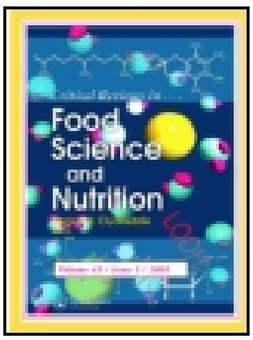

Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Jiayuan Xu, Yongli Ye, Jian Ji, Jiadi Sun & Xiulan Sun (2021): Advances on the rapid and multiplex detection methods of food allergens, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2021.1907736

To link to this article: https://doi.org/10.1080/10408398.2021.1907736

Published online: 08 Apr 2021.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2021.1907736

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REVIEW

Advances on the rapid and multiplex detection methods of food allergens

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ABSTRACT

With the gradually increasing prevalence of food allergy in recent years, food allergy has become a major public health problem worldwide. The clinical symptoms caused by food allergy seriously affect people's quality of life; there are unknown allergen components in novel food and hidden allergens caused by cross contamination in food processing, which pose a serious risk to allergy sufferers. Thus, rapid and multiplex detection methods are required to achieve on-site detection or examination of allergic components, so as to identify the risk of allergy in time. This paper reviews the progress of high-efficiency detection of food allergens, including enhanced traditional detection techniques and emerging detection techniques with the ability high-throughput detection or screening potential food allergen, such as xMAP, biosensors, biochips, etc. focusing on their sensitivity, applicability of each method in food, along with their pretreatment, advantages, limitation in the application of food analysis. This paper also introduces the challenges faced by these high-efficiency detection technologies, as well as the potential of customized allergen screening methods and rapid on-site detection technology as future research directions.

KEYWORDS

Bioinformatics; biosensor; DNA-based detection; food allergen; pretreatment; immunoassay; instrumental analysis

1. Introduction

Food allergens are food components that can induce abnormal reactions in the human immune system. Considered a serious public health issue, food allergy leads to heavy physiological and psychological burden in patients and has recently increased in prevalence in western countries (Koplin, Mills, and Allen 2015; Polloni and Muraro 2020; Teufel et al. 2007). Food allergens usually consist of proteins or glycoproteins with relative molecular masses ranging from 7,000 to 100,000, but food allergens with relative molecular weights up to 360 kDa also exist (Kabasser et al. 2020). According to the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee, more than 380 allergenic food proteins have been identified, and the number of food allergens constantly increase.

Numerous epidemiological studies have reported an increase in food allergy as the environment and human diet change. Food allergy sufferers have increased to 5%-10% of the total population in developed countries. Food allergy has affected almost 32 million of the population in the United States until the end of January 2020 (Pepper et al. 2020). In Chile, among developing countries, the hospitalization rate due to food-induced anaphylaxis in both adult and pediatric patients markedly changed from 0.8 per 1,000,000 inhabitants in 2001 to 3.0 per 1,000,000 inhabitants in 2017. In parallel with rising hospitalization rate, the number of sick leave claims due to food allergy had increased significantly during the 2003-2018 period, which also suggested an increase in food allergy (Hoyos-Bachiloglu et al. 2020). Studies found that children are more susceptible to food allergies than adults. According to two cross-sectional surveys of American households with children, the prevalence of reported children food allergy in the United States has increased from 3.9% in 2007 to 8% in 2011 (Gupta et al. 2011; Branum and Lukacs 2009). Ingestion of a specific food allergen by an allergic person generally induces clinical responses ranging from mild urticaria to fatal anaphylaxis (Dorris 2020). The cross-reactivity of some food allergens with aeroallergens can increase the prevalence of sensitization (Shoormasti et al. 2019). Food allergen avoidance is central to food allergy management (DunnGalvin et al. 2019), requiring reliable techniques in food allergen detection.

The generalized analytical approaches to food allergen detection are traditional enzyme-linked immunosorbent assays (ELISA) and real-time polymerase chain reaction (real-time PCR) (Sena-Torralba et al. 2020), which are high-precision techniques but usually relatively inefficient or unavailable for in situ tests. The presence of allergens in foodstuffs can be incidental to the preparation of different food products on the same production line (Roder et al. 2008). These food allergens can cause economic losses to enterprises and pose a threat to public health. To address this problem and meet the growing needs of allergen

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management, the use of reliable food labels that indicate the presence of allergens has been put into service in some countries. In the United States, the Food and Drug Administration (FDA) has required manufacturers to declare all allergens on the label since 1996. The European Union identifies 13 foods plus sulfur dioxide and sulfites as priority allergens that are required to be declared on the label. Some other countries also specify the priority allergens, like China, Japan, Australia, Canada, Korea, although the labeling regulatory frameworks vary from region to region (Gendel 2012). This approach relies on rapid, multiplex analytical methods for food allergen detection.

Based on an overview of the health effects of food allergens, this article reviews current pretreatment and detection methods for food allergen analysis, with a focus on rapid and multiplex detection methods of allergens published in the last five years; discusses the advantages, disadvantages and application of each method; and offers an outlook on challenges and solutions to allergen analysis and prospects for the application of detection techniques. The examined allergens mentioned in this review were mainly included in the 13 allergenic foods identified by the European Union.

2. Challenges in food allergy management

Food allergy is an abnormal immune response to some given foods or ingredients, including immunoglobulin E (IgE)-mediated, non-IgE mediated or a combination of both. IgE-mediated type-I hypersensitivity is considered to be the most common type of food allergy. Food antigens in the gastrointestinal tract are presented to T cell by dendritic cells (Joeris et al. 2017). For susceptible individuals, these allergens aberrantly activate a Th2-type effector T-cell response, which leads to the secretion of allergen-specific IgE (Figure 1) (Wambre et al. 2017). The specific IgE is bound to the high-affinity IgE receptor on mast cells and basophils. When exposed again to the allergen, the IgE bound to these cells recognizes the allergen, inducing mast cell and basophils activation and the rapid release of preformed and newly synthesized mediators, which lead to various symptoms of clinical allergy, such as diarrhea, urticaria, anaphylactic shock, and death (Kanagaratham et al. 2020; Church et al. 2018; Reber, Hernandez, and Galli 2017). Non-IgE-mediated food allergy is mainly manifested by different forms of intestinal allergic symptoms, such as food protein-induced enterocolitis syndrome and food protein-induced allergic proctocolitis, but the mechanism is not yet well studied (Meyer et al. 2019). An oral immunotherapy called PALFORZIA was approved by FDA in January 2020 to ease allergic reactions caused by accidental exposure to peanut. PALFORZIA could increase tolerance dose of peanut protein in children and adolescents with peanut allergy, with a predictable safety profile that improved with treatment (Hourihane et al. 2020; The PALISADE Group of Clinical Investigators 2018). However, the immunotherapy is to be used in conjunction with a peanut-avoidant diet (FDA 2020), food allergen avoidance continues to be the primary means of preventing food allergies.

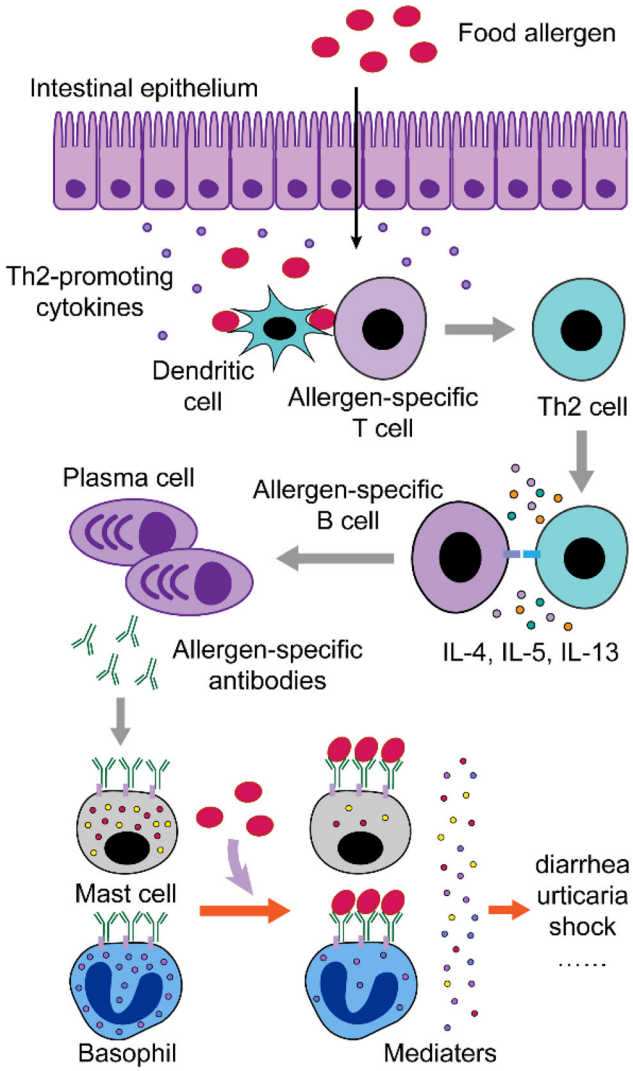

Figure 1. Induction of IgE-mediated food allergy.

Cross-contamination inevitably occurs in food production. It poses a risk of unknown magnitude to food allergy sufferers, which can happen in farms, food production plants, and restaurants (Taylor and Baumert 2010). Various allergenic food proteins are universally recognized as stable to acid denaturation and digestive proteases. Thus, these proteins can potentially remain intact from feed to animal tissues (Toomer et al. 2020). Besides the allergen cross-pollution caused by production-line sharing, the addition of allergenic proteins can present a health risk to allergic individuals. For instance, soybean protein is often added to processed meat products because of its excellent gel formation and water-holding capacity to improve the texture, stability and other quality indicators of meat products. However, the addition of soybean protein may pose a safety risk to consumers suffering from food allergies (Canan et al. 2020). Cross-contamination may also occur during cooking because of the alternate use of kitchenware (Studerus et al. 2018). Cross-contamination can cause foods to be mixed with allergens other than their regular ingredients, creating an obstacle to the timely identification of allergenic foods.

Novel food presents a new challenge for food allergy management. With an increasing demand for sustainable

food sources, novel food proteins or production technologies have been used to replace traditional food sources, such as insects, algae, genetically engineered food, artificial meat. About two billion people consume insects worldwide. However, some insect allergens have been identified, involving arginine kinase and tropomyosin, which have shown cross-reactivity in patients allergic to seafood (De Gier and Verhoeckx 2018). Moreover, using genome sequencing and sequence alignment, researchers found that the red algae and *Fusarium sp*. (as an alternative for Quorn in the United Kingdom) were 35% to 100% identical to food allergens in the AllergenOnline.org database, and proteins that were 70% identical exhibited cross-reactivity to matched food allergens (Abdelmoteleb et al. 2021). In order to avoid health risks to allergic populations and reduce economic losses due to food allergy events, it is important to timely detect cross-contamination in foods and screen for potential allergens in emerging food, indicating that advanced detecting techniques that can quickly identify food allergens need to be explored, multiple allergens should be simultaneously inspected, or latent allergenic proteins in foodstuffs have to be investigated.

3. Pretreatment for allergen detection

The techniques used to inspect allergenic foods can be generally classified into two categories: targeted allergen proteins and targeted allergen DNA fragments. According to the test principle, these two types need different preprocessing methods.

3.1. Targeted allergen proteins

This technique can be subdivided into two main types: affinity-based assays and instrumental analysis. Affinity-based assays, such as immunoassays and biosensors, follow the principle of a specific affinity interaction between biomolecules. Thus, pretreatment for affinity-based assays needs to ensure the suitable structural conformation of targeted allergenic proteins (Galan-Malo et al. 2017). Food sample preparation generally includes buffer extraction, centrifugation, and filtration, with extraction being the most crucial procedure to pretreatment. The efficiency of extraction mainly depends on the composition of the buffer salt solution, extraction duration, and shaking frequency. Various buffer solutions can be used to extract allergens, including Tris-HCl, sodium borate buffer, phosphate buffer saline, and other customized buffers (Jayasena et al. 2019; Polenta et al. 2010; Segura-Gil et al. 2019), and the pH of the buffer is set to approximately 7.2–9.2. The buffers required for different allergen extraction methods are similar, but the ionic strength and pH of these buffers may vary. To improve the extractability of pecan protein with low solubility at low ionic strength, the sodium chloride concentration in the buffer was increased from 0.2 to 4 mol/L that resulted in a 9.2-fold increase in protein solubility (Sathe et al. 2009). The extraction duration usually exceeds 30 min but also depends on the temperature. For thermostable proteins, such as peanut and pecan proteins, increasing the extraction temperature from room temperature to 60 °C could shorten the extraction duration from 1 h to 10 min (Liu et al. 2019). Adequate vibration usually helps to improve extractability but may not benefit some proteins, like egg proteins (Galan-Malo et al. 2017).

The pretreatment procedure for the instrumental analysis of food is similar to that for affinity-based assays, except that instrumental analysis requires enzymatic digestion after extraction; for fatty food matrices, the samples need to be defatted in hexane before extraction (Ma et al. 2020). Protein enzymatic digestion commonly uses trypsin as the specific cleavage enzyme, normally incubated for 12–18 h at 37 °C. To reduce the incubation duration, immobilized trypsin is a suitable alternative to free trypsin, which can decrease the incubation time to 15 min (Qi et al. 2019). Moreover, incorporating solid-phase extraction into pretreatment can further improve the sensitivity of instrumental analysis and shorten the detection time (De Angelis, Pilolli, and Monaci 2017; Gu et al. 2018).

3.2. Targeted allergen DNA fragments

Concerning DNA-based detection methods, the chosen DNA target and DNA isolation technique significantly influence the efficiency of these techniques. DNA targets can be genes that encode allergenic proteins or other specific DNA markers such as chloroplasts and mitochondria (Zhang et al. 2019; Holzhauser 2018). DNA targets are selected based on analytical needs and methods. The general workflow for choosing a suitable DNA target under DNA-based detection techniques is presented in Figure 2. By testing and optimizing the specificity of DNA markers iteratively, applicable DNA primers and probes can be selected. A reliable DNA-based detection method can then be established.

An ideal DNA extraction method for high-efficiency detection of allergenic foods should be rapid, simple, and capable of minimizing experimental interference factors. The traditional DNA isolation method is the cetyltrimethyl ammonium bromide method, which can extract DNA from protein and sugar by forming a complex with DNA. This approach is an efficient means of obtaining highly purified DNA, although the operation involves complexity and requires a toxic reagent such as chloroform (Waiblinger et al. 2019). To simplify DNA extraction, various kits are commercially available. These kits can suitably extract DNA from different food matrices. The frequently used kits reported include DNeasy kits (Plant Mini Kit, mericon Food Kit, Blood and Tissue Kits), the Wizard DNA Clean-up System kit, the NucleoSpin®Food DNA extraction kit, and the AxyPrep Multisource Genomic DNA Miniprep kit. Although one kit can be used in several types of samples, divers DNA extraction kits may have to be assembled for multiplex detection of allergens (Miyazaki et al. 2019). The phenol–chloroform method can also be utilized to extract DNA from peanuts, soybeans, and sesame (Yuan, Fang, et al. 2019). Moreover, to ensure the accuracy of detection results, DNA extraction should be performed with purity, structural integrity and amplification efficiency identification. Determining the UV-Vis absorption at 260/280 nm is

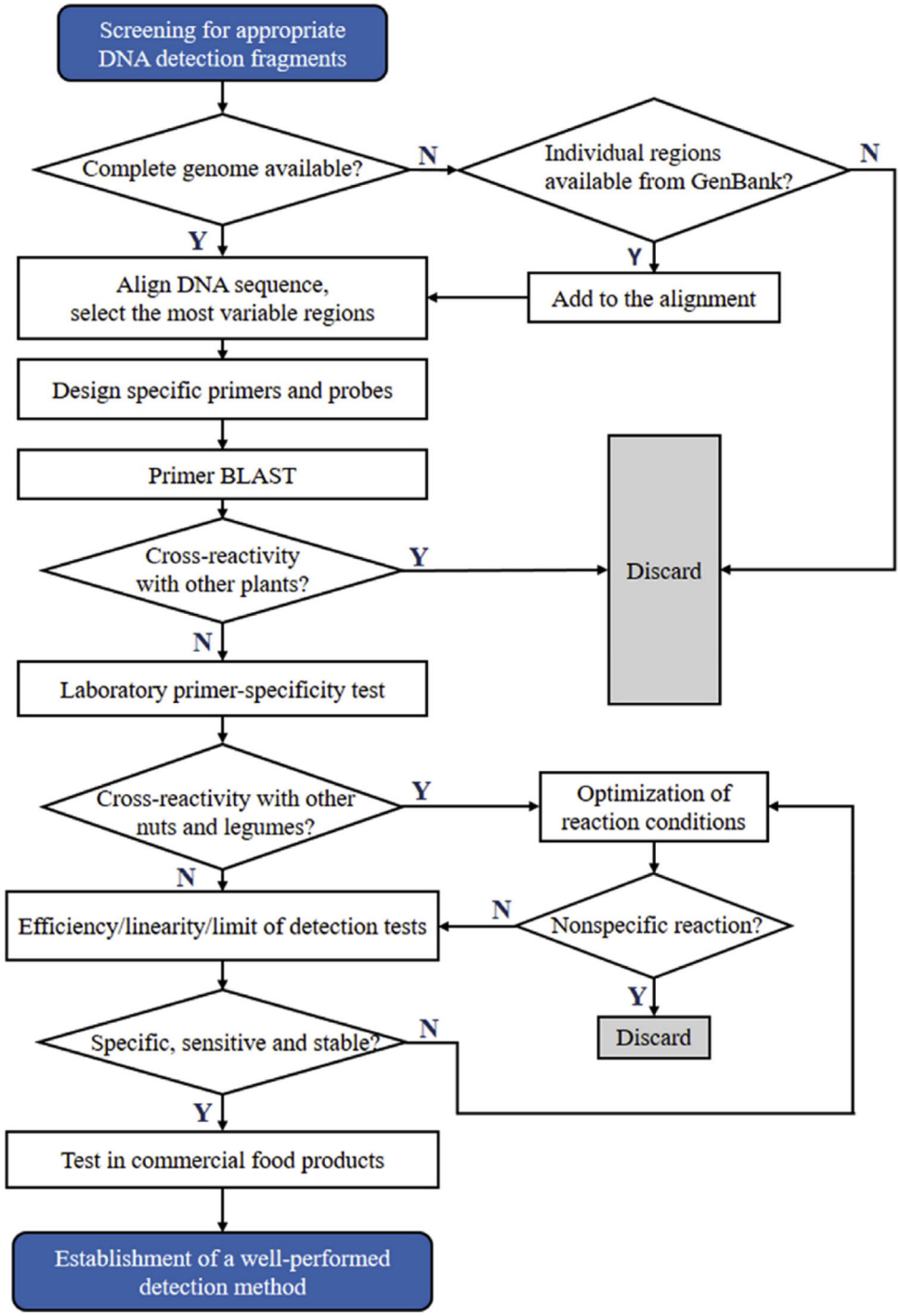

Figure 2. Workflow to identify and select suitable DNA markers for the DNA-based detection methods by Zhang et al. (2019).

the most commonly used method to identify purity. According to European Network of GMO Laboratories (2015), DNA structural integrity is definitized as breakage of genomic (high molecular weight) DNA into smaller DNA fragments. The minimum size of most DNA fragments should be larger than the size of the amplification fragment generated by the PCR module used in the subsequent analysis, which could be checked by comparison with a reference nucleic acid size marker. Amplification efficiency, not applicable to qualitative methods, is calculated from the slope of the standard curve obtained after a decadic semi-logarithmic plot of quantification cycles (Cq) values over the DNA copy numbers/quantity. The average value of the slope should be in the range of −3.1∼-3.6, corresponding to amplification efficiencies of 110% to 90%.

$$Effciency[\%] = \left(10^{\left(\frac{-1}{slope}\right)} - 1\right) \times 100 \quad (1)$$

4. Detection methods of food allergens

4.1. Immunoassays

Based on the high affinity and specific interactions of the antibody with target allergens, immunoassays are widely

Table 1. ELISAs developed for food allergen detection in recent years.

Target protein	ELISA format	Sensitivity level	Detection time	References
fish parvalbumin	immunomagnetic beads-derived ELISA	LOD = 4.29 ng/mL	6 hours	(Yanbo et al. 2020)
	commercial ELISA kit	LOD = 1 mg/kg LOQ = 4 mg/kg	–	(Fernandes et al. 2017)
	sandwich ELISA	LOD = 0.1 mg/mL	8 hours	(Chen and Hsieh 2014)
β-conglycinin	sandwich ELISA	LOD = 0.9 ng/mL LOQ = 2.1 ng/mL	5 hours	(Segura-Gil et al. 2019)
	competitive ELISA	LOD = 0.65 ng/mL	1 day	(Liu et al. 2012)
	direct ELISA	LOQ = 20 ng/mL	1 day	(Zhang et al. 2016)
pecan	competitive ELISA	LOQ = 0.1 mg/g	5 hours	(Polenta et al. 2010)
	sandwich ELISA	LOD = 0.5 mg/kg LOQ = 1.5 mg/kg	2 hours	(Liu et al. 2019)
walnut protein	competitive ELISA	LOD = 1.1 ng/mL LOQ = 2.2 ng/mL	1 day	(Wang et al. 2014)
	quantitative ELISA	LOD = 10 ng/mL LOQ = 25 ng/mL	1 day	(Fang et al. 2015)
Soybean 2S Albumin Gly m 8	sandwich ELISA	LOD = 10 pg/mL LOQ = 65 pg/mL	1 day	(Ueberham et al. 2019)

used in food allergen detection, including ELISA, lateral flow immunoassay (LFIA), and multianalyte profiling (xMAP).

4.1.1. ELISA

Owing to its simplicity and high sensitivity, ELISA is the most commonly employed technique for food allergen detection in food safety risk management. ELISA is based on the recognition and binding of specific regions of antigens to antigen-specific antibodies (Holzhauser et al. 2020). The antibodies are generally labeled with enzymes such as horseradish peroxidase (HRP) and alkaline phosphatase, which can act with specific substrates to produce concentration-dependent color changes (Sena-Torralba et al. 2020). The analytes can be any food allergen or a mixture of several allergens and can be detected in complex food matrices (Holzhauser and Vieths 1999) or on the working surface (Galan-Malo et al. 2017). However, ELISA methods, which rely on the specific interaction between antibodies and antigens, can yield inaccurate results because of the cross-reaction of the antibody with proteins from other foods. Thus, the choice of antibody and the target protein is relevant to the detection efficiency. Ueberham et al. (2019) raised a monoclonal antibody (mAb) against Gly m 8, a soybean 2S albumin, to develop a sandwich ELISA for tracking soybean in processed food. The antibody was highly specific without any cross-reaction with other legume proteins. With the aforementioned technique, Gly m 8 could be detected below 1 ppm in soybean milk and below 10 ppm in texturized vegetable proteins, such as tofu. Liu et al. (2019) evaluated a commercially available mAb-based direct pecan ELISA kit (MonoTrace®) purchased from BioFront Technologies (Tallahassee, Florida). When tested at 100,000 ppm, the detected antibody showed no cross-reactivity with 155 foods. Relying on the high specificity and affinity of the antibody, the ELISA kit showed high specificity and sensitivity, with a limit of detection (LOD) equal to 0.5 ppm of pecan soluble proteins. Table 1 compared the performance of different ELISA reported in the literature.

The results of ELISA detection can be influenced by several factors. The ELISA format affects the determination of food allergens (Table 1) (Montserrat et al. 2015). The sandwich format can recognize the addition of 0.005% soybean proteins in pasteurized sausages, and the competitive format detects only 0.1% of the same (Segura-Gil et al. 2019). Several processing conditions can reduce the estimated value of allergens. Thermal treatment, such as roasting, can reduce the solubility of food proteins and change the local structure of the protein that reacts with the antibody. In dark roasted peanut flour, most commercial ELISA kits failed to quantify peanut allergen below 25 ppm (Jayasena et al. 2019; Perner et al. 2019).

Further research is underway to improve the efficiency of ELISA. Immunomagnetic beads typically exhibit high colloidal stability, abundant binding sites, and sensitive magnetic responsiveness. Combining ELISA with immunomagnetic beads can reduce the operation time (Table 1). Wang, Qi et al. (2020) used amino magnetic beads (MBs) to capture parvalbumin (PV) from fish samples. Depending on the magnetic properties of MB, PV can be easily separated from the sample by an applied magnetic field, reducing the number of steps of sample processing. The sensitivity of ELISA can be enhanced by signal enhancers such as graphene oxide (GO) and gold nanoparticles (AuNPs). GO and AuNPs possess distinct optical, electronic, or structural properties and can bind to biomolecules like antibodies. In a PV ELISA, by combining primary mAb and HRP-labeled secondary antibodies to GO and AuNP, respectively, the signal of allergen binding to specific antibodies could be amplified, thus decreasing the LOD of the method to 4.29 ng/mL of purified fish PV in Tris-HCl (pH 7.5). The method showed feasibility in the detection of PV in real fish samples, and the recovery rates ranged from 89.92% to 115.37% . The application of the novel antibody can avoid the use of live animals and reduce the cost of ELISA. Madrid et al. (2018) engineered an affinity probe for walnut proteins in *Pichia pastoris* to produce the in vivo Juglans regia Biotinylated multimeric recombinant antibody. The recombinant antibody can be used to develop a direct ELISA to detect walnut in food matrices.

4.1.2. Lateral flow immunoassay

LFIA is a semiquantitative tool for food allergen detection, which uses visual signals to indicate test results. Similar to ELISA, LFIA operates on the principle of the specific interaction between allergens and antibodies, but the visual signal is based on the antibody-coated colored particles. The test strip is the most commonly used form of LFIA application and generally consists of a sample pad, a test line (T line), and a control line (C line). After a sample solution is dropped onto the pad, the T line indicates the presence of specific allergens in the sample. To ensure the validity of the test strip, the C line is covered with anti-primary mAb antibodies and shows a red bond regardless of the presence of allergens.

Owing to its quick response (within several minutes), ease of use, visible test results, and sensitivity, LFIA can significantly improve applications for on-site detection of allergens in food (Galan-Malo et al. 2019). As a semiquantitative method, LFIA has a normal LOD of 0.5-5 mg/kg, but its quantification of food allergens is limited. This technique has been used in monitoring different food allergens, such as fish protein, β-conglycinin, casein, and crustacean tropomyosin, among others (Cheng et al. 2017; Jauset-Rubio et al. 2016; Shibahara et al. 2014). Wang, Li, et al. (2017) established an LFIA strip for the rapid detection of the soybean allergen β-conglycinin with the LOD of 1.66 mg/kg. The strip was made in a sandwich format, using the colloidal gold-labeled mouse anti-β-conglycinin mAb and the rabbit anti-β-conglycinin polyclonal antibody (pAb). When the sample was positive, a red bond appeared in the T line, and the color deepened as the β-conglycinin concentration of the sample increased (Figure 3A). Testing was completed after about 10 min. Moreover, LFIA can simultaneously analyze multiple allergens in food samples (Masiri et al. 2016). Galan-Malo et al. (2019) developed a duplex LFIA test strip for the simultaneous detection of milk allergens in food. The assay detected β-lactoglobulin (0.5 mg/kg) and casein (2 mg/kg), with two T lines in the strip (Figure 3B). LFIA can also detect allergenic food residues on a working surface. Galan-Malo et al. (2017) optimized a sampling method based on swabbing to enable LFIA to analyze milk and egg residues on stainless steel and Bakelite surfaces. Milk and egg ingredients were detected at 6 and 0.7 ng/cm^2, respectively. Relying on a device-independent color space, LFIA yields results that can be analyzed by smartphone, suggesting the suitability of LFIA for controlling allergy risk (Ross, Salentijn, and Nielen 2019).

Colored particles affect the sensitivity and potential application of LFIA. AuNPs are also used as labeling particles in LFIA; however, their intrinsic limitations, such as monochromaticity and instability, limit sensitivity and the detection limit (Quesada-Gonzalez and Merkoci 2015). To address this problem, a number of novel materials have been invented as LFIA signal labels, including fluorescent microsphere, magnetic nanoparticles, and quantum dots (QDs). Cheng et al. (2017) established an LFIA test strip with fluorescent microspheres as labels to rapidly detect casein in milk. The strip had the LOD of 100 ng/mL in defatted milk, which was lower than that of the colloidal gold-based LFIA. Using QDs to label anti-tropomyosin pAb, Wang et al. (2019) developed a QD-based fluorescent LFIA strip to detect the major crustacean allergen tropomyosin. The strip followed a competitive format, with the antigen encapsulated at the T line (Figure 3C). QD showed excellent optical properties and stability, significantly enhancing the sensitivity of the test strips. The assay exhibited the LOD of 0.5 μg/mL visually and 0.05 μg/mL with an instrument of purified tropomyosin in PBS (Figure 3D). When used to detect extracts from real food samples, the strips were consistent with validated ELISA kit (Qingdao Marine Food and Nutrition Health Innovation Institute, Qingdao, China), which means that the strips had adequate sensitivity to monitor tropomyosin in foods. Some instruments can also enhance the signal of colloidal gold-based test strips, improve the sensitivity of the test strips, or render them suitable for quantitative analysis. Xi and Yu (2020) modified colloidal gold in β-conglycinin LFIA test strips with molecule 4-aminothiophenol to prepare a Raman immunoprobe. Based on Raman enhancement signals, the strips performed quantitative detection within a practical working range of 160 ng/mL-100 μg/mL of β-conglycinin in PBS. The strips showed good accuracy and recovery (97.5-116%) in the detection of skim milk containing β-conglycinin, suggesting that the test strips had a promising application.

4.1.3. xMAP techniques

The xMAP technique is a sensitive, accurate, and high-throughput immunoassay that can achieve multiplex quantification of different allergens. xMAP is based on fluorescent or MBs conjugating allergen-specific antibodies with biotinylated antibodies and streptavidin-labeled fluorochrome as detectors (Filep et al. 2018; Oliver et al. 2017). Benefiting from its modular feature, the xMAP shows potential for a wide range of applications. Single-laboratory validation and multilaboratory testing verified the superior performance of the xMAP assay under slightly modified conditions or by employing analysts with varying levels of proficiency, suggesting its adequate stability in meeting practical analytical needs (Garber et al. 2020; Nowatzke et al. 2019; Rallabhandi et al. 2020).

Cho et al. (2015) developed an xMAP assay for multiplex detection of food allergens. By conjugating 30 different established antibodies to different color-coded MB sets, the assay could simultaneously quantify 15 food allergens and perform multiple tests for each analyte without additional duplicate testing. The beads were mixed with biotinylated detector antibodies and streptavidin phycoerythrin and then analyzed by the Bio-Plex 200 system to simultaneously examine up to 500 different biomolecules in a single trace sample. Testing was completed in less than 6 h; the LOD of all 15 allergens reached 5 ng/mL in PBST, which was close to that of ELISA. Raw and roasted sesame possess different antigenicity, coupling beads with three different anti-sesame antibodies could make sesame the sixteenth allergenic food detectable by xMAP (Cho et al. 2020). Advances in microsphere technology have also expanded the application of

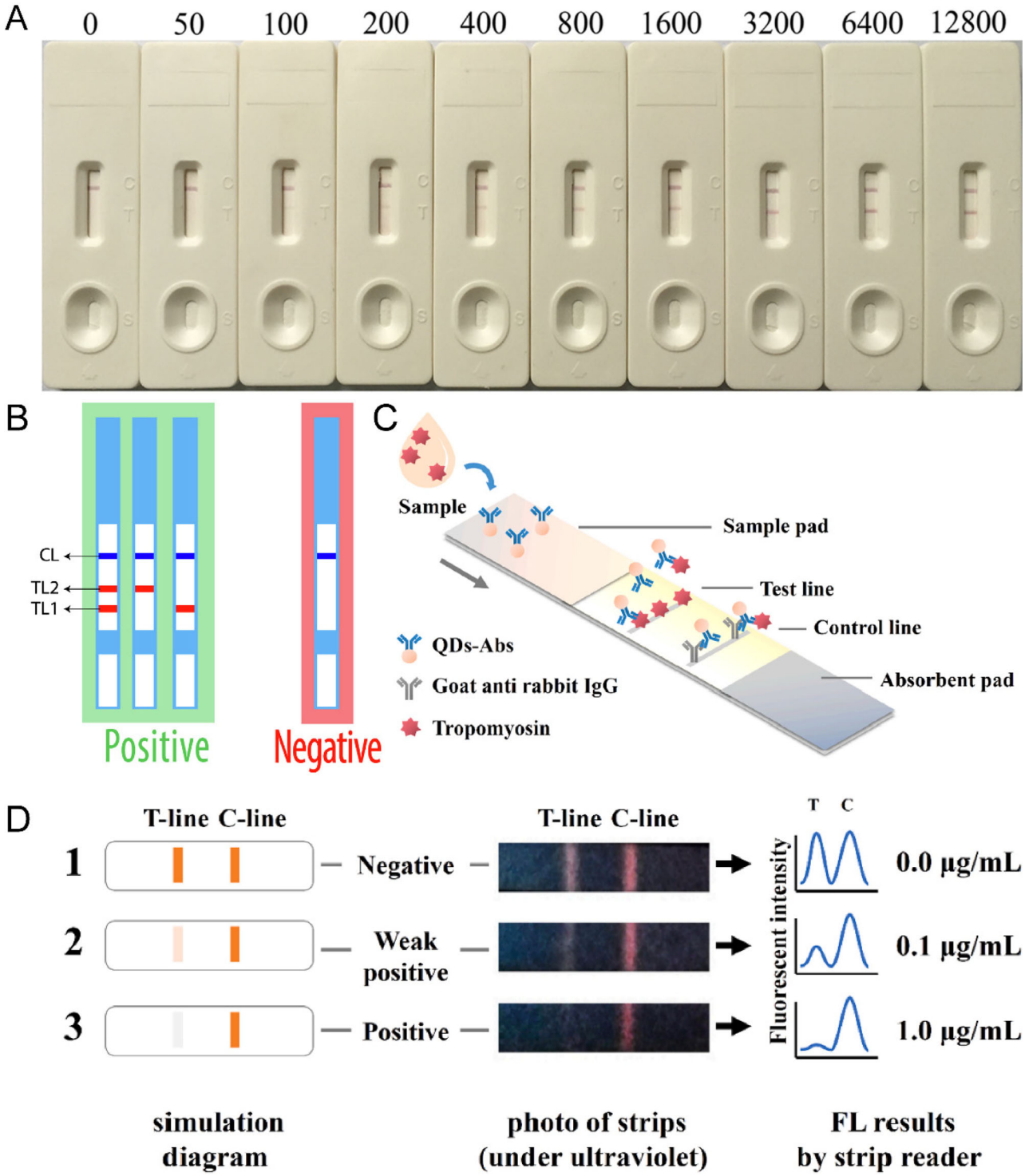

Figure 3. Schematic representation and devices of different lateral flow immunoassay. (A) The real test strips of lateral flow immunoassay to detecting β-conglycinin by Wang, Li, et al. (2017). (B) Scheme of a duplex lateral flow test on the strip by Galan-Malo et al. (2019). (C) Schematic illustration of the QD-based competitive reorganization on the strip. (D) The illustration of hypothetical and real photos of the QD-based competitive lateral flow strip by Wang et al. (2019).

xMAP into a comprehensive approach suitable for most common allergens. Oliver et al. (2017) modified fluorescent beads with allergen-specific mAb for the simultaneous detection of peanut, milk, egg, and shrimp tropomyosin. The target allergens were detected using biotinylated antibodies and streptavidin-conjugated fluorochrome. The assay showed the LOD of 20 pg/mL. By measuring allergen content in different samples, including samples spiked with purified allergens and allergen-induced food matrices, the multiplex method showed a high correlation with allergen specific ELISA.

4.2. Biosensors

Biosensors provide a major alternative for simple, rapid, affordable, and high-specificity allergen detection (Nehra et al. 2020). By the integration of receptors and transducers,

biosensors can transform the interaction between allergens and antibodies or aptamers into a measurable chemical-physical signal, which is proportional to the concentration of target food allergens. Depending on the type of transducer, biosensors for allergen detection are classified into two major groups based on the type of transducer: electrochemical and optical sensors. Moreover, as novel and sensitive receptors, cells have recently been used in biosensors for allergen detection.

4.2.1. Electrochemical sensors

Compared with ELISA methods, electrochemical sensors exhibit high sensitivity, specificity, and simplicity of operation, and their detection time is mostly as short as that of LFIA, showing potential prospects in allergen monitoring in foodstuffs. Electrochemical sensors can be further classified, based on the mode of electrochemical signal transduction, into amperometric, voltammetric, and impedance types. Amperometric sensors for allergen detection are commonly based on magnetic particles and disposable screen-printed carbon electrodes (Montiel et al. 2015, 2016). Angulo-Ibanez et al. (2019) constructed a sandwich-format immunoassay on the surface of MBs to detect shrimp tropomyosin in a complex food matrix. Tropomyosin was captured by mouse anti-tropomyosin antibodies immoblized on MBs and subsequently detected by rabbit anti-tropomyosin antibodies, which were further recognized by HRP-conjugated anti-rabbit antibodies. HRP could catalyze the redox reaction of H_2O_2 with hydroquinone, generating a microcurrent signal. Coupling the MBs onto disposable screen-printed electrodes could sense the current signal, thus enabling tropomyosin detection. The immunosensor had the LOD of 47 pg/mL in PBST containing 1%BSA and could successfully detect tropomyosin in both raw food materials and processed foods within 4 h.

A key procedure in impedimetric sensors is electrochemical impedance spectroscopy (EIS). By applying a mild AC voltage stimulus, EIS can reflect the electrical properties of the sensor electrode surface, allowing the direct monitoring of the interaction between the bioreceptor and its target without labeling (Chen et al. 2012; Trashin et al. 2015). Malvano et al. (2017) demonstrated a label-free impedimetric biosensor, built on a gold electrode, for gluten detection in food products. After cysteamine modification, the gold electrode was immobilized with activated aptamers that could recognize gliadin from gluten sources. Once bound to gliadin, the aptamer changed in structure, consequently altering the electrical properties of the electrode surface. This occurrence allowed gluten detection via EIS. Incubation of markers was unnecessary that significantly improved the detection efficiency. The aptasensor could complete a test within 45 min. The sensitivity of this sensor could be further enhanced by modifying a layer of poly(amidoamine) dendrimers on the electrode before immobilizing the aptamers, exhibiting linearity within the ranges of 5–50 and 50–1000 μg/L in the gliadin standard of Prolamin Working Group, with the LOD of 5 μg/L corresponding to 5 ppm of gluten in foods.

Similar to impedance sensors, voltammetric sensors can monitor the binding of biomolecules on the electrode surface by using voltammetric techniques, such as square wave voltammetry (SWV) and differential pulse voltammetry (DPV) (Eissa et al. 2013; Kilic et al. 2020). Eissa and Zourob (2017) used a selective DNA aptamer to develop a rapid voltammetric biosensor for detecting the milk allergen β-lactoglobulin. The specific aptamers were immobilized on the graphene-modified screen-printed carbon electrodes. When the target was bound to the aptamer, the negatively charged aptamer was detached from the electrode. The process was monitored through changes in the SWV reduction peak signal of the ferricyanide/ferricyanide redox coupling, thereby detecting β-lactoglobulin in the sample. The one-step biosensor with high sensitivity and specificity detected the milk protein β-lactoglobulin within 20 min; the LOD was 20 pg/mL of β-lactoglobulin in binding buffer, which consisted of 50 mM Tris, 150 mM NaCl, and 2 mM $MgCl_2$. When applied to detect β-lactoglobulin in spiked food extracts, the biosensor showed great recovery from 90% to 95%, indicating the promising application of the developed biosensor in the detection of milk allergen in food samples. The testing results of these electrochemical sensors were highly consistent with those obtained using the validated ELISA method.

4.2.2. Optical sensors

Optical sensors usually have two kinds of signal transduction: detection changes in light absorption and detection differences in light output such as fluorescence (Alves et al. 2016). Most optical biosensors for food allergen detection are based on surface plasmon resonance (SPR), which exhibits high selectivity, high sensitivity, real-time detection, and quick response apart from being label-free (Ashley et al. 2017; Zhou et al. 2020). Ashley, D'Aurelio, et al. (2018) developed a biosensor for β-lactoglobulin detection based on SPR. The sensor was sensitive and label-free with the LOD of 0.164 μg/mL, and aimed at monitoring traces of β-lactoglobulin in rinse water samples, and could perform real-time measurement, showing potential for application in on-line systems. Recently, aptamers have been widely used in optical sensors. Compared with antibodies, aptamers have drawn more attention because of their low cost, small size, low cross-reactivity, and ease of production (Khedri et al. 2018). Chinnappan et al. (2020) used an aptamer as the receptor and a fluorescein dye-labeled GO as the signal transducer to demonstrate rapid and sensitive detection of tropomyosin derived from shrimps in various foodstuffs. When the target allergen was present in the samples, the fluorescence was restored owing to the competitive binding of the aptamer to GO. The sensor could complete a tropomyosin test within 30 min, with the LOD of 2 nmol/L in binding buffer (50 mM Tris, 150 mM NaCl, 2 mM $MgCl_2$, pH 7.4), and the short duration was attributable to the use of aptamers. Zhang et al. (2018) conjugated tropomyosin aptamers on the surface of functionalized magnetic nanoparticles (MNPs) to construct aptamer-MNP complexes as detection receptor. Upon interaction with tropomyosin in food matrix, the complex underwent a conformational

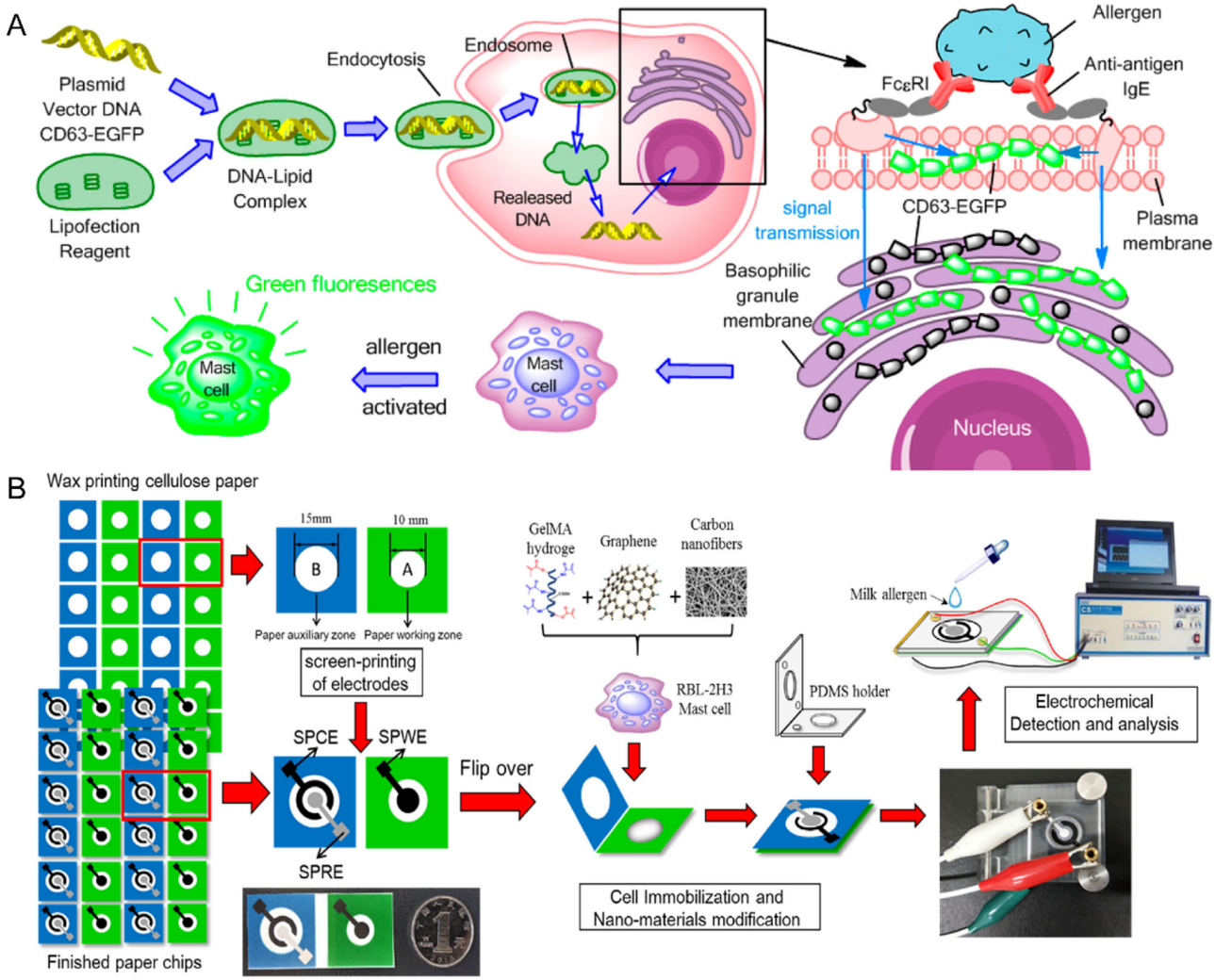

Figure 4. **Figure 4.** The fabrication of two types of cell-based biosensors. (A) Schematic illustration of the working principle of a cell-based fluorescence sensor by Jiang et al. (2014). (B) Schematic representation of the fabrication and assay procedure of the electrochemical mast cell-based paper sensor by Jiang et al. (2019).

change, causing the release of aptamers from the surface of MNPs. The released aptamer reacted with OliGreen dye to produce specific fluorescence, thus reflecting tropomyosin concentration in the sample. The sensor was successfully applied to the detection of tropomyosin in ketchup, mayonnaise, and shrimp paste with the LOD of 77 ng/mL and the limit of quantification (LOQ) of 0.4 μg/mL in the food extracts, and the detection could be completed within 3 h.

Moreover, some novel materials are used as receptors in sensors. Owing to the high affinity and selectivity toward target allergens, molecularly imprinted polymer-nanoparticles (nanoMIPs) can significantly improve the sensitivity of optical biosensors. Ashley, Shukor, et al. (2018) synthesized nanoMIPs with high binding affinity to the bovine protein α-casein by solid-phase blotting. The nanoMIPs were then integrated with a label-free SPR-based biosensor. The simple affinity biosensor had the LOD of 0.127 ppm and recovery from spiked food production line washing wastewater within 87%-120%.

4.2.3. Cell-based sensors

As living organisms, cells used in biosensors have distinct characteristics. Most affinity-based allergen detection methods are based on the interaction between antigenic epitopes and antibodies or aptamers. Thus, these techniques may fail to detect transformed antigenic epitopes despite the existing allergenicity. Some living cells show an immunobiological response to allergenic elements, which can indicate the immunopathological effects of food allergens in various food matrices (Rallabhandi et al. 2019). These cells, such as mast cells and basophils, are generally related to the immune system. Jiang et al. (2014) developed a rat basophil-based fluorescence sensor to detect PV. The cell was transfected with a CD63-enhanced green fluorescent protein (EGFP) plasmid and then activated by anti-PV IgE. Upon PV stimulation, activated basophils degranulated and expressed CD63 on the cell surface and EGFP intracellularly, producing green fluorescence (Figure 4A). The cell-based fluorescence sensors can visualize detection results with the LOD of 0.35 ng/mL in fish PV standard solution. The test results of the sensor for PV content in carp, silver carp, and carp were similar to those reported, indicating that the biosensor could be effectively applied to the detection of actual allergen samples. Igarashi et al. (2018) constructed an allergen detection system based on human mast cells. After being introduced with the nuclear factor-kappa B-responsive

luciferase reporter gene, the human induced pluripotent stem cell line differentiated into mast cells (iPSC-MCs). iPSC-MCs were sensitized with serum from patients with allergies. Histamine, β-hexosaminidase, and luciferase were secreted when sensitized iPSC-MCs were exposed to the corresponding allergen. The presence of allergens in the sample could be determined by detecting the release of these factors.

Cell-based sensors have been employed to detect different allergens, such as casein, gluten, shrimp tropomyosin, PV, ragweed pollen, cedar pollen, mites, and house dust (Jiang et al. 2015; Liu et al. 2008). Cell activation can be evaluated based on cytokine release and changes in fluorescence or impendence. Jiang et al. (2019) developed a disposable electrochemical MC-based paper sensor to detect casein. The paper sensor was modified with a graphene/carbon nanofiber/gelatin methacryloyl hybrid material to enhance its conductivity and biocompatibility. The paper sensor was divided into two parts—A and B. The working electrode and the mast cell were immobilized in part A. Cyclic voltammetry and DPV reflected the state of the cells in the paper sensor; the current peak detected was proportional to the number of cells (Figure 4B). When mast cells were exposed to casein, the current peak showed a significant decrease and was inversely proportional to the casein concentration in the sample within the range of 0.1–1 μg/mL; the LOD of the sensor was 32 ng/mL in PBS. This convenient design provided a new idea for the development of cell-based sensors and made it possible to use them for in situ detection.

Moreover, a cell-based sensor can potentially detect unknown allergens. Jiang et al. (2016) developed a cell-to-cell sensor by co-culturing MCs with macrophages to simulate the allergic response in the organism. In the absence of anti-dinitrophenylated bovine serum albumin (DNP-BSA) IgE activation, the cell sensor could be stimulated by DNP-BSA to achieve changes in impedance and secrete inflammatory factors, providing a method for the application of cellular sensors in allergen detection.

4.2.4. Biochips

Biochips are analogous to the integration of biosensors or biochemical analyses (e.g., ELISA) on the surface of the chip, based on the specific interaction between biological molecules. Owing to their notable advantages—automation, high throughput, rapid response, and multiplex detection—biochips have been widely used in food safety management, including food allergen detection (Su, Zhang, and Peng 2018). The commonly used biochips for allergen detection are the gene chip, protein chip, and microfluid chip.

4.2.4.1. Gene chip. Gene chips generally rely on nucleic acid amplification and hybridization. Relying on the use of visual transduction, such as fluorescent probes and film interference, gene chips can be viable for the optical detection of food allergens. Yuan, Kong, et al. (2019) designed a GO-based paper chip that integrated the hybridization chain reaction and fluorescence resonance energy transfer in one device to simultaneously detect peanut and soybean DNA. In the absence of target DNA, the hairpin DNA probes attached to the GO surface, which quenched fluorescence. Inversely, the probes were combined with complementary target DNA and became double-stranded DNA, which would detach from GO, thereby generating a strong fluorescent signal. The chip exhibited high specificity, with the LOD of 1 nmol/L in a standard solution of the target DNA. Wang et al. (2011) developed an optical thin-film biochip for multiplex inspection of eight food allergens, based on the difference in the interference pattern of light. The method required samples amplified by two tetraplex PCR systems. The nucleic acid hybrids were then transformed into molecular thin films via enzymatic reaction. Varying the molecular weight of the thin films could change the interference patterns used as detection signals (Figure 5A).

4.2.4.2. Protein chip. The protein chip is an integrated technology to determine protein interaction. The technology depends on modified support surfaces, such as glass slides, silicon wafers, and DVDs, which are attached to specific probes. Depending on the detection object, the probes can be allergenic proteins or specific antibodies (Garib et al. 2019). Wang, Zhu, et al. (2017) presented a label-free biochip for the rapid detection of soybean allergen, Gly mBd 30 K (P34), by using a silicon wafer as a support surface and two monoclonal antibodies as capture probes. The inspection results could be reflected directly by a grayscale on the wafer surface with a detection time of 15 min and sensitivity of 1 μg/mL of P34 in PBST. The results of the chip were consistent with those obtained using the Fast Soya ELISA kit (R-Biopharm, Germany) for the detection of P34 in several commercially available foods, indicating that the biochip could be an easy-to-use and effective alternative tool for allergen detection. By immobilizing a set of probes to modify the surface, the chip could be a high-throughput method to simultaneously detect several food allergens. Badran, Morais, and Maquieira (2017) developed a compact disk multiplex competitive immunoassay based on a mixture of specific gold-labeled antibodies for the simultaneous inspection of casein, β-lactoglobulin, gliadin, and ovalbumin. The microarray was performed on a DVD; the allergens were physisorbed on the polycarbonate surface (Figure 5B). Read by a DVD drive, the density of the sediment was related to the concentration of the four allergens. Moreover, a protein microarray, which uses serum from allergic individuals as probes, can potentially determine the latent allergens of specific foods. The portability and flexibility of a protein microarray allow personalized allergy management (Pasquariello et al. 2012).

4.2.4.3. Microfluidic chip. A microfluidic chip (also called "lab-on-a-chip") shows considerable potential in miniaturizing devices, reducing sample/reagent and automation. It can also be an ideal method for the rapid and on-site detection of food allergens (Weng and Neethirajan 2017). Weng, Gaur, and Neethirajan (2016) developed a microfluidic ELISA platform combined with a customized optical sensor

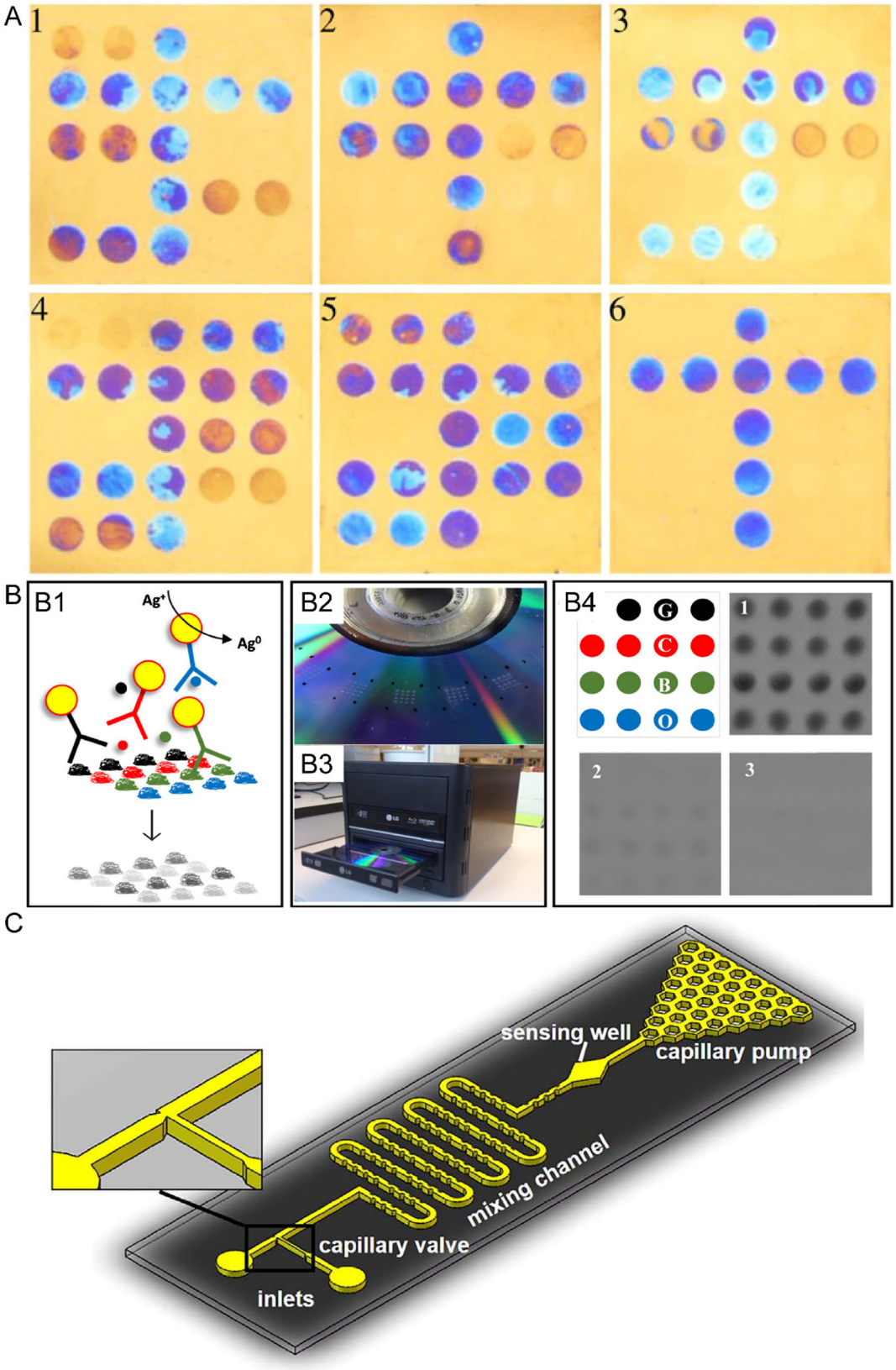

Figure 5. The actual device and schematic illustration of three types of biochips. (A) Detection of allergen components in commercial food products with the optical thin-film biochips. 1, Sesame biscuits; 2, Crispy Oatmeal Cookies; 3, Chocolate Omelet; 4, Hazelnut Wafers; 5, Bread sticks; 6, blank control (ddH2O) by Wang et al. (2011). (B) Scheme of a competitive micro immunoassay on DVD. B1, Schematic illustration of the working principle of compact disk immunoassay. Each array (B2) is localized by four marks made by a waterproof pen and the disk is scanned by the DVD drive (B3). B4, panels 1–3, respectively correspond to the images of the arrays for allergen concentrations of 0, 1.25, and 10 mg/L by Badran, Morais, and Maquieira (2017). (C) Schematic diagram of a graphene oxide and aptamer-functionalized quantum dots based microfluidic chip design by Weng and Neethirajan (2016).

to quantify wheat gluten and Ara h 1. The biochip was designed based on the principle of ELISA, with the detection time reduced from several hours to 15–20 min and the sample/reagent consumption lowered to 5–10 μL with great sensitivity. Weng and Neethirajan (2016) integrated a microfluidic system with a GO nanobiosensor modified by a QD–aptamer probe for rapid, simple, and sensitive food allergen detection (Figure 5C). During interaction with target allergens, the QD–aptamer–GO complexes exhibited a conformational change, altering fluorescence. In a homemade miniaturized optical analyzer, this one-step system was used to complete the quantitative detection of Ara h 1 in 10 min with remarkable selectivity and sensitivity. Moreover, with a selection of complementary aptamers, the biochip can be further used to detect other food allergens.

4.3. Chromatography and mass spectroscopy

Chromatography and mass spectroscopy (MS) essentially represent the combination of separation and detection methods, based on differences in the physical and chemical properties of food components. MS has been widely employed in the identification, characterization, and determination of food allergens, particularly liquid chromatography-mass spectrometry (LC-MS) (Table 2). As shown in Table 2, MS assay is suitable for the multiplex detection of food allergens, and its high accuracy, reproducibility, efficiency, and specificity renders it potentially useful in standard food allergen management (Holzhauser et al. 2020; Costa et al. 2017). When analysis by ELISA shows a significant deviation, MS can replace ELISA. As previously mentioned, ELISA had limitations in tracing almond and hazelnut from nut food roasted at temperatures exceeding 130 °C, whereas MS could detect traces of nut baked at temperatures reaching 150 °C. Based on multiple reaction monitoring methods for several proteolytic peptides of target food proteins, MS can avoid the problems of partial denaturation, decreased solubility, and cross-reactivity of samples in immunoassay, and accurately detect target proteins (Perner et al. 2019).

Using specific tryptic peptides of multiple food allergens as signature peptides, New et al. (2018) developed an LC-MS/MS technique that can simultaneously screen egg white, skim milk, peanut, soybean, and tree nuts (almond, Brazil nut, cashew, hazelnut, pecan, pine nut, pistachio, and walnut). The technique showed high recovery (60–119%), repeatability (RSD, <20%), and sensitivity, with the LOD of 10 ppm for each allergen in incurred cookies and bread, thus meeting the minimum performance requirements set by Association of Official Analytical Chemists International. The selection of signature peptides is important to ensure the accuracy and efficiency of MS analysis and usually depends on the specificity of the peptide to the matrix background and stability during processing. Ogura, Clifford, and Oppermann (2019) selected several significant peptides to monitor 13 food allergens by using the targeted LC-MS/MS approach based on bioinformatics. The performance of the screened peptides was subjected to experimental validation analysis, and the assay was able to detect the target food allergens in thermally processed foods.

Nonetheless, several disadvantages hamper the on-site application of MS analysis, including the need for laboratory equipment and specialized personnel. These shortcomings can be addressed using different techniques. Tan et al. (2019) combined thin-layer chromatography (TLC) with surface-enhanced Raman scattering (SERS) instead of the commonly used MS technique to develop a method allowing histamine detection in tuna fish samples. The method used a TLC plate made of edible-grade diatomaceous earth as the stationary phase to separate histamine from samples. The TLC plate was mixed with colloidal gold by drop-casting. The gold particle can be used as an ultrasensitive SERS substrate to increase the detection sensitivity to 10 ppm. Using a portable Raman spectrometer, the technique can be a rapid, and reliable approach for the on-site detection of seafood allergens.

4.4. DNA-based techniques

DNA-based techniques are indirect methods of food allergen detection generally based on several segments of genes encoding allergens or other specific DNA markers (Zhang et al. 2019). Allergenic food proteins may be affected by food processing or sample extraction, consequently influencing the results detected by protein-based methods (Mattison et al. 2016). The presence of allergenic proteins correlates well with the presence of detectable DNA in the majority of allergenic foods, including fish, crustacean shellfish, peanut, and tree nuts (Sharma et al. 2017). Compared to proteins, DNA molecules have higher stability. Although DNA may still be truncated by food processing, the selection of short-stranded DNA fragments as detection targets could reduce the impact of food processing on detection results (Holzhauser and Röder 2015; Yoshimura et al., 2005). In the detection of allergenic foods, DNA-based could be a complement to immunoassays. For instance, thermal processing can cause a decrease in ELISA recovery in which case DNA-based methods are more suitable (Jayasena et al. 2019). DNA-based techniques for allergen detection mainly include PCR, real-time PCR, and loop-mediated isothermal amplification (LAMP). These methods have been added to the Official Collection of Methods of Analysis as a standard method in some countries. In Japan, the government has made PCR the official method for detecting shrimp and crab in processed foods since November 2002 (Sakai et al. 2013). In Germany, PCR and real-time PCR have been included into the Official Collection of Methods of Analysis for a series of allergenic foods, like wheat, rye, soybean, lupine, almond, hazelnut, celery, mustard, sesame, peanut, and fish (Holzhauser 2018; Waiblinger et al. 2019). DNA-based methods have been applied to detect various allergens and exhibited high sensitivity, specificity, and multiplicity (Table 3).

Traditional DNA-based techniques, such as PCR, real-time PCR, are restricted by the need for of laboratory equipment, e.g. thermocycler, the complexity of the operation,

Table 2. Mass spectroscopy developed for food allergen detection in 2017–2020.

Method	Target	Application	LOD	Reference
LC-MS/MS	sesame allergens (Ses i1-7)	sauce, cookies, cake, candy	0.1 ~ 140.0 fmol/μL	(Ma et al. 2020)
UPLC-MS/MS	4 milk allergens	baked foods	0.38 ~ 0.83 μg/g	(Qi et al. 2019)
LC-MS/MS	13 food allergens: milk, eggs, cod, shrimp, lobster, peanuts, wheat, soybeans and tress nuts	thermally processed foods: bread, cookies, fired fish, frozen pasta	3.9 mg/kg (wheat)	(Ogura, Clifford, and Oppermann 2019)
UPLC-MS/MS	10 food allergens: milk, wheat, egg, soy, peanut, and tree nuts	flours, beers, heat-treated food stuffs	0.5-10mg/kg	(Henrottin et al. 2019)
LC-MS/MS	egg white, skim milk, peanut, soy and tree nuts	bread and cookies	10 mg/kg	(New et al. 2018)
LC-MS/MS	milk, soy, peanut, tree nuts	chocolate	0.2 ~ 2.5 μg/g	(Gu et al. 2018)
LC-MRM/MS	3 milk allergens: α-lactalbumin, β-lactoglobulin, α_{S1}-casein	daily foods	0.48 ~ 0.97 μg/mL	(Ji et al. 2017)
HPLC-MS/MS	lupine, pea, and soy proteins	meat	2 ~ 5 mg/kg	(Hoffmann et al. 2017)
LC-MS/MS	egg and milk allergens	wine	0.036 ~ 0.05 μg/mL	(De Angelis, Pilolli, and Monaci 2017)

and the degree of expertise required. Using specific LAMP primers, DNA amplification is efficiently performed under isothermal conditions, rendering a laboratory thermal cycler unnecessary. In a LAMP assay for the detection of pistachios, the reaction system was maintained at 65 °C for 1 h in a Mini Heating Dry bath (Major Science, China). The amplified products were visually observed by adding SYBR Green I dye, or analyzed by DNA electrophoresis. The LAMP could detect a minimum of 10 pg of pistachio nuts genomic DNA, which was equivalent to 10 mg/kg of pistachios in wheat flour, compared to the LOD of 100 mg/kg for conventional PCR when the same extraction method was used (Mao et al. 2020). Sheu et al. (2020) developed a LAMP assay for the specific detection of mango in food products. The method showed high sensitivity toward the mango DNA without cross-reactivity to other species. The detection duration was shorter than 60 min, rendering it viable for the rapid detection of mango in actual food markets. A novel on-site PCR assay referred to as ultrafast PCR has been developed to monitor allergenic foods. The method can directly detect target DNA without complex DNA extraction, substantially reducing the testing time. Using a portable device, Kim et al. (2019) explored an ultrafast real-time PCR system for the rapid field detection of shrimp DNA with the LOD of approximately 3.2 pg. The assay required less than 30 min—from the preparation to the analysis of results—to quantify shrimp DNA in raw and processed foods. The high sensitivity, quick response, and efficiency of the technique suggest its potential for field detection of allergens in various food products. Coupling DNA-based methods with some label particles, such as AuNPs, can improve their performance, including enhancing sensitivity, simplifying operation, and transducing the detection results into visible signals. Yuan, Fang, et al. (2019) coupled a hybridization chain reaction (HCR) with AuNPs to detect gene fragments of allergens in soybean, sesame and peanut. Two hairpin probes (H1 and H2) were designed for each of the three allergenic foods. When the target DNA was present, the probes underwent a hybridization chain reaction with the target DNA and thus failed to adsorb to the surface of AuNPs. AuNPs without probes would aggregate in the NaCl solution, causing the solution to change from red to blue-violet and a significant decrease in absorbance at 520 nm. The assay was performed at 37 °C without the use of enzymes, and the LOD for DNA of all three allergenic foods was 0.5 nmol/L in the standard solution. The method successfully detected the target DNA from cookies and candies, suggesting its feasibility in food detection.

4.5. Bioinformatic approaches

As an interdisciplinary field that integrates mathematics, biology, and computer science, bioinformatics has considerably promoted the progress of omics research. With the development of high-throughput methods for allergen detection, concepts of allergomes and allergomics, which refer to the repertoire of allergens in a certain food, have emerged (Chardin and Peltre 2005; Christensen et al. 2011). Allergomics can facilitate the management of food allergens, particularly potential allergenic food.

Bioinformatics is an important tool for identifying latent allergenic proteins in a novel food by screening homologous amino acid sequences to known allergens. Kulkarni, Ananthanarayan, and Raman (2013) collected known allergen sequences in the *Fabaceae* family from the database WHO/IUIS (http://allergen.org) and used Basic Local Alignment Search Tool (BLAST) to identify latent and cross-reactive allergens from chickpea (*Cicer arietinum* Linn). According to the bioinformatics comparison results, seven potential allergens from chickpea were found, and four of them might have cross-reactivity with existing allergens, which provided a basis for comprehensive management of allergenic food containing chickpea. The accuracy of bioinformatics are relevant to allergen databases. Nowadays, the commonly used databases in allergomics include the most comprehensive allergen database

Table 3. DNA-based methods developed for food allergen detection in 2019–2020.

Method	Target	Primer	Application	Cross-activity	LOD	Reference
real-time PCR	milk proteins	12S rRNA:916-P-FAM-TCTAGAAGGATATAAAGCACCGCCA AGT-BHQ1 cytb gene: Bos-P-FAM-CCGATACATACACGCAAACGGAGCTT CAA-BHQ1	meat products	minor reactivity with two species above 36 cycles of amplification	0.05 %	(Villa et al. 2020)
multiplex PCR	tropomyosin from 4 shellfish	oyster, 150 bp; mussel, 119 bp; abalone, 98 bp; clam, 76 bp	seafood products	no cross-reaction with shrimp, crab, squid, and mackerel	16 pg (DNA)	(Suh et al. 2020)
LAMP	mango allergens	outer primer:5′-TCGAACCTGTCGAGCAGA-3′ 5′-TCC TTG GCG CAA TTCGC-3′ inner primer:5′-AGGGCGCGCAAGCACAAG-ACGACCCGTGAACTTGTTG-3′′ 5′-CGCGTTGGGCTTTCGTTGC-GGGGTTGGTTAAGGCACC-3′′	commercial mango foods	no cross-reactions with other species	1 ng (DNA)	(Sheu et al. 2020)
LAMP	pistachio allergen Pis V 1	5′-CGTACTTCTCCTATCTGCC-3′ 5′-CTAGCCACACACTAACAACG-3′	commercial nuts and processed foods	no cross-reactions with cashew, mango, walnut, almonds, macadamia nut, hazelnut, peanut, and soybean	10 pg (DNA) 10 mg/kg (pistachio nuts)	(Mao et al. 2020)
multiplex PCR	tomato, apple, peach and kiwi	Cyclophilin (tomato), Mdtl 1 (apple), Pru p 2.01 A (peach) and Pectin methylesterase inhibitor (kiwi)	commercial fruit products	not test	0.08 ng (DNA)	(Suh et al. 2019)
real-time PCR	wheat, buckwheat, and peanuts	wheat: 5′-CATGGTGGGCGTCCTCGCTTTATCAATGCAGTGCAT CCGGCGCCCAGCTGGCATTATGGCCTTT-3′; buckwheat: 5′-CGCCAAGGACCACGAACAGAAGCGCGTCCCGAGCCTCCCGGT CCCCGGGCGGCACGGCGGCGTCGCGTCGTTTCTACCAAACAGA ACGACTCTCGGCAACG-3′; peanut: 5′-TTGGTTCAAAGAGACGGGCTCTTCGTGGGGAGCGGCACCGCGGCAGATGG TGGTCGAGAACAACCCTCGTG-3′	rice, juice, soup, sauce, chicken meatballs, clams	no cross-reactions with 79 common food materials and some of their relatives	10 mg/kg	(Miyazaki et al. 2019)
PCR	shrimp allergen tropomyosin	5′-TGTTGGTTGAGCACCTCCTA-3′ 5′-GCTTCATCGCCTGCATCTTC-3′	raw and processed seafoods	not test	3.2 pg (DNA)	(Kim et al. 2019)

Table 4. Comparison of different techniques for food allergen detection.

Technique	Advantage	Disadvantage
ELISA	High sensibility; Commercialization; Validation test.	Sensitive to some processing condition; False positive; Relatively low efficiency.
LFIA	Quick response; ease to use; Portability; On-site detection.	Limit of quantification; Relatively low-throughput.
xMAP	High-throughput; High sensibility; Flexibility and modularity.	Relying on the antibody activity.
Chromatography and Mass Spectroscopy	High accuracy and reproducibility; Multiplex detection; Without the problem of cross-reactivity; Validation test when ELISA shows deviation.	Generally, require laboratory equipment; Need for specialized personnel; Time-consuming.
Biosensor (biochip not included)	Quick response; Simplicity of operation; Wide usage; On-site quantitative detection; Able to detect allergenicity (cell-based).	Low-throughput; Some suffer from instability.
Biochip	High-throughput; Quick response; Portability; Avoid cross-contamination; Achieve individual allergy management.	Some need customized analyzers.
DNA-based methods	High accuracy and sensitivity; Multiplicity; Possible to achieve on-site detection.	Indirect methods of food allergen detection; Some require laboratory equipment and complex DNA extraction.
Bioinformatics	High-throughput; Able to screen out latent allergens in novel foods.	Require further validation tests; Accuracy depends on the databases; Lack standardized operating procedures.

Allergome (www.allergome.org), the allergen protein family database AllFam (http://www.meduniwien.ac.at/allfam/), the allergen nomenclature database WHO/IUIS, and the allergen structure database SDAP (http://fermi.utmb.edu/SDAP/index.html) (Mari and Scala 2006; Sircar et al. 2014). Universal databases, such as UniProt (https://www.uniprot.org/) and NCBI (https://www.ncbi.nlm.nih.gov/), can provide more information of known allergenic species, including proteomes, transcriptomes, and genomes, as well as alignment tools, including BLAST.

Bioinformatics can screen out latent allergens from numerous amounts of data in a relatively short time and reduce the cost of further validation. Bioinformatics results can be verified by traditional assays (Wang, Wang et al. 2020). However, the deficiencies of current allergen databases have limited the application of bioinformatics. As an *in silico* analysis, bioinformatics requires verification tests but lacks standardized operating procedures (Herman and Song 2019).

4.6. Comparison of the allergen detection methods

We conducted a comparison of different detection methods for allergenic foods (Table 4). Immunoassays exhibit high sensitivity on the basis of the specific interaction between allergens and antibodies. As one of the official methods or a validation test for novel detection techniques, ELISA has been well developed and commercialized. However, because of the cross-reaction of the antibody with non-target proteins, ELISA may present false-positive results. Its low efficiency restricts its application in modern allergy management. By contrast, LFIA and xMAP techniques have broad application potential because of their quick response and flexibility. LFIA is suitable for on-site detection, such as monitoring cross-contamination in production lines, although its quantification is limited. xMAP presents a considerable advantage in detection throughput and expandability and thus is a comprehensive detection technique for the simultaneous inspection of most common food allergens.

Due to their independence from biomolecular interactions, chromatography, mass spectroscopy, and DNA-based methods can replace ELISA when ELISA shows an obvious deviation. Nonetheless, chromatography, MS and conventional DNA-based methods require unportable equipment and specialized operators, which limit their application in in situ detection. As an indirect allergen detection method, the DNA-based detection technique is based on the premise that the presence of DNA is directly related to the presence of protein of allergenic food. Although there is a great correlation between proteins and DNA in most allergenic foods, the current studies show that DNA-based methods are not suitable for the detection of eggs or for the detection of single allergenic proteins, such as gluten and soybean protein added to meat products (Sharma et al. 2017; Holzhauser and Röder 2015). The amount of DNA in eggs is too low to develop DNA-based methods with sufficient sensitivity, and the DNA in eggs can be interfered with by chicken, resulting in poor specificity (Holzhauser et al. 2020). In addition, there are difficulties in quantifying the amount of specific DNA fragment into the amount of allergenic protein due to the lack of a universal conversion factor from target DNA to total protein (Holzhauser 2018).

As a sensitive, rapid, easy-to-use measurement technique, biosensors have received much attention in recent years.

Given their different receptors and transducers, biosensors can be widely applied in allergen detection, including multiplex detection, on-site quantitative detection, and even unknown allergens detection. However, restricted by the device format, biosensors detect flux limitedly. As the integration of biotechnology and microarray technology, biochips show superior high throughput, quick response, and portability, which endow them with the ability to monitor cross-contamination, investigate potential allergenic proteins, or achieve personalized allergy management, which traditional assays fail to efficiently accomplish. However, owing to variations in format, biochips may demand customized analyzers or entail difficulty in sample pretreatment. In addition, it is important to note that all detection techniques based on the principle of antigen-antibody reactions may be affected by cross-reactivity, resulting in inaccurate results.

Relying on comprehensive allergen databases and appropriate algorithms, bioinformatics can screen out most allergens, including unknown allergens, of a specific food within a relatively short time. Thus, bioinformatics can suitably identify latent allergenic proteins in a novel food and is typically used as a screening assay in the initial stages of allergomics research. However, as a data-driven method, bioinformatics is dependent on the quality of databases and generally requires validation tests.

5. Conclusions and perspectives

This paper reviews the research progress of rapid detection methods of food allergens in recent years. Allergen detection has significantly improved in diversity, quick response, and high throughput. Integrated with advanced nanoparticles, immunomagnetic beads, or portable devices, the detection capabilities of traditional detection techniques, like ELISA and PCR, can be enhanced, such as reducing the testing time or enabling on-site detection. The development of bioresponsive molecules, including antibodies and aptamers, also provides various choices for the establishment of allergen detection assays. However, different techniques have respective intrinsic advantages and disadvantages owing to variations in the principle underlying the detection techniques. Appropriate detection methods need to be selected based on application scenarios.

Although numerous technologies are available for efficient detection of allergens, the methods used in actual food samples are still restricted to traditional assays, including ELISA, PCR, and MS. Most emerging detection techniques are still in the laboratory stage. The performance of these methods in complex actual food samples requires further evaluation to ensure stability in different food matrices. Moreover, in terms of sample pretreatment, these rapid and multiplex assays are not significantly different from traditional assays. The detection results can be influenced by factors such as protein solubility, environmental pH or sample heat treatment. Pretreatment methods should be optimized for different sample matrices and assay technique characteristics to ensure assay stability and detection efficiency.

Most existing allergen detection techniques are targeted at antibody-binding epitopes in specific allergen proteins or DNA fragments in allergenic foods and do not allow for allergen screening for individual allergies. Few detection skills can potentially achieve personalized allergen screening, such as biochips. According to European Food Safety Authority, individuals' allergy changes with their physiological status and food allergies need to be reevaluated periodically to avoid unnecessary dietary restrictions (EFSA Panel on Dietetic Products, Nutrition and Allergies 2014). With the increasing demand for personalized health management and minimizing risk while ensuring the quality of life, comprehensive allergen monitoring technology combining personalized allergen screening with allergen content detection is a possible future research direction. In addition, developing rapid smart phones-based on-site detection of allergens, particularly for consumers, may also be adopted as an approach.

Disclosure statement

No potential conflict of interest was reported by the authors.

Abbreviations

AuNP	Gold nanoparticle
BLAST	Basic Local Alignment Search Tool
Cq	quantification cycles
DNA	Deoxyribonucleic Acid
DNP-BSA	Dinitrophenylated bovine serum albumin
DPV	Differential pulse voltammetry
EFSA	European Food Safety Authority
EGFP	Enhanced green fluorescent protein
ELISA	Enzyme-linked immunosorbent assays
EIS	Electrochemical impedance spectroscopy
FDA	the Food and Drug Administration
GO	Graphene oxide
HCR	hybridization chain reaction
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
IgE	Immunoglobulin E
iPSC	Induced pluripotent stem cell
IUIS	International union of immunological societies
LAMP	Loop-mediated isothermal amplification
LC	Liquid chromatography
LFIA	Lateral flow immunoassay
LOD	Limit of detection
LOQ	Limit of quantification
mAb	Monoclonal antibody
MC	mast cell
MB	Magnetic bead
MNP	Magnetic nanoparticle
MS	Mass spectroscopy
NCBI	National Center for Biotechnology Information
pAb	Polyclonal antibody
PV	Parvalbumin
QD	Quantum dot
SDAP	Structural Database of Allergenic Proteins
SERS	Surface-enhanced Raman scattering
SPR	Surface plasmon resonance
SWV	Square wave voltammetry
TLC	Thin-layer chromatography
UniProt	Universal Protein Resource
UPLC	Ultra performance liquid chromatography
UV-Vis	Ultraviolet visible

WHO/IUIS	the World Health Organization and International Union of Immunological Societies
xMAP	Multianalyte profiling

Funding

This work was supported by the Fundamental Research Funds for the Central Universities (1022050205205500), national first-class discipline program of Food Science and Technology (JUFSTR20180303), Collaborative Innovation Center of Food Safety and Quality Control.

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