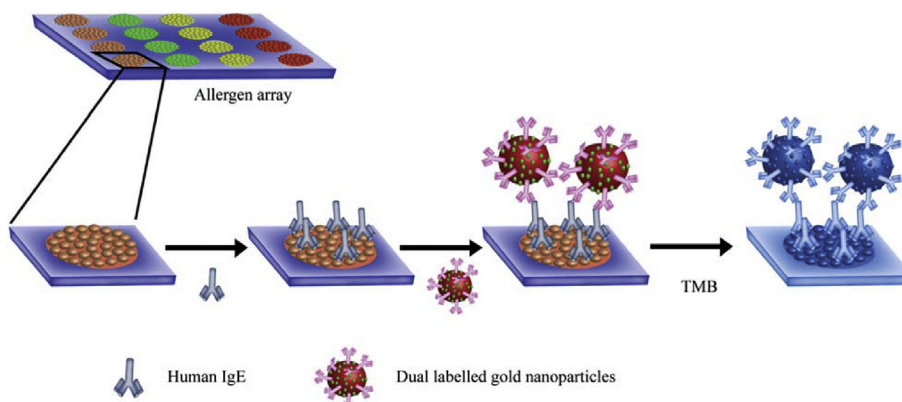


Fig. 3. Schematic overview of paper-based lateral flow allergen microarrays [24].

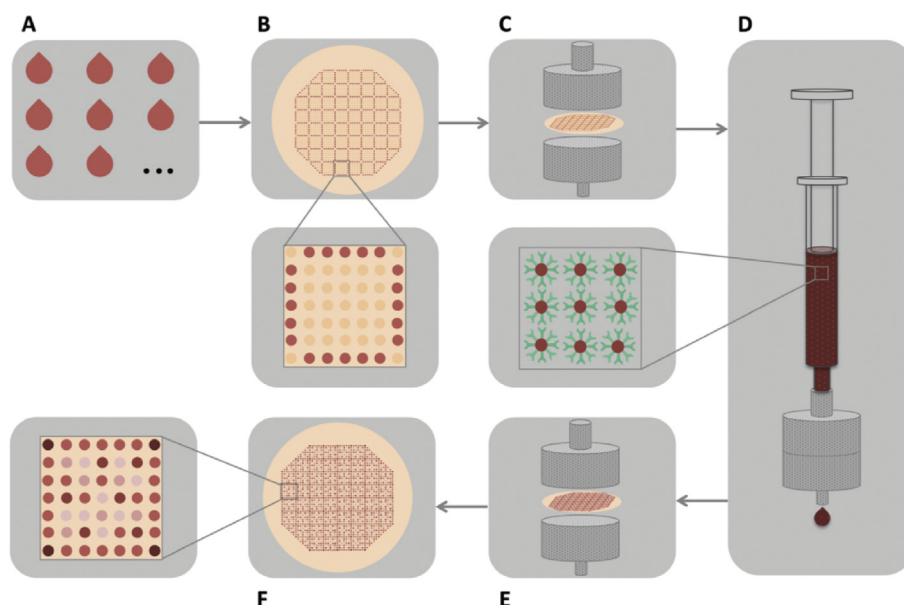


Fig. 4. Concept picture of the paper-based Vertical Flow Microarray (VFM) assay [25].

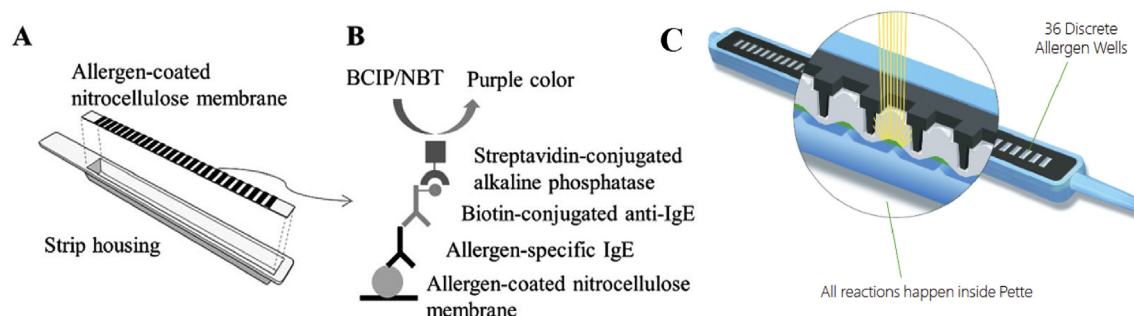


Fig. 5. (A) Allergen-coated strip used for the AdvanSure AlloScreen [26]. (B) Scheme of the AdvanSure AlloScreen assay principle. (C) Zoomed view of the inside of a Pette [37].

4.2. Electrochemical biosensors

The bioelectrochemical detection of IgE antibodies is an interesting approach that might provide a simple cost-effective alternative compared to classic IgE detection methods. However, after a thorough scientific literature search, it can be stated that electrochemical detection is a scarce analytical strategy for determining specific and/or total IgE. In fact, very few examples are reported that deal with the determination of specific IgE against food allergens based on electrochemical detection.

A recent paper describes the fabrication of an allergen epitope peptide layer on a gold-based nanoparticle sensor surface that provides a sensitive biorecognition platform to detect an antipeanut antibody [28]. In particular, non-faradaic impedance with a biocatalytic amplification approach accomplished a LOD of 5 pg/mL for IgY in serum. The authors concluded that according to the assay's sensitivity, this approach could be used to detect specific IgE. However, this was not experimentally demonstrated.

There is a more recent report about a sensitive and selective electrochemical biosensor based on macroporous Au and a zwitterionic peptide for IgE detection [29]. Its improved sensitivity is due to a larger specific surface area and it accomplishing a LOD of 42 fg/mL of IgE in 5% serum. Interestingly, the zwitterionic peptide present on the biosensing interface allows the target IgE to be detected with diluted biological samples due to the interface's

antifouling property. The biosensor appears to be prone to non-specific recognition when working with raw serum. Papamichael reported the development of a disposable aptamer-based electrochemical sensor (aptasensor) that uses differential pulse voltammetry as the transduction mode [30]. The biosensor was tested to detect total IgE in a competitive format with a biotinylated specific probe and sensitivity above 300 ng/mL, the equivalent to 125 kU/L. However, this limited the biosensor to determine specific IgE for food allergens.

4.3. Array technology

Most of the biosensing systems described for determining food-specific IgE perform a singleplex approach. However when considering clinical laboratories' needs, the modern diagnosis of allergic diseases demands the parallel (multiplex) and highly sensitive determination of different specific IgE in a single assay. A useful approach is assays in a microarray format because they allow the identification of a plurality of targets in a single analysis with a small amount of sample. The literature describes several types of protein arrays for sIgE assays.

Planar chips. In a first category, biochips are planar with allergens immobilised directly onto the surface. The conventional approach is the nano-dispensation of each allergen (isolated protein or recombinant protein) on a glass slide or nitrocellulose pad.

After immobilisation, the sample is incubated on the chip and the spot intensities produced by fluorophores coupled to the secondary antibody are recorded. Several examples have been published [31–33].

A colorimetric microarray assay based on using a substrate of alkaline phosphatase as the developer reagent has also been developed [34]. A peptide array is also applied as an alternative to attach the entire protein to the chip. Thus, the chip is constructed with linear peptide fragments, reported as IgE-binding epitopes for cow's milk allergy [35]. This strategy allows the IgE epitope mapping of food allergens by the immobilisation of a huge set of overlapping peptides [36]. This led to the discovery of an additional important epitope of Ara h1.

One issue with these methods is the limited availability of the specific allergenic protein in its recombinant or natural origin form. Renault et al. developed a microarray test based on protein extracts of food components [37]. The reported results indicate good discrimination between challenge responders in atopic and non-atopic individuals.

Another innovative approach for high-sensitivity assays in the microarray format on glass employs alternative substrates, such as silicon coated with a functional polymer [38]. The authors found that silicon significantly enhances the performance of the allergen microarray compared to glass.

Hitachi Chemical Diagnostics, Inc. developed a technology for allergy diagnoses that uses the CLA® and OPTIGEN® allergen-specific IgE assay, which enables multiple allergen testing. The device consists of three injected moulded parts: a pette body, a coverslip and a partition. The pette is a small plastic containing a serum channel for fluid flow with up to 36 cellulose threads, each bound with a selected allergen. The coverslip contains wells on the channel side to bind allergens and lenslets on the outer side for light collimation. The partition separates individual wells to block light emission from adjacent wells. The three parts are assembled to create an enclosed device (Fig. 5C). This technology includes a luminometer that simultaneously measures the IgE content of a patient's allergic reaction to up to 36 different allergens using a single 0.6–1.5 mL serum sample. The system's sensitivity and specificity are good with 88% and 94%, respectively [39].

The drawbacks of planar biochips are uncontrolled immobilisation efficiency and denaturation of proteins on the carrier surface, which result in loss of protein activity and reduce analytical sensitivity. To improve variation in probe immobilisation and other experimental variations inherent to the array technology, the calibrated fluorescence enhancement technique has been proposed [40]. The authors evidenced a strong linear dependence of the amount of bound protein to the resulting fluorescence signal of the secondary antibody for allergen-specific IgEs to Ara h1 (peanut major allergen) and other allergens in human serum.

Our research group is investigating an innovative protein array-based method that combines an innovative substrate and reader, the direct immobilisation of extracts and colorimetric detection (unpublished results). This system is based on digital versatile technology that uses an audio–video compact disc as an analytical substrate for allergen immobilisation and an optical detector (DVD player). Morais et al. reviewed the principles of disc-based microarrays [41].

Gel chips. The second category is three-dimensional gel-based microchips. ImmunoCAP ISAC microchip (Thermo Fischer) includes 103 natural and recombinant allergens, which are immobilised on such substrates. This microchip enables specific IgE levels to be detected in a sample in accordance with the four classes of allergic reactions: negative, low, medium, high.

At the lab level, the German company Dr. Focke Laboratorien GmbH developed hydrogel biochips for the simultaneous

detection of specific IgE and total IgE [42,43]. This microarray contains elements with allergens, including recombinant proteins, which belong to the main groups: pollen, household, epidermal, food, fungi, and insect venoms. Fluorescence signals are recorded using a two-wavelength microarray analyser based on the digital wide-field fluorescence microscopy concept. Recently, the same research group described the preparation of a microarray gel pad from glass slides treated with bind silane for a similar purpose [44].

4.4. Nanomaterial-based methods

In recent years, the advances made in nanotechnology open up a novel path to develop highly sensitive and selective biosensors for the future generation of diagnostic tools. A wide range of nanomaterials and their nanocomposites have become widespread and offer promising results in the biosensing field [45,46]. Micro- and nanoparticles have been frequently used in immunoassays as mobile substrates on which the target antigen is captured as detection labels, or simultaneously as substrates and labels. In particular, magnetic beads can be manipulated and separated using a magnet, which strongly facilitates the assay protocol.

A recent review article reports the new advances made in developing IgE antibody assays based on nanotechnologies [47]. In this section, we discuss in depth the most relevant approaches applied to food allergy and those that can be extrapolated to this field.

Nanoparticle-based capture. Several researchers have used nanoparticles as a support to capture specific immunoglobulins. Thus, a sandwich assay is performed using magnetic polyacrylamide-agarose beads labelled with anti-IgE antibodies for human IgE separation [48]. An electrochemical immunosensor has also been developed using gold nanoparticles covalently coated with an Ara h2 peptide with a well-characterised IgE binding site [28]. Ashraf et al. used functionalised magnetic nanoparticles coated with a peanut allergen to isolate specific IgEs [49] and, by quantifying by ELISA, to improve the LOD (0.1 kU/L) and to cut the total assay time.

A recent approach involves the broad optical detection of pigmented microparticles in suspension [50]. Beads, which act as supports, react with the serum of an allergic patient and perform a magnetically assisted competitive immunoassay, similarly to indirect ELISA. This method is presented as an inexpensive compact benchtop device for the quantitative detection of different antibodies in serum.

Several papers have focused on IgE detection using a magnetic nanoparticle core shell in a microchip device [51,52]. As later discussed, the objective is to reduce both the assay time and sample/reagent use.

Nanomaterials-based detection. Different nanomaterials demonstrate interesting properties related to the detection of food allergies in biosensing systems. Indeed signal enhancement using nanoparticles has emerged as a potent approach to be used in clinical immunology. Several authors have exploited the use of anti-IgE aptamers as receptors to anchor to particle surfaces. For instance, gold nanoparticles coated with aptamers have been applied in a system that relies on SPR [53], which considerably increases analytical sensitivity. Silver nanoparticles have also been used for enhancing fluorescence signals (Fig. 6A) [54]. At the same time, this principle has been demonstrated with polystyrene beads coated with an IgE aptamer for chemiluminescence detection [55]. The approach consists of a sandwich assay format that employs magnetic beads functionalised with a capture anti-IgE antibody and aptamer-modified beads coupled to fluorescent dyes (Fig. 6B) to enhance the analytical response.

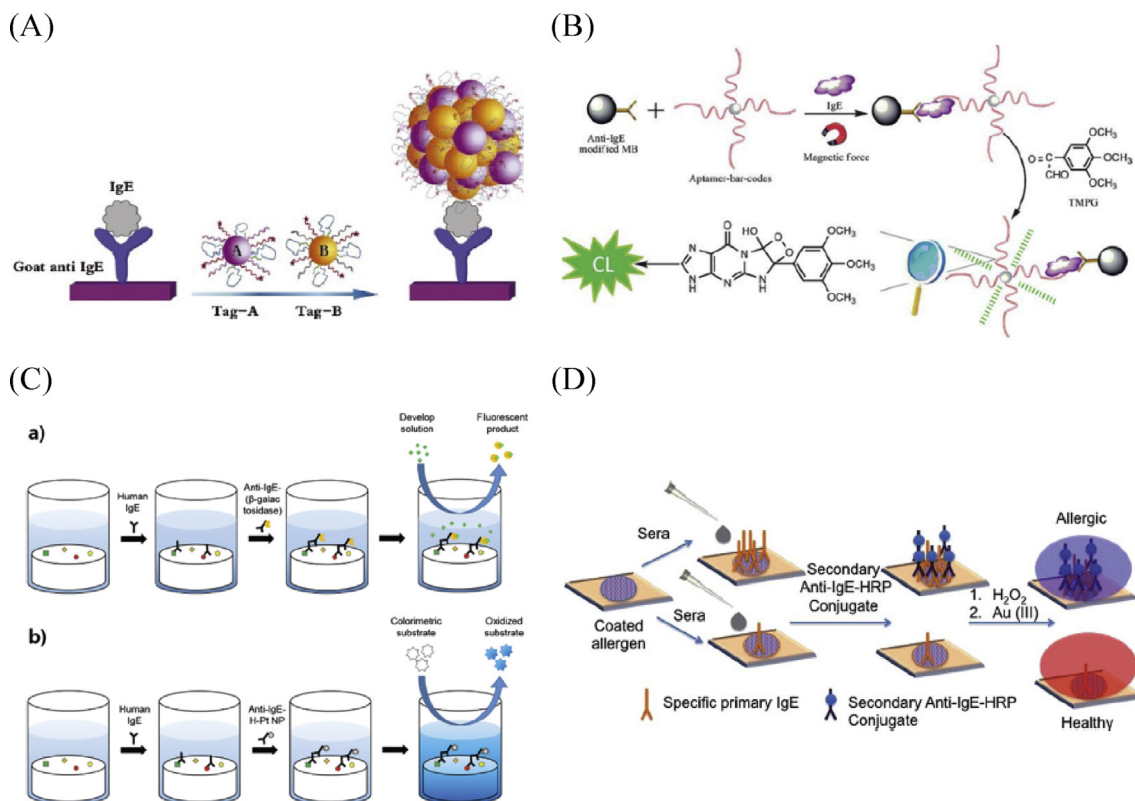


Fig. 6. Schematic illustrations of approaches using nanomaterials-based detection. (A) Procedure of the immunoassay and detection strategy by sandwich-type with hybrid probes [54]. (B) Aptamer-barcode-based immunoassay for the instantaneous detection of IgE coupled to MB [55]. (C) (a) conventional and (b) peroxidase mimicking H-Pt NP-based ImmunoCAP diagnostic systems for the quantification of human IgE [57]. (D) Multiplexed assay with five different allergens and five distinct patients [59].

Another alternative material to nanoparticles is quantum dots. They are nanometre-sized semiconductor particles with optical properties in tune with size. Compared to traditional fluorescent reporters, quantum dots display much more brightness and photostability. Thus, Platt et al. developed a sandwich assay for the detection of anti-casein IgEs based on capturing immunoglobulins on a chip surface and using streptavidin-coupled QD to amplify the signal [56].

Nanomaterials that exhibit enzyme-like characteristics, also called nanozymes, attract considerable attention given their potential to overcome the intrinsic limitations of natural enzymes; e.g. low stability and relatively high preparation and purification costs. A very efficient colorimetric immunoassay was built using the peroxidase-mimicking activity of hierarchically structured platinum nanoparticles [57]. They were successfully integrated into a conventional ImmunoCAP allergy diagnostic test as the replacement with natural signalling enzymes (Fig. 6C). Better analytical performance was reported for allergy with a LOD of 0.17 kU/L for specific IgE.

Nanomaterials for visual detection. The use of plasmonic nanomaterials to develop diagnostic tools has been a crucial breakthrough that favours the determination of a wide variety of clinical biomarkers [58]. In this way, gold nanoparticles become the most important markers for the visual detection of specific IgE [59]. In each assay platform well, a sandwich assay is run using a secondary anti-IgE-HRP (horseradish peroxidase) antibody. Then gold (III) chloride is added, and the colour change from red to blue is proportional to the concentration of the remaining hydrogen peroxide that triggers the aggregation of gold nanoparticles. The generated visible blue colour indicates the presence of allergy, while the intensity at 550 nm measures the amount of serum-specific IgE.

Colour differences are distinguishable by the naked eye (Fig. 6D). Along this line, a rapid visual protein detection method has been developed for 96-well microplate arrays by using aptamer to modify silver nanoparticles. This enhancement technique achieves visual dots depending on the concentration of total IgEs [60].

4.5. Label free biosensors

The advances made in nanofabrication have allowed technologies and structures to be developed capable of detecting bio-interactions without resorting to markers. This approach offers interesting advantages for biosensing and allows research into the underlying physico-chemical features of target species, which depend mainly on their intrinsic physico-chemical properties.

Technical innovations have led to highly sensitive and robust label-free detection schemes. Thus, many label-free optical biosensors with interesting characteristics have emerged in recent years. Of these, surface plasmon resonance (SPR) is one of most successful label-free sensing techniques because it allows real-time detection and monitors biomolecular events, and provides quantitative information. The recognition of the target molecule brings about a change in the reflection index of the material and, consequently, a change in the resonance angle, which is measurable in real time without labelling (Fig. 7A).

A recent paper reviews the use of SPR-based methods for immunochemical allergen detection in food [61]. Regarding its clinical interest, several authors have described the quantitation of total human IgE [62]. In addition, IgE-specific aptamers, which can specifically interact with IgE at different epitopes, have been investigated for their specific interaction (Fig. 7B) [63,64]. As detection signals were weak, biofunctionalised gold nanoparticles

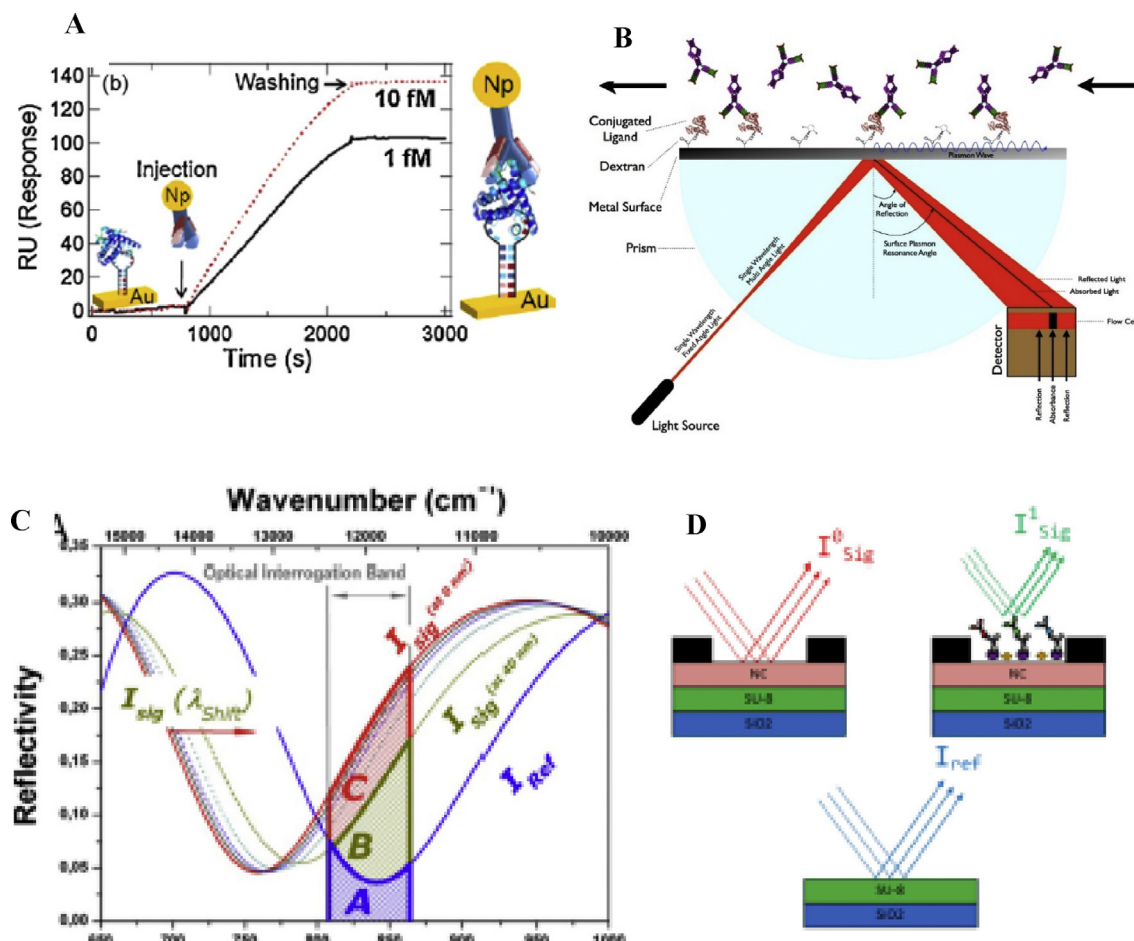


Fig. 7. (A) Adsorption of anti-IgE coated Au-Nps onto 10 fM (dotted line) and 1 fM (bold line) IgE complexed with surface-bound IgE-aptamers [63]. (B) Scheme of SPR detection principle [68]. (C) Theoretical calculation of reflectivity according to the wavenumber and wavelength for the reference interferometer and for the signal interferometer. (D) Optical scheme of BICELLS without biomolecules (top-left) after the recognition step (top-right), and the optical power of the reference interferometer (bottom) [72].

were employed to generate sandwich complexes. SPR has also been used to study inhibitors against the complex between IgE and its cell receptor (high-affinity IgE Fc receptor (FcεRI) [65,66]. The described bispecific protein is an interesting alternative to mAb because it is able to co-ligate it with FcεRI-bound IgE on allergic

effector cells, and it represents an efficient dual modality to interfere with allergic hypersensitivity reactions.

On the other hand, the IgE-mediated activation of human basophils has also been monitored [67]. Basophils of patients with allergy were fixed on the sensor chip and exposed to antigens. This

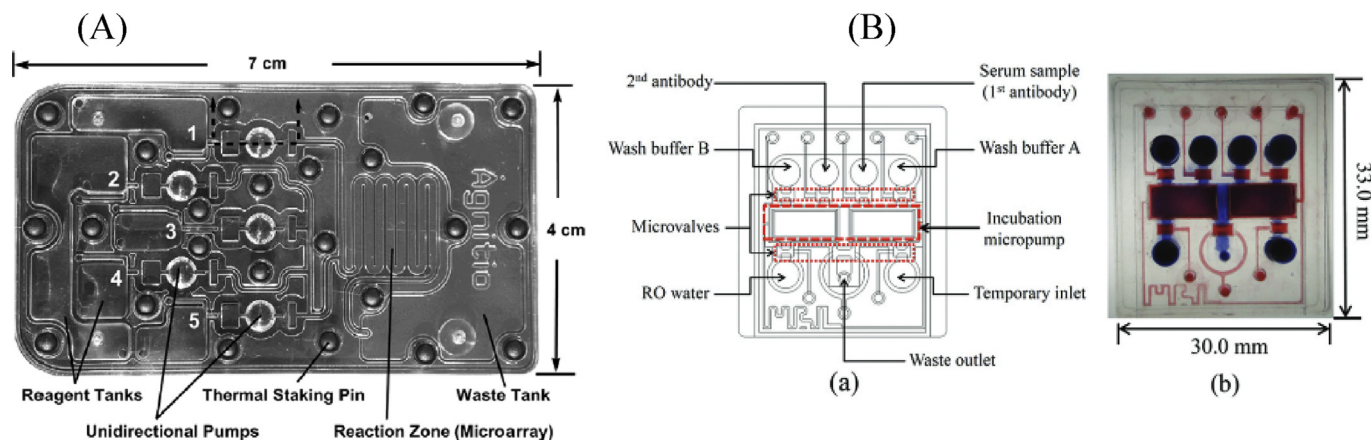


Fig. 8. Examples of microfluidic systems with the potential to determine specific IgE. (A) Injection moulded microfluidic cartridge for automated immunoassays [82]. (B) A photograph of the integrated microfluidic chip (33.0 × 30.0 mm) equipped with multiple microdevices, including six chambers (designed for loading with antibodies/serum, wash buffers and RO water prior to reactions), an incubation micropump and several microvalves [83].

Table 1

Comparison of the biosensing methods employed for food-specific IgE determinations.

Biosensor type	Format	Allergen Target	LOD ^a (ng/mL)	Sample	Comment	Ref.
Immunochemical	Paper-based assay	Egg white, cow's milk	2.4	Serum	Enhanced sensitivity due to the implementation of dual-labelled GNPs and an enzymatic amplification step.	[24]
	Vertical flow assays	Total IgE	1900	Serum	Rapid 8-min colorimetric alternative for the reverse phase microarray analysis	[25]
	Allergen-coated strips	Soyabean, milk, egg white, crab, shrimp, peach	0.84	Serum	Efficient automation of processing a line blot enzyme immunoassay	[26]
Electrochemical	Gold nanoparticle film on a graphite electrode	Peanut	0.005	Serum	Impedimetric sensor configuration featuring the HPR-catalysed precipitation of the enzyme product	[28]
	Porous gold-modified electrode-based aptasensor	Total IgE	$1 \cdot 10^{-4}$	5% serum	Biosensor based on macroporous Au and zwitterionic peptide and IgE aptamer	[27]
	Aptasensor	Total IgE	300	Serum	Disposable electrochemical sensor based on an aptamer receptor	[30]
Arrays	Competitive ELISA	Peanut	0.84	Serum	Biotin–streptavidin amplification and fluorescence detection using Cy5 labelled conjugate	[37]
	Indirect assay with bound allergens	β -lactoglobulin, peanut	49.3	Serum	Silicon chip engineered to enhance a particular fluorophore	[40]
	Multiplex solid-phase immunoassay	Egg white, cow's milk, wheat, flour, peanut, hazelnut	0.84	Serum	Hydrogel biochip manufactured by photoinduced copolymerisation and fluorescence detection	[43]
Arrays	Indirect assay on gel pads	Egg white, Cow's milk, α -lactalbumin, β -lactoglobulin, casein, codfish, wheat flour, peanut hazelnut, carrot, apple, peach	0.36	Serum	A CCD camera was used to record the fluorescence intensities of gel elements.	[44]
	Magnetic capture	Peanut, hazelnut	24	Serum	Detection based on a competitive immunoassay	[50]
	Quantum dots	Casein	84	Serum	Detection based on DPI	[56]
Nanomaterial-based	Gold particle	Specific IgE	NR	Serum	Visual detection based on plasmonic ELISA	[59]
	Silver particle	Undefined	20	Serum	Visual detection based on plasmonic array	[60]
	SPR	Total IgE	190	Buffer solution	Sandwich complexes with biofunctionalised gold nanoparticles	[64]
Label-free	SPR imaging	Undefined	0.001	Buffer solution	Precaptured onto magnetic beads	[69]
	Miniaturised array	Peanut	27	Serum	Chemiluminescence detection	[80]
	Miniaturised array	Specific IgE	2.4	Serum	Chemiluminescence detection	[82]
Microfluidics	Miniaturised immunodiffusion	Multiplex	1	Serum	Chemiluminescence detection	[51]
	Miniaturised array	α -Lactalbumin	NR	Serum	Magnetic concentration and fluorescence detection	[83]
	Miniaturised array	Specific IgE	NR	Serum	Fluorescence detection	[83]

^a LOD: limit of detection (1 ng/mL $\equiv$ 0.417 kIU/L), NR: not reported.

approach shows an increased resonance angle in response to either an anti-IgE antibody or the corresponding antigens. Studying the histamine release test can support basic scientific research, but can also serve the purpose of clinical examinations. The method to visualise individual basophil activation by an SPR imaging sensor is also described [68].

Regarding the detection of specific IgEs, SPR imaging, utilising both peptide and carbohydrate epitopes from peanut allergen glycoprotein *Arachis hypogaea* h2 (Ara-h2), is reported [69]. To enhance sensitivity and minimise non-specific binding, IgEs are precaptured onto magnetic beads loaded with polyclonal anti-IgE antibodies. The analysis of serum samples from allergic patients to peanuts confirmed the method's reliability. Later research by the same research group focused on epitope-resolved detection by immobilising more biomolecules [70]. SPR imaging has also been proven for α -lactalbumin, β -lactoglobulin and caseins [71]. On

gold-activated surfaces, covalent binding is promoted for the immobilisation of major milk allergens.

The interferometric signal generated by binding events has been estimated by a read-out platform that works in a label-free format [72]. The authors introduced a new photonic concept, biophotonic sensing cells (Fig. 7C and D) to determine total IgE levels in serum. This interferometric nitrocellulose sensing system shows good sensitivity to be applied to food allergies and achieves a LOD of 0.7 KU/L. The system's multiplex capability makes it an interesting approach to deal with allergy diagnosis. This equipment and technology will soon be commercialised.

4.6. Microfluidic systems

Miniaturised systems for integrated assays have drawn much attention in recent years. The key element is a microfluidic chip

Table 2
Analysis of the approaches proposed to determine specific food-allergy IgEs.

Approach	Advantages	Drawbacks
Immunochemical	Conventional instruments Low-cost Cost-effective	Low multiplexing capability Limited sensitivity Large amount of sample
Electrochemical	Simple transducers Easy to miniaturise Possibility of mass production	Few studies Low multiplexing capability
Arrays	Multiplexing capability Low reagent use Small amount of sample High-throughput	High-cost instrumentation
Nanomaterial-based	Modulable properties Improved sensitivity Easier miniaturisation Versatility	Partially explored field
Label-free	Low reagent use Lower cross reactivity Fast response	Limited sensitivity Sophisticated instrumentation Low multiplexing capability
Microfluidics	High automation Miniaturisation Low reagent use Small amount of sample Portability Simple for end users	Complicated design Difficult selection of materials Reagent storage difficulties
All techniques	Demonstrated for serum samples	Need for adequate sample treatment Lack of clear validation Lack of standards

with a set of microchannels etched or moulded in a material to provide throughput processing, enhance the transport and mixing of different reagents, and reduce samples and reagent volumes (down to microlitres). The integration of microfluidic and biosensor technologies confers the ability to merge sample preparation and detection on a single platform. Its small dimensions offer advantages for biosensing applications, such as multiplexing, integrating all assay steps and automating the process because it is demanded in point-of-care approaches [73].

Microfluidic designs have been developed for many different applications. Chips are often fabricated in glass, silico and polymers, with polymers drawing more attention. The most frequent detection method is fluorescence, followed by electrochemistry. As biological samples often complicate sample pre-treatment or require system modifications for reliable detection purposes, an interesting review paper compares the immune-based separations performed in miniaturised formats [74]. These microfluidic systems offer selective target isolation with minimal reagent use and short analysis times for a wide range of applications.

Lab-on-a-chip and lab-on-a-disc, which rely on micro-fabrication, have been designed for allergy testing. Ho et al. published an article that summarises these systems [75]. They highlight microfluidic systems as a tool test for automation by identifying IgE-mediated and non-IgE-type allergies.

In the present paper, we extend the compared microfluidic systems by focussing on food allergy, and classify them according to assay type.

The first category is cell-based assays, where microfluidics present solutions to overcome the limitations of conventional methods when performing the basophil activation test. The drug effects in eosinophils under flow conditions are examined [76]. To do so, a microchannel system is coated with recombinant human VCAMs to study the adhesion profile of resting and GM-CSF-stimulated human eosinophils by microscopic imaging. Chen et al. developed a centrifuge-based lab-on-a-disc system to

examine the allergic responses of live basophils using acridine orange as a fluorescent probe to report degranulation for allergen testing [77].

More recently, a novel test capable of capturing basophils directly from whole blood followed by *in vitro* activation and quantification of CD63 expression appeared [78]. This system is based on coating the chip with anti-CD203c and activation by an anti-FcεRI antibody, followed by the optical detection of this degranulation marker.

The second category is immunoassay, where a microfluidic system facilitates the recognition of allergen-specific antibodies. One relevant example is the detection made in a microfluidic chip with a protein microarray format and chemiluminescence signal imaging [79]. In this approach, β-lactoglobulin and peanut lectin were immobilised on a polydimethylsiloxane elastomer (PDMS) and integrated SU-8/glass microfluidic reaction chambers were developed. The LODs were 33 pM and 6.6 pM, respectively. The results obtained from artificial samples agreed well with the sera reactivity displayed by the standard ImmunoCAP system. In a later paper, the performance of this automated microfluidic immunoassay system was closely compared by analysing serum samples of 212 children [80]. A similar approach for detecting allergies to hazelnut, cherry and apple is described [81]. In this case, the associated recombinant proteins were immobilised on a copolymer of N,N-dimethylacrylamide and N,N-acryloyloxysuccinimide to coat the glass slide inside the microfluidic cell with PDMS etched structures.

A more sophisticated microfluidic cartridge to determine food allergies was proposed (Fig. 8A). The chip comprised three layers of injection-moulded plastic, sealed together by a thermal staking technique [82]. A full-frame CCD sensor captured the image of the chemiluminescence signals in the reaction zone. The tested panel included 20 target allergen extracts, including food and environment allergies. The complete analysis time was 27 min with a LOD of about 0.5 kU/L.

The synergistic combination of the properties and characteristics of homogeneous (species diffusion) and heterogeneous (separation and pre-concentration) immunoassays is reported [51]. Magnetic core-shell nanoparticles were employed with the selective capture of IgE in a single-channel microchip. The system was demonstrated to immobilise α-lactalbumin antigen on 7 μm-ferromagnetic iron beads by incubating with spiked serum mixtures. Its advantages are its short assay time (20 min), its low LOD (about 0.42 kU/L), and lower sample and reagent uses (5 μL). A similar approach based on magnetic beads has been proposed, which was carried out with the GRAVI-Cell immunoassay device from DiagnoSwiss (Switzerland) [52]. In this case, polyimide chips composed of eight independent microchannels allow the performance of eight tests in parallel. This platform offers the possibility of using a robot to enable a fully automated assay. Only 1.5 μL of serum is required.

A recent paper reports an advanced chip for detection in an array format (Fig. 8B) [83]. The device includes a temperature control module and 30 electromagnetic valves used to control the flow of both samples and reagents. In the chip, microwalls surround the reaction chamber and liquid channels on the backside of the thick PDMS layers that come into contact with the microarray slide. The polymethylmethacrylate loading design serves as a reagent reservoir and cover. Each chip contains 20 kinds of allergens and control immunoglobulin G in triplicate spots. Although the system has been demonstrated in serum sample, very little evidence for analytical performances is reported.

In summary, more microfluidic-based systems, including cartridge and the complete micro-pumping and imaging detection systems, are expected to be developed given their reliability

and highly adaptable utility in healthcare and point-of-care systems.

The following Tables 1 and 2 compare IgE determination methods and the pros and cons of different approaches, respectively.

5. Conclusions and future trends

This article reviews the current analytical approaches based on biosensing technologies that focus on the *in vitro* determination of food-specific immunoglobulins E. Individuals produce this biomarker during an immunological response due to a food allergy episode.

Many allergic food components can potentially develop an immunological response mediated by food-specific IgEs. Several countries have made mandatory protocols to declare the level of food allergens in foods due to their strong impact on health (e.g., EU 14 allergens).

One first conclusion is that the targeted analyte, IgE in blood serum, is determined mainly by immunochemical recognition approaches. In general, the biosensors mostly used to determine food-specific IgE employ optical or electrochemical detectors, the former being preferred. This remark is not clearly justified, but is real. Label-free detection strategies are also reported and offer rapid assays by reducing the number of assay steps and frequently providing good sensitivity.

As far as the assay format is concerned, the most widely employed one is based on the direct determination of food-specific IgE using an allergic determinant (raw allergic protein or peptides) immobilised on the biosensing substrate. With this format, an interesting point is the development of multiplex assays. Many authors propose using microarray technology because it offers advantages, such as the simultaneous determination of different specific IgEs, saving reagents and reaching high throughput capability. Such interesting devices are already available on the market [39].

An important issue, and one not well resolved in many articles, is sample treatment. However, this issue is no main issue because nearly all the developed biosensors herein reported are able to determine food-specific IgE in raw or diluted serum. Nevertheless, a formidable challenge related to determining levels of specific IgEs is the low concentration of these immunoglobulins present in serum. For this reason, the sensitivity of assays must be very high and reach a level up to 0.1 kUA/L, the equivalent to 0.24 ng IgE/mL. To overcome this challenge, a highly sensitive assay is required, which is achieved only when bioreagents and the others counterparts of the biosensor (support, detector, etc.) offer good analytical performance. Another key point, related to the methodology validation and interpretation of analytical results, is to acquire a representative pool of blood sera with positive and negative responses (control samples), including patients' clinical history. The development of any kind of biosensor without using representative samples for validation purposes ends all the possibilities of it reaching the market, even though the biosensor might show exceptional performance and other analytical features, such as robustness, portability or low cost.

Calibration is not well addressed in the biosensing applied to allergies because no specific standards are available for each allergen to date. To overcome this limitation, all biosensors use the WHO standard as a calibrator. This standard is a pooled human non-food-specific IgE. However, from the analytical point of view, the calibrator should be a food-specific IgE with the same affinity for the determinant as the IgE produced by patients who have suffered an allergic event. The development of similar specific IgE to the human-produced type is challenging, and it is very important

to obtain conclusive analytical and clinical results because the WHO standard is now an indirect and non-specific calibrator.

Regarding assay simplicity, most reported biosensors hardly include automation to achieve a format to be used to directly load low volumes of sample and go. This goal is very interesting when attempts are made to establish an assay concept with the capacity to be accepted by the end-user community. In line with this, microfluidics are suitable for simplifying device operations and process samples in parallel. However, major challenges as regards expected performance remain. One of the most significant one is dispensing and/or storing reagents. Many approaches have been studied, ranging from simple one-sample-chip to sophisticated platforms by simultaneously analysing several samples and targets.

Biosensing approaches are often published but, for different reasons, they have not yet reached the market. Our recommendation is to previously consider the development of the biosensor core, the final assay format, and the possibility of manufacturing the whole device (hardware and consumables) at a reasonable cost.

Further considerations must be examined to deliberate between automatic and manual assay formats. The first approach is the option requested by huge hospitals with the economical resources to buy automatic analyzers because many samples per day are processed. However, for the doctor-office or personal users, manual biosensors are desirable because they do not need sophisticated readers or high investments made in detectors or auxiliary equipment. It is also worth mentioning that the allergist must finally evaluate the use of the information displayed by biosensors to recommend the correct nutritional practice to allergic patients to live a healthy life.

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