Journal of Smart Sensor and Materials
Volume 4, No. 1, 2026 (14-47)

DESIGN STRATEGIES FOR MULTI-MYCOTOXIN ANALYSIS: A FOCUS ON ELECTROCHEMICAL BIOSENSOR APPROACH

Nur Azura Mohd Said*, Norhafniza Awaludin, Suria Mohd Saad, Erna Mutiara Masdek, Sahira Akmar Zulkepli and Syah Noor Muhammad Ramli

Biodiagnostic-Biosensor Programme, Biotechnology & Nanotechnology Research Centre, Malaysian Agricultural Research & Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, MALAYSIA
**Corresponding author email: nazurams@mardi.gov.my*

Abstract

The simultaneous co-occurrence of multiple mycotoxins in agricultural commodities, such as grain corn and animal feed, poses a severe threat to global food and feed safety. This multi-mycotoxin contamination exacerbates toxicological risks to both humans and livestock, resulting in substantial economic losses when contaminated products exceed regulatory maximum residue limits (MRLs) are rejected from the market. Although conventional analytical methods offer high sensitivity detection, they remain laboratory-bound, expensive and unsuitable for both rapid and on-site multiplex monitoring. These limitations have driven the transition from single-mycotoxin analyte detection toward advanced multi-mycotoxin analysis platforms. This review provides a comprehensive evaluation of design strategies for multi-mycotoxin detection, focusing specifically on electrochemical biosensor approaches. Key considerations discussed include the selection and engineering of biorecognition elements (antibodies, nanobodies, aptamers and molecularly imprinted polymers), nanomaterial-assisted signal amplification, assay formats suitable for small-molecule toxins and transducer configurations for multiplexed readout. The integration of electrochemical sensing with portable devices and intelligent data processing is also evaluated for field deployment application. Overall, electrochemical biosensors demonstrate strong potential for sensitive, cost-effective and miniaturized multi-mycotoxin screening tool. However, challenges related to extraction compatibility, cross-reactivity, reproducibility and regulatory validation remain critical hindrances to commercialization. Strategic design optimization and standardization efforts will be essential to successfully translate laboratory-scale multiplex biosensors into robust point-of-need monitoring systems.

Keywords: Multi-mycotoxins, Electrochemical biosensor, Food safety, Multiplex detection, Point-of-need testing

Article History:- Received: 6 January 2026; Revised: 4 April 2026; Accepted: 6 April 2026; Published: 30 April 2026

Introduction

Climate variability, elevated humidity and poor post-harvest storage conditions significantly increase the susceptibility of agricultural commodities and cereal grains to fungal contamination. These fungi then produce hazardous secondary metabolites known as mycotoxins, which compromise global food safety. To date, researchers have identified more than 400 mycotoxins from filamentous fungi, primarily belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium*. Among these, aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FUMs), T-2/HT-2 toxins, deoxynivalenol (DON), zearalenone (ZEN), citrinin (CIT), patulin (PAT), sterigmatocystin (STC) and ergot alkaloids (EAs) command the highest regulatory concern due to their frequent

occurrence in the food supply chain and severe toxicity (Wang et al., 2022). Table 1 outlines the primary fungal sources and toxicological profiles of these major mycotoxins.

Dietary exposure to mycotoxins leads to both acute and chronic health complications. The International Agency for Research on Cancer (IARC) classifies Aflatoxin B1 (AFB1) and STC as Group 1 carcinogens (carcinogenic to humans), whereas OTA, FUMs and CIT belong to Group 2B (possibly carcinogenic). Beyond carcinogenicity, these toxins induce distinct pathological effects. For instance, DON causes gastrointestinal distress and growth retardation, whereas ZEN acts as a potent endocrine disruptor. Additionally, CIT and PAT are linked to nephrotoxicity and genotoxicity, respectively, while the trichothecene T-2 toxin acts as a potent protein synthesis inhibitor that presents distinct biosecurity concerns (Janik et al., 2021). A critical factor in their persistence is their remarkable heat stability. Most mycotoxins remain chemically stable during standard food processing temperatures (i.e. boiling, baking, or pasteurization), meaning that once a commodity is contaminated, conventional thermal treatments are insufficient to eliminate the risk.

Table 1. Major mycotoxins, fungal sources, and toxicological effects

Mycotoxin Category	Primary Fungal Species	Primary Toxicological Effects
Aflatoxins (AFB1, B2, G1, G2)	*Aspergillus flavus, A. parasiticus*	Hepatotoxicity, liver cancer, immunosuppression, growth stunting
Ochratoxin A (OTA)	*Aspergillus ochraceus, Penicillium verrucosum*	Nephrotoxicity, Balkan endemic nephropathy, teratogenicity
Fumonisins (FB1, FB2)	*Fusarium verticillioides, F. proliferatum*	Esophageal cancer (humans), Leukoencephalomalacia (horses), pulmonary edema (swine)
Deoxynivalenol (DON)	*Fusarium graminearum, F. culmorum*	Gastrointestinal distress ("vomitoxin"), feed refusal, protein synthesis inhibition
Zearalenone (ZEN)	*Fusarium graminearum, F. culmorum*	Endocrine disruption, hyperestrogenism, reproductive disorders
T-2 / HT-2 Toxins	*Fusarium sporotrichioides, F. poae*	Potent protein synthesis inhibition, hematotoxicity, biosecurity concern
Patulin (PAT)	*Penicillium expansum, Aspergillus spp.*	Genotoxicity, gastrointestinal hemorrhaging, neurotoxicity
Citrinin (CIT)	*Penicillium citrinum, Aspergillus spp.*	Nephrotoxicity, synergism with OTA
Sterigmatocystin (STC)	*Aspergillus versicolor, A. nidulans*	Precursor to AFB1, hepatocarcinogenicity

The global distribution of mycotoxins is largely influenced by regional climatic conditions. In temperate regions (e.g., North America and Europe), DON and T-2 toxins are more prevalent, whereas tropical regions frequently report higher occurrences of aflatoxins and fumonisins. While

the Food and Agriculture Organization (FAO) traditionally estimated that 25% of global crops were affected, recent meta-analyses suggest that detectable levels of mycotoxins may exist in up to 60–80% of agricultural commodities (Eskola et al., 2020). This widespread contamination results in massive economic losses, potentially reaching hundreds of billions of dollars annually due to crop destruction, trade rejections, and the resulting healthcare burden (Ameh et al., 2011).

Multi-mycotoxin Contamination

In recent years, the focus of food safety surveillance has shifted significantly from single-toxin contamination to the complex co-occurrence of multiple mycotoxins within a single commodity. An alarming amount of evidence indicates that food and feed matrices are no longer contaminated by a single toxin; instead, they are often contaminated with “mycotoxin cocktails,” creating complex exposure scenarios (Fusilier et al., 2022). Such contamination has been widely reported in animal feed and grain corn across continents (Atnafu et al., 2024; Nji & Mwanza, 2024; Yamashita et al., 1995). In Brazil, surveys of beef cattle rations have revealed that FUMs and ZEN frequently coexist with AFs and trichothecenes (Pires et al., 2025). Beyond these commodities, multi-mycotoxin contamination also extends to everyday consumer goods, including cow’s milk, wheat, herbal medicines, and processed foods (El-Khatib et al., 2025; Hamid et al., 2025; Jonard et al., 2025). In Malaysia, the reported co-occurrence of multiple mycotoxins represents an escalating food and feed safety concern. In 2019, a total of 194 grain corn samples intended for animal feed in Peninsular Malaysia were evaluated, and the analysis revealed that more than 50% of the samples were contaminated with *Aspergillus* and *Fusarium* mycotoxins above their respective limits of detection (LODs). *Fusarium* toxins, namely FUMs, ZEN and DON, were widely distributed across all regions (Wan Syahidah et al., 2021). Another study conducted in grain corn plantations in Terengganu supported this broad fungal profile, reporting high incidence rates of both *Fusarium verticillioides* (83%) and *Aspergillus niger* (50%) in grain corn samples (Yazid et al., 2021). These findings underscore the urgent need for multiplex analytical systems capable of simultaneously detecting multiple toxins.

The presence of multiple toxins can induce interactive effects that are categorized as additive, synergistic or antagonistic in both animals and humans. Their accumulation increases the overall toxic burden and introduces uncertainty into health risk assessments. Nevertheless, susceptibility to combined mycotoxin exposure varies among animal species. In particular, swine are highly vulnerable to DON and ZEN, often exhibiting severe growth suppression, feed refusal and reproductive disorders. In contrast, poultry generally demonstrate moderate tolerance, whereas ruminants exhibit partial and variable resistance due to the detoxification and biotransformation activities of their rumen microbiota. Along the food chain, these toxins may also be transferred to humans through the consumption of contaminated animal products. For instance, dairy cows that ingest AFB1-contaminated feed can excrete AFM1 into their milk, thereby posing a significant food safety concern, particularly for infants and young children who consume milk-based products (Zentai et al., 2023).

The increasing occurrence of multi-mycotoxin contamination in food and feed has created an urgent need for analytical tools capable of delivering high sensitivity while enabling rapid

response and simultaneous multi-analyte detection at the point of need. Conventional chromatographic techniques such as liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) and high-performance liquid chromatography (HPLC) remain the gold standard for mycotoxin analysis due to their exceptional sensitivity, selectivity and quantitative accuracy. However, their routine implementation for continuous and on-site monitoring is limited by high operational costs, bulky instrumentation, laborious sample preparation involving organic solvents and the requirement for skilled technical personnel.

Another widely used approach, enzyme-linked immunosorbent assay (ELISA), offers a simpler and more accessible alternative through the use of 96-well plate formats that accommodate the simultaneous analysis of multiple samples. Nevertheless, most commercially available ELISA formats are designed primarily for single-analyte detection, which limits their suitability for comprehensive multi-mycotoxin screening. In this context, electrochemical biosensors have attracted considerable attention as rapid screening platforms. These systems offer several advantages including portability, rapid signal readout, low detection limits and minimal operator requirements, making them highly suitable for on-site and point-of-need testing applications.

While existing review articles primarily focus on the broader field of multi-mycotoxin analysis, this review specifically examines the evolution and strategic design of electrochemical biosensing approaches for multi-analyte detection. The fundamental architecture of electrochemical sensors is evaluated, including biorecognition elements such as antibodies, aptamers and molecularly imprinted polymers, as well as the pivotal role of nanomaterial integration and advanced functional materials in enhancing signal amplification and interface stability. The review also discusses the transition toward multiplexed transducer platforms and the emerging integration of IoT-enabled smart sensing systems for real-time data management. In the final section, the commercial readiness of these devices is assessed by comparing experimental sensor performance with current regulatory standards in order to identify the technical advancements required for future practical field deployment.

Analytical Challenges and Regulatory Frameworks in Multi-Mycotoxin Analysis

As mentioned previously, mycotoxin monitoring strategies have traditionally focused on single-analyte detection, in line with regulatory frameworks that govern individual mycotoxins. However, increasing toxicological evidence and global occurrence data have confirmed that the co-occurrence of multiple mycotoxins is highly prevalent. In a wide range of food and feed commodities, the simultaneous presence of major mycotoxins such as AFs, OTA, FUMs and ZEN is frequently reported. This shift has redefined analytical priorities and created a need for platforms capable of rapid and simultaneous multi-target detection without compromising analytical performance (Hu et al., 2026). Conventional detection methods can no longer fully satisfy the demand for high-throughput, rapid, simple and real-time mycotoxin surveillance required for routine monitoring of large sample numbers (Li et al., 2021). The capabilities and reliability of conventional chromatographic and immunoassay-based systems

are increasingly challenged by the growing demand for on-site, routine and point-of-need analysis.

Nevertheless, multiplex detection presents several inherent analytical challenges that must be addressed. Extracts obtained from food and feed samples often contain interfering compounds that may compromise signal accuracy. Cross-reactivity among structurally similar toxins further complicates multiplex immunoassays. In addition, the physicochemical diversity of mycotoxins, ranging from highly polar FUMs to relatively non-polar ZEN (Nakhjavan et al., 2020), creates substantial difficulties in achieving balanced extraction efficiency and uniform detection sensitivity. Variations in regulatory limits and the required dynamic detection ranges also contribute additional complexity to simultaneous quantification.

Table 2. Comparison of mycotoxin Maximum Residue Limits (in µg/kg or ppb) for animal feed in the UK, US, China, and Malaysia

Mycotoxin	**Animal Category**	**UK (EU Assimilated)**[1]	**US (FDA)**[2]	**China (GB 13078)**[3]	**Malaysia (Act 648)**[4]
Aflatoxin B1 (AFB1)	All Feed Materials	20	20 – 300	10 – 50	20 – 50
	Dairy Animals	5	20	10	5
Ochratoxin A (OTA)	Pig/Poultry Feed	50 – 100	*No specific limit*	100	100 (Codex)
DON	Swine/Young Animals	900	5,000	1,000	2,000
	Beef Cattle/Poultry	5,000	10,000	3,000 – 5,000	5,000
Zearalenone (ZEN)	Piglets/Gilts	100	No specific limit	100	100
	Beef Cattle	500	No specific limit	500	500
Fumonisins (FUMs)	Equine	5,000	5,000	5,000	5,000
	Beef Cattle	60,000	60,000	60,000	60,000

[1] Following Brexit, the UK enforces Assimilated EU Regulations (EC) 1881/2006

[2] U.S. Food and Drug Administration (FDA)

[3] China's GB 13078-2018

[4] Malaysia (Feed Act 2009)

In the development of rapid detection systems, compliance with strict regulatory criteria defined by MRLs is essential. These limits vary considerably across commodities and geographical regions. MRL guidelines for livestock feed are generally higher than those established for human food products. Furthermore, feed regulations commonly distinguish between complete feed formulations and raw feed ingredients such as grain or corn. Table 2 summarizes the maximum permitted levels across the UK, US, China and Malaysia. Among the regulated mycotoxins, AFB1 is the most strictly regulated in these countries because of

its carcinogenic properties and its potential carry-over into milk as AFM1. In Malaysia, the Department of Veterinary Services (DVS) implements guidelines under the Feed Act 2009, while Codex and EU standards are commonly adopted for emerging toxins such as ZEN.

While aflatoxin limits are relatively harmonized globally, toxins like ZEN and FUMs exhibit significant regional variability. Consequently, there is a pressing need for adaptable, highly sensitive analytical platforms capable of maintaining robustness across diverse matrices. In this context, biosensor-based technologies have emerged as promising candidates. By enabling rapid, portable and cost-effective preliminary screening prior to confirmatory "gold-standard" analysis, biosensors can significantly reduce the analytical burden and support timely decision-making in global food safety management.

Biosensor-Based Approaches for Multi-Mycotoxin Detection

Biosensors are analytical devices that integrate a biological recognition element with a physicochemical transducer to generate a measurable signal proportional to analyte concentration. Depending on the signal transduction mechanism, biosensors are broadly classified into electrochemical, optical, piezoelectric and thermal platforms. Among these classes, electrochemical biosensors are the most widely favored for multi-mycotoxin analysis (Figure 1).

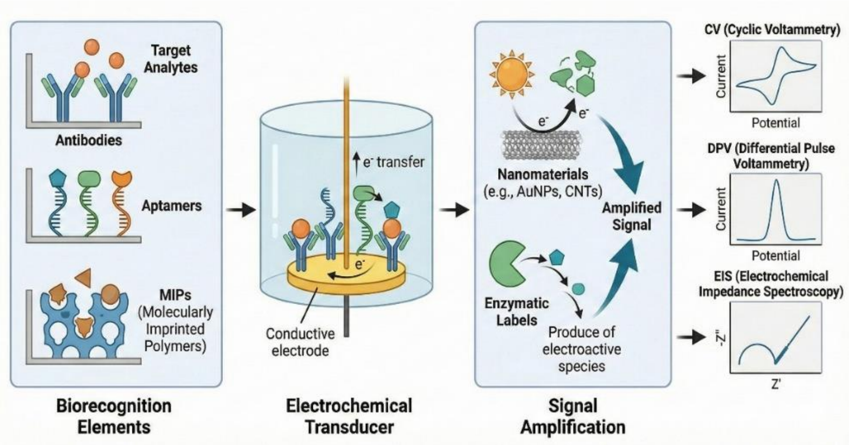

Figure 1. Schematic diagram of an electrochemical biosensor.

Electrochemical biosensors offer several advantages over other biosensor classes, including greater stability, reduced susceptibility to interference such as light, low power consumption, rapid response time, portability and miniaturized instrumentation suitable for on-site applications. Techniques such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) are commonly used for electrochemical measurements. Label-free detection systems enable highly sensitive

detection while simplifying assay design and reducing procedural complexity. Most importantly, electrochemical systems readily support multielectrode array configurations, which are well suited for multiplexed detection strategies.

The development of next-generation multi-mycotoxin biosensors requires a multidisciplinary approach that integrates nanomaterial synthesis and application, immunology, molecular biology, surface chemistry and electrochemistry. For field-deployable applications, electronic hardware engineering is also required. Recent breakthroughs in functional nanomaterials have significantly improved signal amplification in biosensing, surface immobilization chemistry and interfacial electron transfer rates. These material advancements ultimately lead to lower detection limits and improved capabilities for multiplexed screening.

Bioreceptors: The Front-Line for Sensor Selectivity

Bioreceptors or biorecognition elements are essential components in biosensor development. The selection of appropriate bioreceptors, along with their binding affinity and specificity, plays a major role in determining analytical performance. In multi-mycotoxin analysis, the key challenge lies in identifying suitable bioreceptors that can withstand surface functionalization and diverse extraction solvents while maintaining high sensitivity toward targeted small-molecule haptens.

Antibodies: From Immunoglobulin G (IgG) to Nanobodies

Antibodies are the most widely used biorecognition elements in mycotoxin detection. They can be classified into polyclonal antibodies (pAbs), monoclonal antibodies (mAbs) and recombinant formats. Both pAbs and mAbs are characterized by the approximately 150 kDa Y-shaped IgG structure (Figure 2). These antibody types have long served as the foundation for commercial immunoassays such as enzyme-linked immunosorbent assays (ELISA) and lateral flow immunoassays (LFIA).

Polyclonal antibodies are produced by immunizing animal hosts with the target mycotoxin conjugated to a carrier protein, such as bovine serum albumin (BSA), ovalbumin (OVA) or keyhole limpet hemocyanin (KLH), to induce an immune response against these low-molecular-weight haptens. pAbs recognize multiple epitopes on the antigen surface, which can enhance signal generation and assay robustness. Despite considered as an old-fashioned approach, pAbs remain valuable and highly applicable due to their relatively straightforward production and multi-epitope binding capacity, which occasionally yields superior analytical sensitivity compared with monoclonal antibodies (Ascoli & Aggeler, 2018).

Conversely, mAbs target a single, specific epitope with high precision. They are typically generated using hybridoma technology, where antibody-producing B cells from an immunized animal are fused with immortal myeloma cells to create stable cell lines capable of producing identical antibody molecules indefinitely. This high specificity and batch-to-

batch consistency makes mAbs particularly suitable for diagnostic assays and multiplex detection platforms. For instance, mAbs have been integrated into mass-sensitive microarray biosensors to achieve concurrent, real-time and label-free tracking of T-2 toxin, ZEN and FB1 (Nolan, 2019).

More recently, recombinant antibodies have emerged as attractive alternatives to conventional antibody formats. These proteins are produced through genetic engineering techniques in which antibody genes are cloned and expressed in host systems such as *Escherichia coli*, yeast, insect or mammalian cells. This methodology permits the custom synthesis of specialized fragments, such as single-chain variable fragments (scFv), Fab fragments and nanobodies, with precisely calibrated binding affinity, structural stability and target selectivity. Additionally, recombinant platforms improve manufacturing scalability, ensure batch reproducibility and mitigate ethical concerns by eliminating the requirement for ongoing animal immunization. These properties make recombinant antibodies particularly promising for integration into next-generation biosensor platforms and multiplex detection systems for mycotoxin monitoring.

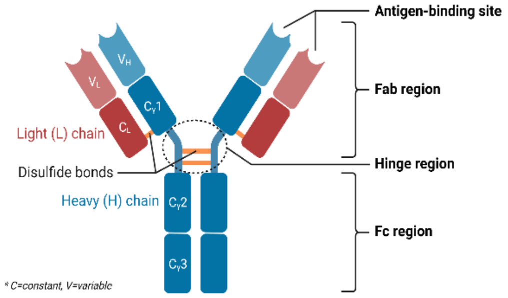

Figure 2. Basic antibody structure. Depicted is a standard Immunoglobulin G (IgG) molecule.

Numerous studies have reported the development of antibodies targeting major mycotoxins for biosensing. For instance, pAbs for mycotoxin detection have been produced by Mohd Said and co-workers for AFB1, OTA and ZEN (Said et al. 2023, 2025). Meanwhile, Elliott and colleagues have extensively developed highly sensitive mAbs against several mycotoxins, including aflatoxins (Oplatowska-Stachowiak et al., 2016), OTA (Meneely & Elliott, 2014) and sterigmatocystin (Oplatowska-Stachowiak et al., 2018). Monoclonal antibodies targeting FUMs and ZEN have also been reported (Tang et al., 2014; Wang et al., 2022; Zhang et al., 2018), while monoclonal antibodies against the T-2 and HT-2 toxins were described by Molinelli et al. (2008).

A detailed comparison of polyclonal, monoclonal and recombinant antibodies for the detection of small molecules such as mycotoxins was presented by Kavanagh and co-workers (2015). The authors highlighted that animal-derived polyclonal (pAb) and monoclonal (mAb) antibodies have traditionally served as the primary binding elements in most immunoassays.

However, recombinant antibodies (rAbs), typically isolated from in vitro display libraries, are emerging as a promising alternative. For the past two decades, single-chain variable fragments (scFv) have been the most widely used recombinant antibody format. Nevertheless, other formats such as antigen-binding fragments (Fab) and single-domain antibodies (sdAb) are gaining increasing attention due to their improved expression efficiency and enhanced tolerance to organic solvents. Although recombinant antibody technologies have not yet consistently surpassed the analytical performance of conventional pAb- and mAb-based formats, engineered rAbs are widely expected to become next-generation high-performance reagents for the rapid and selective detection of haptenic biotoxins, including fungal, marine, and aquatic contaminants.

Recent advances in bioreceptor engineering have introduced nanobodies (VHH), single-domain antibodies derived from camelid heavy-chain-only antibodies, as promising alternatives to conventional IgG antibodies (Alexander & Leong, 2024; Muyldermans, 2013). With a molecular weight of ~12–15 kDa, nanobodies are approximately 10–15 times smaller than full-length antibodies (~150 kDa) (Figure 3), enabling improved access to hidden or sterically hindered epitopes. Unlike conventional antibodies, nanobodies are genetically encoded and can be recombinantly produced in microbial systems, facilitating scalable and cost-effective production. They also exhibit enhanced thermal stability and solvent resistance, making them particularly attractive for biosensor applications that require robustness under variable environmental conditions. For example, anti-ochratoxin A (OTA) nanobodies have been reported to retain more than 50% binding activity after prolonged heat exposure, such as 75 minutes at 95 °C or 40 minutes at 80 °C, whereas conventional monoclonal antibodies typically lose activity rapidly under similar conditions (He et al., 2014; Liu et al., 2017; Zhang et al., 2019).

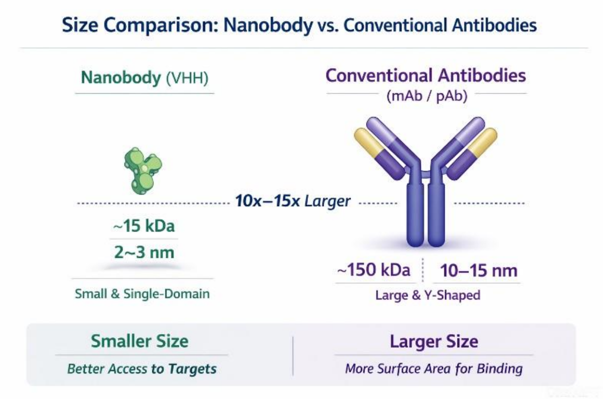

Figure 3. Molecular weight and size comparisons for nanobody and conventional antibodies.

Despite these advantages, several practical challenges remain. Nanobody development often requires camelid immunization, which introduces logistical constraints. Additionally, their small molecular size may lead to steric hindrance during surface immobilization and can

complicate the recognition of small-molecule haptens such as mycotoxins. These limitations must be carefully addressed to fully realize their potential in multiplex electrochemical biosensors for multi-mycotoxin detection. The following Figure 4 illustrates a comparison and production overview of pAbs, mAbs, recombinant antibodies and nanobodies.

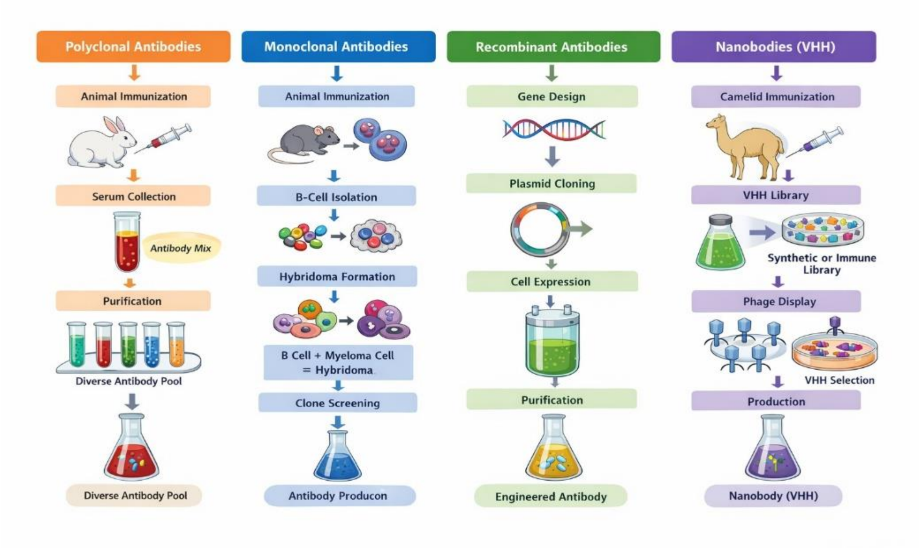

Figure 4. Production pathways for polyclonal, monoclonal, recombinant and VHH nanobody antibodies. Polyclonal antibodies are gathered from immunized animal serum to create a diverse antibody pool. Monoclonal antibodies use hybridoma technology to create a single, highly specific type. Recombinant antibodies bypass standard animal steps by using gene cloning and cell expression for targeted sequence engineering. Nanobodies are selected from camelid immune or synthetic libraries via phage display before final manufacturing.

Aptamers for Mycotoxin Detection

As an alternative to protein antibodies, aptamers function as highly selective, animal-free recognition elements. These short single-stranded DNA or RNA sequences (10 to 100 nucleotides) adopt specific three-dimensional conformations to bind targets tightly and selectively. Researchers synthesize them using SELEX (Systematic Evolution of Ligands by Exponential Enrichment) or computer-aided designs to tailor their exact binding properties. Distinct from conventional antibodies, these molecules feature reliable chemical stability, cost-effective manufacturing, low immunogenicity and reversible binding mechanisms ideal for sensor reuse. The first aptamer specifically developed for a mycotoxin was reported by Cruz-Aguado and Penner (2008) for the detection of OTA. This foundation led to the generation of specialized aptamers for AFB1 (Shim et al., 2014), AFM1 (Malhotra et al., 2014), FB1 (Yue et al., 2014), ZEN (Azri et al., 2020), DON (Ong et al., 2020; Wen et al., 2021), PAT (Wu et al., 2016), T-2 toxin (Chen et al., 2014) and ergot alkaloids (Rouah-Martin et al., 2012). The sequences and specific binding profiles of these common mycotoxin aptamers are outlined in Table 3.

Table 3. Sequences and dissociation constants (K_D) of selected mycotoxin aptamers used in biosensing. Adapted from Wang et al. (2022).

Target	**Sequence (5’-3’)**	**K_D**
AFB1	GT TGG GCA CGT GTT GTC TCT CTG TGT CTC GTG CCC TTC GCT AGG CCC ACA	n.a. *
AFM1	ACT GCT AGA GAT TTT CCA CAT	n.a.
OTA	GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA	0.2 µM
FB1	ATA CCA GCT TAT TCA ATT AAT CGC ATT ACC TTA TAC CAG CTT ATT CAA TTA CGT CTG CAC ATA CCA GCT TAT TCA ATT AGA TAG TAA GTG CAA TCT	100 ± 30 nM
ZEN	TCATCTATCTATGGTACATTACTATCTGTAATGTGATATG	41 ± 5 nM
DON	GCATCACTACAGTCATTACGCATCGTAGGGGGGATCGTTAAGGAAGTGCCCGGAGGCGGTATCGTGTGAAGTGCTGTCCC	n.a.
PAT	GGCCCGCCAACCCGCATCATCTACACTGATATTTTACCTT	21.83 ± 5.022 nM
T-2	GTATATCAAGCATCGCGTGTTTACACATGCGAGAGGTGAA	20.8 ± 3.1 nM
Ergot alkaloids	ACTCATCTGTGAAGAGAAGCAGCACAGAGGTCAGATGTCCGTCAGCCCCGATCGCCATCCAGGGACTCCCCCCTATGCCTATGCGTGCTACCGTGAA	44 nM

*n.a: data not available

Recent studies have highlighted the effectiveness of aptamer-based electrochemical biosensors for mycotoxin detection, particularly for ZEN. Azri et al. (2020) developed a label-free competitive electrochemical aptasensor, in which a gold electrode is modified with immobilized ZEN, and the aptamer competes between binding to the surface-bound toxin and free ZEN molecules in the sample. This competitive mechanism enables rapid and highly sensitive detection, demonstrating the versatility of aptamer-based strategies. Similarly, Hassani et al. (2022) reported an ultrasensitive electrochemical aptamer biosensor for ZEN, emphasizing the design of highly selective aptamer interfaces to enhance signal response and detection performance. Aptamers also offer additional advantages in biosensing due to their sequence programmability and their ability to reduce false-positive responses in complex food matrices. Single-stranded DNA binding protein-assisted aptamer systems have been reported for the simultaneous detection of AFB1, OTA and ZEN in maize, demonstrating enhanced sensitivity and specificity compared with conventional ELISA-based approaches (Fan et al., 2023; Xing et al., 2020). Overall, these studies highlight the potential of aptamer-functionalized electrochemical platforms for robust, sensitive and selective detection of prevalent mycotoxins in food matrices.

Despite the promising outlook in the literature, commercial aptamer kits for mycotoxin detection are not readily available. In addition to difficulties in securing strong intellectual property for SELEX-derived sequences, the current market is dominated by established antibody-based diagnostic tools. Nevertheless, aptamers have strong potential for biosensor development in multiplexed, high-throughput and on-field applications.

Molecularly Imprinted Polymers (MIPs) as Synthetic Receptors

MIPs, often referred to as artificial antibodies, represent a synthetic alternative to natural bioreceptors. These materials consist of polymer matrices engineered with molecular cavities that complement the shape, size and chemical functionality of the target molecule, thereby enabling selective recognition of specific analytes such as mycotoxins. One of the key advantages of MIPs is their strong chemical and thermal stability, which makes them highly suitable for biosensing applications under harsh environmental conditions that would typically denature protein-based receptors. Wang and co-workers (2024) successfully demonstrated the integration of MIPs into electrochemical and optical sensing platforms for the detection of OTA and AFB1. These synthetic receptors provide reproducible and chemically stable binding sites, which are well suited for long-term monitoring and field-deployable sensing systems.

Like aptamers, MIP-based biosensors have yet to move beyond the laboratory stage. The main challenges include relatively lower binding affinity compared with natural bioreceptors and difficulties in achieving efficient and consistent template removal during fabrication, which may lead to residual template leakage. Overcoming these limitations is necessary to advance MIP-based platforms toward commercialization as reliable and scalable tools for multi-mycotoxin detection.

In conclusion, selecting suitable bioreceptors, including antibodies, nanobodies, aptamers and MIPs, requires careful consideration. A balance must be achieved among target specificity, structural stability, assay compatibility and multiplexing potential. The integration of these recognition elements with appropriate transduction technologies is essential for the development of sensitive, rapid and cost-effective detection systems for multi-mycotoxins in food and feed samples. Moving these systems from laboratory setups to commercial production lines for on-field testing also requires a realistic assessment of manufacturing costs, particularly in relation to large-scale production compared with lab-scale fabrication.

Transducer Platforms and Signal Readout Strategies

In biosensors, a transducer converts a biorecognition binding event into a measurable signal, for example when a bioreceptor such as an antibody, aptamer or MIP interacts with its target analyte such as a mycotoxin. For multi-mycotoxin analysis, the choice of transducer plays a key role in determining analytical sensitivity for trace detection, simultaneous multi-detection and feasibility for on-site or point-of-need (PON) deployment. As highlighted in

the previous section, most biosensor designs utilize one of three primary physical operating mechanisms: optical, mass-sensitive (piezoelectric) or electrochemical biosensing.

Optical biosensors, among the most widely reported class of biosensors, measure changes in light properties. However, this type of biosensor is susceptible to external factors and performance can be affected by interference from ambient light or sample turbidity (Yoo & Lee, 2016). Surface plasmon resonance (SPR) is one of the prominent optical techniques that offers real-time and label-free detection. However, SPR typically requires bulky instrumentation and presents challenges for miniaturization (Homola, 2008). Combined approaches such as surface-enhanced Raman scattering (SERS) integrated with lateral flow immunoassays (LFIA) also provide a strong alternative for multi-mycotoxin detection. SERS identifies distinct spectral fingerprints that enable highly sensitive multiplexing, allowing detection of up to six toxins simultaneously within 20 minutes (Zhang et al., 2020). Mass-sensitive piezoelectric biosensors, such as quartz crystal microbalances (QCM), measure small mass changes on the sensor surface when binding events occur between toxins and their receptors. This approach enables high sensitivity and label-free detection. However, interference from complex food matrices can distort or obscure the target signal (Becker & Cooper, 2011).

To date, electrochemical biosensors remain the most widely reported platform for multiplexed mycotoxin detection (Li et al., 2021). This preference is driven by key advantages such as high sensitivity, low manufacturing cost and natural compatibility with microelectronic circuits. Most importantly, their system architecture can be miniaturized into portable or handheld devices, making them well suited for high-throughput field testing in resource-limited settings (Mejri Omrani et al., 2016). While these sensors offer excellent sensitivity and selectivity along with strong potential for miniaturization and on-site applications, their fabrication often requires complex surface modification procedures, which can compromise reproducibility and reduce resistance to interference in complex sample matrices. Compared with optical detection platforms, electrochemical systems have yet to demonstrate a decisive advantage for multiplexed detection strategies. The important parameters of electrochemical biosensor platforms for multi-mycotoxin determination, as reported in recent years, are summarized in Table 4.

Electrochemical Biosensors: The Primary Focus

Electrochemical biosensors have emerged as the leading platform for multiplexed mycotoxin detection, primarily due to their high sensitivity, low-cost instrumentation and inherent compatibility with miniaturized, portable devices. Unlike optical or mass-sensitive sensors, which can be bulky or sensitive to environmental light, electrochemical transducers convert biochemical recognition events (such as antibody-toxin or aptamer-toxin binding) directly into electrical signals (i.e. current, potential, or impedance) at the interface of the working electrode. This direct digital readout is particularly advantageous for integration with Internet of Things (IoT) sensors and "in-field" diagnostic kits (Vidal et al., 2013).

Voltammetry techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) involve applying a varying potential to the electrode and measuring the resulting current. While CV is indispensable for characterizing sensor surface modifications and understanding redox behavior, DPV is the most frequently reported technique for quantitative multi-toxin detection. DPV excels by pulsing the potential, which effectively minimizes background charging current. This results in sharper peaks and significantly higher sensitivity, often reaching the pg/mL range. These sharp, resolved peaks are essential for identifying multiple toxins in a single scan when using different redox labels or potential-resolved strategies (Chen et al., 2023; Dong et al. 2022). Differential pulse voltammetry (DPV) has become an important technique in label-free biosensing. The peak current of a solution-phase redox probe decreases as toxins bind to the electrode surface and block electron transfer. These current changes can be correlated with toxin concentration for standard curve development.

Table 4. Summary of electrochemical sensor platforms for detecting multi-mycotoxins. Adapted from Li et al. (2021)

Target	Method	Principle	Electrode	Sample	LOD
FB1/DON	Immunosensor	DPV	Indium tin oxide electrode integrated with PDMS microfluidic channel	Corn	97/35 pg/mL
ZEN/FB1	Aptasensor	DPV	Co-reduced molybdenum disulfide/AuNPs-modified glass carbon electrode	Maize	0.5 pg/mL
OTA/FB1	Aptasensor	DPV	Gold electrode	Beer	0.47/0.26 pg/mL
AFB1/OTA	Ratiometric aptasensor	DPV	Gold electrode	Corn and whear	4.3/13.3 pg/mL
OTA/FB1	Magneto-controlled aptasensor	SWV	Glassy carbon electrode	Maize	20/5 pg/mL

**DPV- differential pulse voltammetry*
SWV- square wave volatmmetry

Another widely used technique is electrochemical impedance spectroscopy (EIS). EIS measures changes in charge transfer resistance (R_{ct}) or capacitance (C) at the electrode–electrolyte interface. Upon binding of mycotoxins to the bioreceptor, a physical barrier or

dielectric layer is formed that blocks electron transfer from a redox probe such as $[Fe(CN)_6]^{3-/4-}$ to the electrode surface. The resulting increase in resistance is proportional to the target toxin concentration (Gu et al., 2015). Like DPV, EIS offers effective label-free detection and provides reliable quantitative information on film stability. Nevertheless, researchers typically employ EIS as a supportive validation tool or complementary technique rather than a primary field-testing method, as interpretation of raw data requires complex equivalent circuit modeling.

Multi-Electrode Arrays for Multiplexing

For multiplex biosensing, electrochemical biosensor platforms utilizing single-electrode configurations must shift to multi-electrode arrays. To construct multiple working electrodes on a single substrate, electrode arrays can be fabricated using screen printing or photolithography. Each working electrode in the array must then be functionalized with bioreceptors specific to individual toxins, allowing simultaneous and spatially resolved detection of multiple mycotoxins from a single sample droplet. This architectural shift, often referred to as a lab-on-a-chip or micro-total analysis system (µTAS), enables electrochemical platforms to achieve the throughput required for industrial food safety standards. An MXene-based electrochemical aptasensor arrays have been developed with independent electrodes targeting AFB1, OTA and ZEN, enabling simultaneous signal readout and quantification on a compact portable device (Wang et al., 2024). Spatially resolved array electrodes have also been used to concurrently detect ZEN and FB1 using electrochemical biosensor platforms (Zou et al., 2025). These dedicated configurations increase throughput for dual or multi-mycotoxin detection while bringing portable electrochemical testing closer to the performance standards of instrumental chromatography.

In cases where electrodes employ inert or non-conductive materials such as carbon on the working electrode, the surface must be functionalized and modified to enable immobilization of bioreceptors and to enhance electrochemical performance. Typical localized nanomaterial deposition strategies include electrodeposition or drop-casting of materials such as gold nanoparticles (AuNPs), carbon nanotubes (CNTs), chitosan (Jiang & Wu, 2019) and graphene quantum dots (Jebril et al., 2024) onto individual electrode sites. These approaches are widely reported in the literature and are commonly used to improve electron transfer kinetics, increase surface area and facilitate biomolecule attachment. This modification has been shown to significantly increase the electroactive surface area and to provide a biocompatible interface for the subsequent immobilization of thiolated aptamers or antibodies (Arduini et al., 2016). These nanoscale modifications have been demonstrated to significantly enhance analytical sensitivity and lower detection limits while maintaining the compact and portable nature of electrochemical biosensor platforms for field deployment.

Currently, several commercially manufactured multi-working electrode (multi-WE) systems are available for multiplex electrochemical biosensing applications. Metrohm DropSens (Spain) produces dual- and four-working electrode screen-printed platforms. These include the dual carbon working electrode system (product code X1110), a dual carbon

working electrode readily modified with gold nanoparticles (product code X1110 GNP) and a four-working electrode carbon configuration (product code DRP-4W110-U20). The DropSens screen-printed electrodes integrate multiple individually addressable working electrodes on a single ceramic chip that share common reference and counter electrodes. This configuration enables spatially isolated modification, allowing each electrode to be functionalized with distinct bioreceptors targeting analytes such as AFB1, DON, OTA or ZEN for simultaneous screening within a single droplet. Biogenes Technologies Sdn. Bhd. (Malaysia) also provides four-working electrode screen-printed platforms suitable for multiplex electrochemical sensing applications (Figure 5). These commercially fabricated arrays offer improved reproducibility, batch-to-batch consistency and standardized geometries compared with custom laboratory-fabricated electrodes, making them particularly useful for research applications.

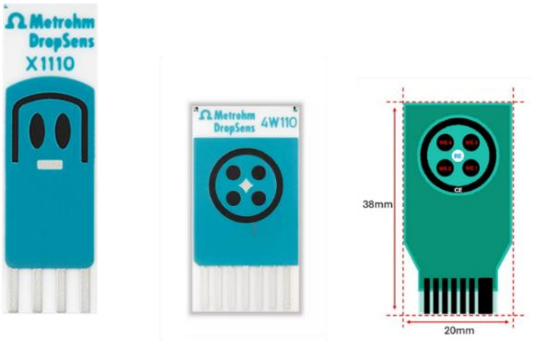

Figure 5. Commercial multi-electrode configurations showing dual working electrode (X1110) and four working electrode (4W110) ceramic-based screen-printed electrodes (SPEs) from DropSens (Spain), along with a four working electrode plastic-based SPE (green, far right) from Biogenes Technologies (Malaysia).

It is important to note that operation of multi-working electrode systems requires dedicated multi-channel cable connectors or custom adapter interfaces for connection to commercial potentiostats such as those from Metrohm Autolab or Metrohm DropSens. These connectors enable independent addressing of each working electrode while maintaining a shared reference and counter electrode configuration.

Nanostructured Interfaces and Signal Amplification Strategies

Nanomaterials are widely incorporated into electrochemical biosensors for surface functionalization, signal amplification and bioreceptor immobilization. Nanoscale materials offer several physicochemical advantages, including high surface-to-volume ratios, excellent electrical conductivity and strong biocompatibility. As a result, electrochemical biosensors are able to achieve lower LOD and improved assay stability for contaminant analysis (Eissa et al., 2022). On planar electrodes, trace mycotoxin detection is often constrained by the limited number of available active binding sites. Carbon-based nanomaterials such as

graphene and CNTs address this limitation by serving as highly conductive scaffolds that increase the electroactive surface area. Their π–π stacking interactions, structural defect sites and oxygen-containing functional groups support stable immobilization of antibodies or aptamers, thereby improving target capture efficiency. Owing to these properties, graphene- and CNT-modified electrodes frequently achieve sub-nanogram per milliliter detection limits in electrochemical biosensing applications.

In addition to carbon allotropes, two-dimensional MXenes, particularly transition metal carbides, have emerged as promising materials for electrochemical biosensing because of their metallic conductivity and hydrophilic surface chemistry, both of which facilitate rapid electron transfer and improved sensor response (Amara et al., 2023). Materials such as $Ti_3C_2T_x$ exhibit metallic-level conductivity together with hydrophilic terminal functional groups including –OH, –O and –F, which enhance interfacial charge transport. Although MXenes are often combined with carbon nanomaterials to improve structural and electrochemical stability, incorporation into hybrid nanocomposites has been shown to enhance biosensor sensitivity and shorten response times. These improvements are partly attributed to their large interlayer spacing, which accommodates a high density of immobilized bioreceptors.

Signal amplification efficiency also depends strongly on nanomaterial integration. Metal nanoparticles such as AuNPs enhance electron transfer kinetics and increase effective surface conductivity. These nanoparticles act as conductive nanobridges and electrocatalytic centers that facilitate redox reactions, producing sharper and more defined peaks during differential pulse voltammetry (DPV) measurements. Nanomaterials can also function as carriers for electroactive or redox-active labels in multiplex signal-on assays. For example, magnetic nanoparticles, mesoporous silica nanoparticles and carbon nanostructures can be functionalized with distinct redox reporters to enable simultaneous electrochemical readout of multiple analytes within a single scan.

Besides AuNPs, graphene quantum dots (GQDs) are increasingly employed as nanostructured materials for signal enhancement in biosensing platforms. In optical LFIA, GQDs have demonstrated substantially lower LOD compared with AuNP-based systems because of their strong fluorescence characteristics (Foubert et al., 2017; Li et al., 2018). Similarly, fluorescent microspheres have shown improved analytical sensitivity in several mycotoxin assays (Liu et al., 2020). However, these observations cannot be directly translated to electrochemical biosensors because signal generation relies on different transduction mechanisms.

In electrochemical biosensor fabrication, nanomaterials affect not only signal amplification but also the orientation and stability of bioreceptor immobilization. AuNPs readily support antibody immobilization through passive adsorption mediated by electrostatic and hydrophobic interactions. More stable attachment can also be achieved through thiol-gold chemistry or carbodiimide coupling on functionalized AuNP surfaces. In contrast, GQDs provide abundant surface carboxyl groups that facilitate covalent antibody immobilization through carbodiimide chemistry involving 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

(EDC) and N-hydroxysuccinimide (NHS). This strategy generally provides stronger attachment and improved surface stability, although additional activation steps are required.

The selection of AuNPs, GQDs or other nanomaterials therefore depends on the sensing strategy and targeted performance requirements. AuNPs provide excellent conductivity and straightforward bioconjugation, whereas GQDs offer a high density of functional groups and greater flexibility for controlled surface modification. Although quantum dots are capable of generating highly sensitive optical signals, their application in electrochemical biosensors usually requires integration with electroactive mediators or redox-active systems to produce measurable electrical responses. Consequently, the analytical performance of electrochemical biosensors is determined not only by the intrinsic properties of the nanomaterial but also by the effectiveness of its integration within the overall signal transduction architecture.

Surface Functionalization for Working Electrodes in Multi-Electrode Arrays

Surface functionalization and modification strategies for multi-electrode arrays differ substantially from those used for single working electrodes and several important factors must be considered. In densely packed electrode arrays, the distribution and uniformity of the modification layer play a critical role in bioreceptor immobilization to ensure signal consistency and repeatability. It is also important to minimize factors such as the coffee-ring effect, protein denaturation and electrochemical cross-talk between adjacent electrodes. Surface engineering has therefore become an essential design consideration in multiplex electrochemical biosensors. Unlike single-analyte platforms, multi-electrode systems require high spatial precision to independently functionalize each working electrode (WE) with distinct bioreceptors. Several surface functionalization techniques that are applicable to multiplex biosensors for multi-analyte detection are discussed below.

Drop-Casting

Drop-casting remains one of the most straightforward approaches for electrode surface modification. This method involves depositing a microliter-scale droplet of nanomaterial or bioreceptor solution onto the electrode surface either manually or through automated dispensing systems such as BIODOT dispensers. The technique is particularly suitable for materials that are difficult to polymerize, including CNTs, MXenes and graphene-based materials. Despite its simplicity, drop-casting is highly operator-dependent and often produces inconsistent surface coatings. The method is also associated with the coffee-ring effect, where non-uniform solvent evaporation causes nanomaterial accumulation at the droplet perimeter, resulting in poor signal reproducibility (Deegan et al., 1997). In compact arrays containing multiple working electrodes on a single chip, droplet spreading may extend to adjacent electrodes and cause cross-contamination of bioreceptors. Furthermore, the resulting films generally exhibit weak adhesion because immobilization relies primarily on physical adsorption. As a result, the coatings may peel or detach during fluid flow or stirred

electrochemical measurements, leading to inconsistent signal generation and significant batch-to-batch variation.

Electropolymerization

Electropolymerization utilizes an applied electrical potential to initiate polymer growth directly from a monomer solution onto a targeted electrode surface. Conducting electroactive polymers (CEPs) such as polyaniline (PANI), polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT) are commonly employed for this purpose. These polymers form strongly integrated layer on the electrode surface with superior adhesion and mechanical durability compared with drop-cast films. Electropolymerization also provides excellent spatial control and uniform film distribution without the risk of physical spreading across adjacent electrodes. Another major advantage of this technique is the precise control of film thickness, which can be tuned at the nanometer scale depending on the total charge passed during the polymerization process (Bard et al., 2022). Despite these advantages, electropolymerization requires careful optimization of parameters such as monomer concentration, pH, applied potential window and scan rate. In addition, some biomolecules may not tolerate the electrochemical conditions involved during polymer formation. Under such circumstances, a two-step strategy is commonly employed in which polymer deposition is first performed, followed by subsequent bioreceptor immobilization through EDC/NHS coupling or affinity-based surface chemistry (Cosnier, 1999; Gerard et al., 2002).

Emerging and Alternative Surface Patterning Strategies

Other approaches to improve spatial resolution and scalability include inkjet printing, bioprinting, self-assembled monolayers (SAMs), microcontact printing and microfluidic patterning. Inkjet printing enables non-contact, digitally controlled deposition of nanomaterial-based inks with micrometer-scale precision. This high-throughput method accommodates industrial mass manufacturing while allowing individual electrodes to receive distinct bioreceptors without contamination. Bioprinting adapts this principle by dispensing bio-inks under mild conditions that safeguard biological activity, making both techniques highly viable for scalable multiplex sensor production (Derby, 2010).

Self-assembled monolayers, particularly alkanethiol SAMs on gold electrodes, establish highly ordered and controllable surface chemistry via strong gold-sulfur bonds. These monolayers allow oriented immobilization of antibodies and aptamers, improving binding efficiency and minimizing nonspecific adsorption. This precise structural control makes SAMs especially useful for building impedance-based biosensors (Love et al., 2005).

Microcontact printing utilizes elastomeric stamps, typically made of polydimethylsiloxane (PDMS), to transfer patterned biomolecules or functional inks onto target surfaces with sharp spatial boundaries. This approach is useful for creating multiplexed recognition zones without solution spreading (Whitesides et al., 2001). Microfluidic channels can be aligned over multi-electrode arrays to selectively deliver different functionalization

solutions to individual electrodes. This approach eliminates droplet spreading and enables highly controlled surface modification. It is especially attractive for integrated lab-on-chip systems.

Although drop-casting remains widely used for rapid single-target assays, its poor spatial control restricts its utility in high-density arrays. When using drop-casting, minimizing repeated droplet deposition, avoiding excessive multilayer formation and optimizing surface coverage are crucial to preserve electroactive surface accessibility and ensure consistent current responses. Electrodeposition and advanced patterning methods address these limitations by preserving spatial resolution, minimizing cross-contamination and increasing mechanical durability.

Immobilization Strategies for Bioreceptors

Proper immobilization and correct orientation of bioreceptors are essential to ensure high loading capacity and optimal analytical performance. Control of bioreceptor orientation on the electrode surface directly influences sensitivity, as a higher number of accessible binding sites leads to an increased number of binding events and stronger signal generation (Turner, 2013). In antibody-based immobilization, orienting the antigen-binding fragments (Fab) upright on the surface ensures that they remain accessible to the target analyte (Hermanson, 2013; Welch et al., 2017). If Fab regions are improperly oriented, steric hindrance may occur, reducing binding efficiency and overall assay performance. To address this limitation, Protein A has been widely used in biosensor design due to its ability to bind the Fc region of antibodies, thereby promoting oriented immobilization with Fab regions directed away from the surface and available for antigen recognition (Caroselli et al., 2018; Trilling et al., 2013).

Various immobilization strategies can be used to control bioreceptor orientation on electrode surfaces, as illustrated in Figure 6. Although simple and straightforward, physical adsorption relies on weak interactions and often results in random bioreceptor orientation. This can lead to bioreceptor leaching during operation and reduced binding efficiency. Alternatively, covalent immobilization using EDC/NHS chemistry provides a robust and chemically stable linkage (Hermanson, 2013). Anchoring amine-functionalized antibodies or aptamers onto carboxylated carbon nanomaterials or AuNPs creates a stable surface layer. These covalent bonds are generally stable under a range of pH conditions commonly encountered in food matrices such as wine or maize extracts.

Self-assembled monolayers (SAMs), particularly those based on gold–thiol (Au–S) chemistry, have been reported as highly effective platforms for biosensor fabrication (Love et al., 2005). SAMs regulate the lateral spacing between bioreceptors and reduce steric hindrance at the electrode interface. Bioaffinity-based immobilization strategies, including biotin–streptavidin coupling, enable controlled alignment of antibodies or aptamers on the sensor surface, thereby preserving biological activity and enhancing sensitivity (Alves et al., 2013; Hanson & Whelan, 2024; Trilling et al., 2013). This bioaffinity approach provides a modular framework for multi-toxin screening, offering stable binding and uniform orientation

that improves the electrochemical signal-to-noise ratio.

Entrapment methods, in which bioreceptors are encapsulated within polymer or porous matrices, help to protect fragile biomolecules from environmental degradation (Cosnier, 1999). However, this approach has several limitations, including the need to balance physical protection with efficient electron transfer to maintain clear electrochemical signals, as signal attenuation may occur due to diffusion barriers within the matrix.

Overall, the selection of an immobilization strategy requires careful balancing of stability, sensitivity, fabrication complexity and compatibility with multiplex electrochemical platforms. This choice ultimately governs the analytical performance of the biosensor and determines whether a multi-mycotoxin electrochemical system can meet the requirements for reliable and practical food safety applications.

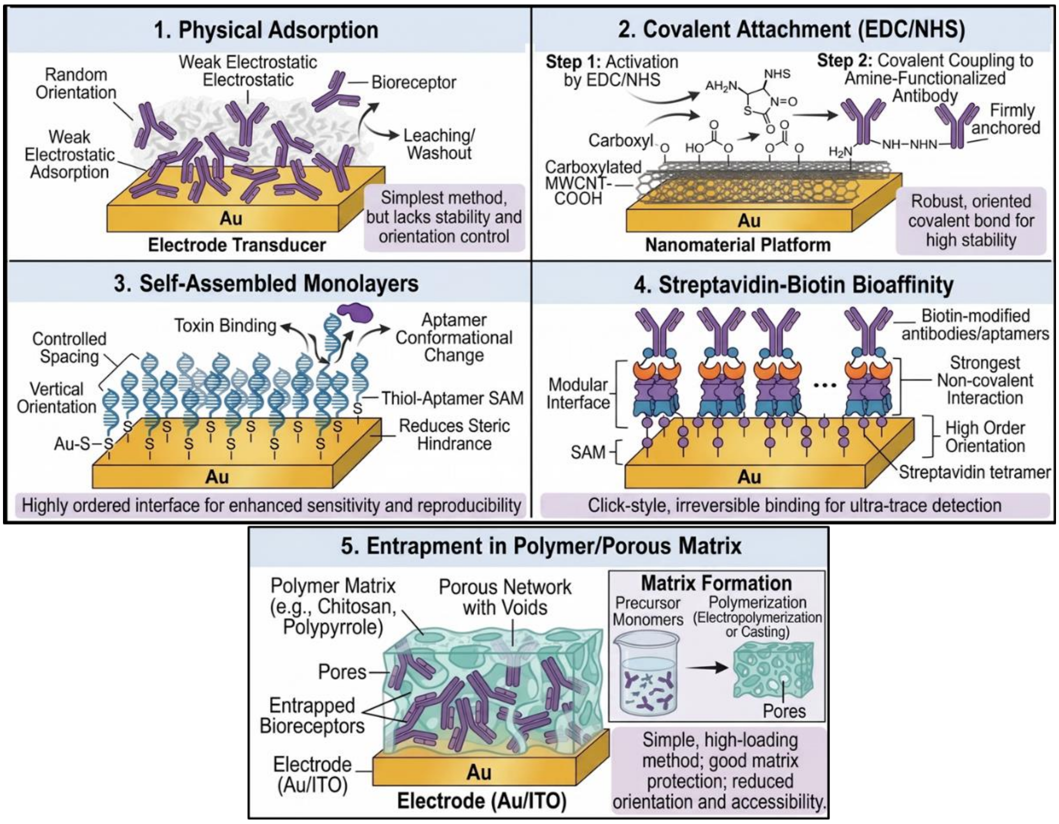

Figure 6. Overview of bioreceptor immobilization strategies for biosensing platforms.

Matrix Effects and Key Analytical Considerations

Another important aspect that must be addressed in biosensor applications is matrix effects in real samples. From the high protein and lipid content in milk to the particulate-rich nature of fibrous feed extracts, these matrices can trigger nonspecific adsorption, surface fouling and nanomaterial aggregation. As a result, the analytical reproducibility of biosensor

systems is compromised. For instance, AuNPs which exhibit high stability in buffered laboratory reagents, may rapidly develop a protein corona in crude extracts, potentially attenuating the electrochemical response. Current biosensor engineering therefore increasingly integrates antifouling strategies to mitigate these effects. Common methods include polyethylene glycol (PEG) grafting, bovine serum albumin (BSA) blocking, zwitterionic coatings or the use of composite nanostructures to improve colloidal stability.

Mycotoxins' Polarity and Solubility

The wide variation in polarity and solubility among different mycotoxins creates a significant challenge in the development of multiplex electrochemical biosensors for mycotoxin detection (Stroka & Maragos, 2016). For example, AFB1 is relatively hydrophobic and poorly soluble in water, so it often requires organic solvents such as methanol or acetonitrile for efficient extraction. In contrast, DONs and FUMs are more polar and dissolve more readily in aqueous or aqueous–organic mixtures. ZEN and OTA fall in between and are commonly extracted using methanol- or acetonitrile-based systems. Because of these differences, multi-mycotoxin extraction methods typically rely on acetonitrile–water mixtures such as 80:20 or 50:50 (v/v), or modified QuEChERS protocols that can handle a broader range of chemical properties (Sulyok et al., 2020; Turner et al., 2009).

These solvent differences also create a practical challenge for biosensor design. The sensing environment must keep all target analytes in solution while still preserving the stability of the biological recognition elements and maintaining a stable electrode interface. Although organic solvents help prevent hydrophobic toxins like AFs from precipitating, high solvent levels can damage protein-based receptors such as antibodies, disrupt aptamer folding and weaken nanomaterial-modified surfaces. To manage this, many biosensor designs use more robust covalent immobilization strategies such as EDC/NHS coupling, which provide stronger resistance to solvent-related loss of biomolecules compared with physical adsorption. In practice, electrochemical biosensors often keep organic solvent content below 10 to 20 percent (v/v) to maintain biomolecular activity, since higher levels can reduce antibody binding efficiency and increase background electrochemical noise (Nguyen et al., 2019; Valera et al., 2007). Overall, careful tuning of extraction solvents, buffer conditions and surface chemistry is essential for reliable simultaneous detection of multiple mycotoxins with different polarity characteristics.

Sensitivity Requirements and Regulatory MRLs

The sensitivity of biosensor systems relative to established MRLs for specific mycotoxins is a critical factor for effective multi-mycotoxin detection. As shown in Table 2, mycotoxins in animal feed are subject to varying MRLs established by respective national regulatory bodies. For food matrices in particular, Table 5 outlines typical European Union (EU) MRLs and the corresponding analytical performance required for compliance. Achieving the low-level detection required by these MRLs, which are frequently in the µg/kg (ppb) range,

presents a significant challenge in biosensor development. This requirement necessitates a high signal-to-noise (S/N) ratio to distinguish weak analytical signals from background interference inherent in complex food and feed matrices.

Table 5. EU Maximum Residue Limits (MRLs) for key mycotoxins in food matrices

Mycotoxin	**Typical Matrix**	**EU MRL Range (µg/kg or ppb)**	**Analytical Demand**
AFB1	Groundnuts, maize, dried fruit, spices	0.1 – 12.0	Ultra-trace detection
OTA	Cereals, dried vine fruits, coffee, wine, grape juice	0.5 – 10.0	High sensitivity
FB1 and FB2	Maize and maize-based foods	200 – 4000	High troughput
ZEN	Maize, cereals, breakfast cereals, snacks	200-400	Moderate sensitivity
DON	Unprocessed cereals, pasta, bread, baby food	200 – 1750	Broad range detection

A major obstacle in the simultaneous electrochemical detection of multiple mycotoxins is the wide variance in their regulatory MRLs. For instance, the EU has established a maximum level of 0.1 µg/kg for AFB1 in processed cereal-based foods and baby foods intended for infants and young children, whereas DON is regulated at substantially higher concentrations, with limits reaching 750 µg/kg in certain cereal-based products. This variation requires multiplexed biosensors to feature an expansive linear dynamic range. A platform optimized solely for the ultra-sensitive detection of AFB1 can suffer from premature signal saturation when encountering much higher concentrations of co-occurring toxins like ZEN or DON. To circumvent this, the nanomaterial-bioreceptor interface must be precisely engineered. Modulating the surface density of the bioreceptor allows the sensor to maintain analytical validity across three orders of magnitude within a single assay run.

Beyond sensor sensitivity, sample heterogeneity presents an additional challenge. Mycotoxins rarely distribute evenly throughout a commodity; instead, they often cluster in localized "hotspots" within grain lots and food matrices (Kerry et al., 2024; Wagner, 2015). Overcoming this limitation requires robust pre-analytical sampling protocols, including thorough homogenization and sufficient sample sizes, to ensure the final electrochemical readout accurately reflects the true contamination level of the entire batch.

Strategic Analyte Pairing for Dual-Electrode Biosensors

Selecting analyte combinations with compatible physicochemical properties is critical for maximizing the efficiency of multiplexed electrochemical detection. For example, AFB1 and OTA represent an excellent analytical pair because both partition into organic extraction phases and share similarly low MRLs. This alignment enables the use of a unified sample

preparation protocol and a consistent linear sensing range. Conversely, pairing a highly polar toxin like DON with a lipophilic species like AFB1 introduces a distinct solubility conflict. In these scenarios, the electrolyte composition must be carefully optimized to prevent the precipitation of the lipophilic analyte while ensuring the dissolution of the polar target. Resolving this issue often requires introducing organic modifiers at concentrations of 10% to 20% (v/v), a threshold that can compromise the structural stability of protein-based bioreceptors.

To guide the development of dual-sensing platforms, Table 6 presents common mycotoxin pairs based on their chemical compatibility and regulatory status. These classifications highlight how large differences in polarity and variations in maximum residue limits can create significant challenges for the simultaneous extraction and detection of multiple mycotoxins.

Table 6. Compatibility categorization for mycotoxin pairs in dual-electrode systems

Category	Mycotoxin Pair	Rationale	Polarity & MRL Status
Ideal	AFB1 + OTA	Highly compatible. Both are lipophilic and regulated at similar trace levels.	Similar polarity & MRL
Acceptable	AFB1 + ZEN	Similar organic solvent requirements (methanol/acetonitrile), however ZEN has higher MRLs.	Partial polarity match and different MRL
Not Ideal .	AFB1 + DON	Solubility Paradox. AFB1 is lipophilic, DON is polar. Simultaneous extraction is a major bottleneck.	Conflicting polarity
Not Ideal	FB1 + T-2	Extreme polarity difference. FB1 requires aqueous/acidified solvents while T-2 is lipophilic.	Extreme polarity gap

Ultimately, how well a dual-electrode biosensor performs is not only determined by the sensitivity of the functionalized electrode surface itself but also by how well the selected analytes fit together chemically. Choosing target pairs with similar solubility behaviour and comparable regulatory limits is key to making reliable one-pot electrochemical measurements.

Commercialization Barriers and Translational Challenges

Although there are many reports on rapid and portable mycotoxin detection in the academic literature, a clear gap remains between laboratory proof-of-concept systems and industrial-scale production. Most biosensors show strong analytical performance under controlled conditions, but this does not always translate to reliable large-scale manufacturing.

A key reason for this gap is the lack of standardized fabrication methods, which leads to batch-to-batch variation, inconsistent electrode surfaces and signal drift. In multiplex electrochemical arrays, even small changes in nanomaterial deposition, bioreceptor immobilization or drying conditions can noticeably affect performance. When these systems are integrated with portable potentiostats, microfluidic components and simplified readout devices, additional engineering challenges arise that are often not fully addressed in early-stage studies.

In the same way, techniques such as manual drop-casting and small-scale surface modification work well for prototyping but are not suitable for large-scale production due to limited precision and repeatability. To achieve consistent quality across large numbers of devices, fabrication needs to shift toward scalable methods such as automated dispensing, inkjet printing or screen printing. This transition from manual preparation to automated manufacturing is essential to reproduce laboratory-level sensitivity in commercially produced sensors.

For real-world use, reproducibility and long-term stability are also critical. Many current biosensors rely on fragile biological recognition elements such as antibodies, which require cold storage and careful handling. This creates challenges for storage, transport and field deployment. More extensive stability testing under real operating conditions, including changes in temperature, humidity and mechanical stress during transport, is necessary to ensure reliable shelf life. Developing sensors that remain stable under ambient conditions without refrigeration is particularly important for tropical regions, where high temperature and humidity can accelerate both sensor degradation and mycotoxin formation.

Finally, strong validation against established reference methods such as liquid chromatography–tandem mass spectrometry (LC–MS/MS) is essential. New platforms should be tested using naturally contaminated samples rather than relying only on spiked laboratory matrices. Evaluating accuracy, precision and robustness under field conditions is a key step for regulatory acceptance and eventual commercial use. Overall, moving from laboratory innovation to market-ready devices requires a shift in focus from achieving peak analytical performance to ensuring manufacturability, robustness and regulatory compliance.

Future Perspectives and Emerging Trends

Future advancements in multi-mycotoxin monitoring will likely be defined by the convergence of material science, data analytics and digital connectivity. The following sections outline the primary technological directions driving this field.

Artificial Intelligence and Machine Learning–Enhanced Sensing

Multiplexed electrochemical detection frequently encounters overlapping redox peaks, baseline drift and matrix-dependent interference. These phenomena are particularly pronounced when analyzing complex agricultural commodities such as maize, milk and grain corn. Incorporating machine learning algorithms, chemometric tools like principal component

analysis and neural network modeling provides an effective route for signal deconvolution and accurate pattern recognition. Emerging analytical platforms are shifting from basic concentration reporting toward adaptive, self-calibrating systems. Training algorithms to recognize matrix-specific interference signatures permits real-time correction of electrochemical signal distortions. This computational processing improves quantification accuracy under field conditions and increases the overall analytical reliability of multiplexed arrays.

Hybrid Biorecognition Systems

A primary constraint in sensor development is the historic compromise between the high specificity of biological receptors and the chemical robustness of synthetic alternatives like MIPs. Although antibodies and aptamers yield excellent target affinity, they exhibit limited thermal stability and require strict storage conditions. Conversely, MIPs provide exceptional mechanical and chemical durability but can lack optimal binding precision. To address these limitations, contemporary research focuses on hybrid bioreceptors, including combined MIP-aptamer dual-recognition platforms (Chi et al., 2025; Hu et al., 2025; Yang et al., 2025). Merging the selective affinity of oligonucleotide aptamers with the structural integrity of a polymer matrix yields a sensor with lock-and-key specificity that simultaneously withstands adverse environmental conditions (Roushani et al., 2022). These hybrid architectures provide a viable route toward robust, field-ready diagnostic tools that do not require refrigeration.

Internet of Things Infrastructure and Networked Food Safety

Multi-mycotoxin monitoring is gradually moving away from standalone devices toward connected digital systems. When miniaturized electrochemical cells are paired with smartphone-based potentiostats, analytical results can be transmitted directly to cloud platforms for immediate processing. This Internet of Things (IoT) approach enables near real-time tracking of contamination and supports more predictive forms of surveillance.

A clear example of this shift is the MARDIsense system reported by Said et al. (2023), which uses a cloud-connected electrochemical biosensor for AFB1 detection. Platforms such as this allow field measurements to be uploaded and shared with central databases, turning food safety monitoring from a reactive process into a more proactive and data-informed system. In another development, Yang et al. (2025) demonstrated a smartphone-based visual sensing platform that uses a combined MIP-aptamer recognition strategy for simultaneous detection of AFB1 and OTA. This approach helps bridge laboratory-level analytical performance with practical field usability.

Together, these connected systems allow regulators and supply chain stakeholders to observe contamination patterns across regions more effectively. As field data are continuously uploaded to cloud-based mapping tools, emerging hotspots such as Fusarium contamination in grain corn can be identified earlier. This type of distributed data collection

supports faster decision-making, including targeted recalls and improved storage management, before contaminated products move further along the supply chain.

Conclusion

The increasing occurrence of multiple mycotoxins in agricultural products has created an urgent need for rapid, sensitive and multiplex detection technologies. Electrochemical biosensors have emerged as a promising solution due to their high sensitivity, relatively low production cost and suitability for portable field applications. This review discussed the main design factors that influence biosensor performance, including nanomaterial selection, surface functionalization and multi-working electrode configurations that allow simultaneous detection of several targets. Successful development of reliable multiplex systems also depends on a clear understanding of the chemical behaviour and regulatory limits of target analytes. Maximum residue limits vary considerably among different mycotoxins; therefore, sensor interfaces must be carefully optimized to achieve a broad linear response range while preventing early signal saturation. The selection of analyte combinations should also consider differences in solubility during extraction. At the same time, effective sample preparation and homogenization remain essential to minimize variability caused by the uneven distribution of mycotoxins in real agricultural samples. Although significant progress has been achieved at the laboratory level, translating these technologies into large-scale industrial production continues to be a major challenge. Many currently reported systems still depend on manual fabrication procedures, which can affect reproducibility and limit scalability. Future progress will likely depend on the adoption of automated and standardised manufacturing approaches such as screen printing and automated dispensing techniques. Further advances in artificial intelligence, hybrid recognition strategies and IoT-based sensing platforms are expected to improve analytical performance and support real-time monitoring capabilities. These developments will contribute to the creation of more practical, reliable and field-ready biosensors for multi-mycotoxin detection throughout the food supply chain.

Acknowledgment

The authors acknowledge the Ministry of Agriculture and Food Security (KPKM) for the financial support provided under grant numbers K-RB167, P-RB502 and P-RB606. Gratitude is also extended to the research team at Biogenes Technologies Sdn. Bhd. for providing technical insights regarding the development of the MARDIsense platform.

Author Contribution

Nur Azura Mohd Said – Conceptualization, Writing - original draft preparation, Visualization.
Norhafniza Awaludin –Writing – review & editing.
Suria Mohd Saad –Writing – review & editing.
Erna Mutiara Masdek –Writing – review & editing.
Sahira Akmar Zulkepli –Writing – review & editing.
Syah Noor Muhammad Ramli –Writing – review & editing.

Conflict of Interest

The authors declare no conflict of interest.

Declaration on the Use of Generative AI

Generative Artificial Intelligence (AI) tools (ChatGPT and Gemini) are used in their manuscript preparation for language enhancement. However, their use does not compromise the originality, intellectual contribution, or scientific integrity of the work. The authors then reviewed and edited the content as needed and take full responsibility for the content of the published article. Conceptual illustrations and graphical elements were generated using Gemini (Nano Banana 2/Nano Banana Pro).

References

Alves, N. J., Mustafaoglu, N., & Bilgicer, B. (2013). Oriented antibody immobilization by site-specific UV photocrosslinking of biotin at the conserved nucleotide binding site for enhanced antigen detection. *Biosensors and Bioelectronics*, *49*, 387-393.

Alexander, E., & Leong, K. W. (2024). Discovery of nanobodies: A comprehensive review of their applications and potential over the past five years. *Journal of Nanobiotechnology*, *22*(1), 661.

Amara, U., Hussain, I., Ahmad, M., Mahmood, K., & Zhang, K. (2023). 2D MXene-based biosensing: a review. *Small*, *19*(2), 2205249.

Ameh, J. B., Atawodi, S. E., Negedu, A., Tanko, H. Y., & Umoh, V. J. (2011). Economic and health perspectives of mycotoxins: A review. *Continental Journal of Biomedical Sciences*, *5*(1), 5–26.

Arduini, F., Micheli, L., Moscone, D., Palleschi, G., Piermarini, S., Ricci, F., & Volpe, G. (2016). Electrochemical biosensors based on nanomodified screen-printed electrodes: Recent applications in clinical analysis. *TrAC Trends in Analytical Chemistry*, *79*, 114-126.

Ascoli, C. A., & Aggeler, B. (2018). Overlooked benefits of using polyclonal antibodies. *BioTechniques, 65*(3), 127-136.

Atnafu, B., Garbaba, C. A., Lemessa, F., Migheli, Q., Sulyok, M., & Chala, A. (2024). Multiple mycotoxins associated with maize (Zea mays L.) grains harvested from subsistence farmers' fields in southwestern Ethiopia. *Mycotoxin Research, 40*(3), 389-399.

Azri, F. A., Eissa, S., Zourob, M., Chinnappan, R., Sukor, R., Yusof, N. A., Raston, N.H.A., Alhoshani, A., & Jinap, S. (2020). Electrochemical determination of zearalenone using a label-free competitive aptasensor. *Microchimica Acta, 187*(5), 266.

Bard, A. J., Faulkner, L. R., & White, H. S. (2022). *Electrochemical methods: Fundamentals and applications*. John Wiley & Sons.

Becker, B., & Cooper, M. A. (2011). A survey of the 2006–2009 quartz crystal microbalance biosensor literature. *Journal of Molecular Recognition*, *24*(5), 754-787.

Caroselli, R., Garcia Castello, J., Escorihuela, J., Bañuls, M. J., Maquieira, Á., & García-Rupérez, J. (2018). Experimental study of the oriented immobilization of antibodies on photonic sensing structures by using protein a as an intermediate layer. *Sensors*, *18*(4), 1012.

Chen, X., Huang, Y., Duan, N., Wu, S., Xia, Y., Ma, X., Zu, C., Jiang, Y., & Wang, Z. (2014). Screening and identification of DNA aptamers against T-2 toxin assisted by graphene oxide. *Journal of Agricultural and Food Chemistry*, *62*(42), 10368-10374.

Chen, X., Wu, H., Tang, X., Zhang, Z., & Li, P. (2023). Recent advances in electrochemical sensors for mycotoxin detection in food. *Electroanalysis*, *35*(2), e202100223.

Chi, H., Xiao, Y., Wen, X., & Tang, J. (2025). An aptamer–MIP dual-recognition electrochemical sensor utilizing Au-Pt bimetallic nanoflowers and CMCS@ COF-300 nanocomposites for the ultrasensitive detection of aflatoxin B1. *Analytica Chimica Acta*, 344605.

Cosnier, S. (1999). Biomolecule immobilization on electrode surfaces by entrapment or attachment to electrochemically polymerized films. A review. *Biosensors and Bioelectronics*, *14*(5), 443-456.

Cruz-Aguado, J. A., & Penner, G. (2008). Determination of ochratoxin A with a DNA aptamer. *Journal of Agricultural and Food Chemistry*, *56*(22), 10456-10461.

Deegan, R. D., Bakajin, O., Dupont, T. F., Huber, G., Nagel, S. R., & Witten, T. A. (1997). Capillary flow as the cause of ring stains from dried liquid drops. *Nature*, *389*(6653), 827-829.

Derby, B. (2010). Inkjet printing of functional and structural materials: fluid property requirements, feature stability, and resolution. *Annual Review of Materials Research*, *40*(1), 395-414.

Dong, S., Yan, J., Zhou, S., & Zhou, Q. (2022). Mycotoxins detection based on electrochemical approaches. *Electroanalysis*, *34*(2), 132-147.

Eissa, S., Tlili, C., Mounir, B. A., & Kanoun, O. (2022). Nanomaterials-based electrochemical biosensors. *Frontiers in Bioengineering and Biotechnology*, *10*, 1091592.

El-Khatib, A. H., Bahlmann, A., Hutzler, C., & Weigel, S. (2025). A validated LC–MS/MS multi-method for the determination of 110 mycotoxins and plant toxins in cow milk and application to samples from Germany. *Analytical and Bioanalytical Chemistry, 417*(22), 5047-5059.

Eskola, M., Kos, G., Elliott, C. T., Hajšlová, J., Mayar, S., & Krska, R. (2020). Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate'of 25%. *Critical Reviews in Food Science and Nutrition*, *60*(16), 2773-2789.

Fan, Y., Li, J., Amin, K., Yu, H., Yang, H., Guo, Z., & Liu, J. (2023). Advances in aptamers, and application of mycotoxins detection: A review. *Food Research International*, *170*, 113022.

Foubert, A., Beloglazova, N. V., & De Saeger, S. (2017). Comparative study of colloidal gold and quantum dots as labels for multiplex screening tests for multi-mycotoxin detection. *Analytica Chimica Acta*, *955*, 48-57.

Fusilier, K., Chilvers, M. I., Limay-Rios, V., & Singh, M. P. (2022). Mycotoxin co-occurrence in Michigan harvested maize grain. *Toxins, 14*(7), 431.

Gerard, M., Chaubey, A., & Malhotra, B. D. (2002). Application of conducting polymers to biosensors. *Biosensors and Bioelectronics*, *17*(5), 345-359.

Gu, W., Zhu, P., Jiang, D., He, X., Li, Y., Ji, J., Zhang, L., Sun, Y., & Sun, X. (2015). A novel and simple cell-based electrochemical impedance biosensor for evaluating the combined toxicity of DON and ZEN. *Biosensors and Bioelectronics*, *70*, 447-454.

Hamid, H. B., Foong, J. T., Kong, C. Z. Y., Ng, Y. P., Tan, B. S., & Ng, K. W. (2025). Advances and challenges in multi-mycotoxin detection: Ensuring safety in herbal medicinal products. *International Journal of Food, Drug and Cosmetics*, *1*(1), 1-13.

Hanson, E. K., & Whelan, R. J. (2024). Combining the benefits of biotin–streptavidin aptamer immobilization with the versatility of Ni-NTA regeneration strategies for SPR. *Sensors*, *24*(9), 2805.

Hassani, S., Maghsoudi, A. S., Akmal, M. R., Shoeibi, S., Ghadipasha, F., Mousavi, T., Ganjali, M.R., Hosseini, R., & Abdollahi, M. (2022). A novel approach for designing electrochemical aptamer-based biosensor for ultrasensitive detection of zearalenone as a prevalent estrogenic mycotoxin. *Current Medicinal Chemistry, 29*(37), 5881-5894.

He, T., Wang, Y., Li, P., Zhang, Q., Lei, J., Zhang, Z., Ding, X., Zhou, H., & Zhang, W. (2014). Nanobody-based enzyme immunoassay for aflatoxin in agro-products with high tolerance to cosolvent methanol. *Analytical Chemistry, 86*(17), 8873-8880.

Hermanson, G. T. (2013). *Bioconjugate techniques*. Academic Press.

Homola, J. (2008). Surface plasmon resonance sensors for detection of chemical and biological species. *Chemical Reviews*, *108*(2), 462–493.

Hu, R., Guo, Z., Wang, J., Zhou, M., Zhu, K., & Xu, Y. (2026). Research advances in rapid detection technologies for multiple mycotoxins. *Journal of Food Protection*, 100702.

Hu, X., Yang, Y., Wang, K., & Liu, Y. (2025). Dual recognition strategies using aptamer-molecularly imprinted polymers for enhanced detection of food safety hazards: A review. *Polymer Bulletin*, *82*(10), 4351-4373.

Janik, E., Niemcewicz, M., Podogrocki, M., Ceremuga, M., Stela, M., & Bijak, M. (2021). T-2 toxin-The most toxic trichothecene mycotoxin: Metabolism, toxicity, and decontamination strategies. *Molecules*, *26*(22), 6868.

Jebril, S., Fredj, Z., Saeed, A. A., Gonçalves, A. M., Kaur, M., Kumar, A., & Singh, B. (2024). Nanomaterial-based electrochemical chemo (bio) sensors for the detection of nanoplastic residues: trends and future prospects. *RSC Sustainability*, *2*(4), 832-851.

Jiang, Y., & Wu, J. (2019). Recent development in chitosan nanocomposites for surface-based biosensor applications. *Electrophoresis*, *40*(16-17), 2084-2097.

Jonard, C., Chandelier, A., Eylenbosch, D., Pannecoucque, J., Godin, B., Douny, C., Scippo, M-L., & Gofflot, S. (2025). Multi-mycotoxin analyses by UPLC-MS/MS in wheat: The situation in Belgium in 2023 and 2024. *Foods, 14*(13), 2300.

Kavanagh, O., Elliott, C. T., & Campbell, K. (2015). Progress in the development of immunoanalytical methods incorporating recombinant antibodies to small molecular weight biotoxins. *Analytical and Bioanalytical Chemistry, 407*(10), 2749-2770.

Kerry, R., Ingram, B., Abbas, H. K., & Ahlborn, G. (2024). An investigation of the spatial arrangement of mycotoxin build-up in corn stored under different environmental conditions. *Toxins*, *16*(12), 508.

Li, S. J., Sheng, W., Wen, W., Gu, Y., Wang, J. P., & Wang, S. (2018). Three kinds of lateral flow immunochromatographic assays based on the use of nanoparticle labels for fluorometric determination of zearalenone. *Microchimica Acta, 185*(4), 238.

Li, R., Wen, Y., Wang, F., & He, P. (2021). Recent advances in immunoassays and biosensors for mycotoxins detection in feedstuffs and foods. *Journal of Animal Science and Biotechnology, 12*(1), 108.

Liu, Z., Hua, Q., Wang, J., Liang, Z., Li, J., Wu, J., Shen, X., Lei, H., & Li, X. (2020). A smartphone-based dual detection mode device integrated with two lateral flow immunoassays for multiplex mycotoxins in cereals. *Biosensors and Bioelectronics*, *158*, 112178.

Liu, X., Tang, Z., Duan, Z., He, Z., Shu, M., Wang, X., Gee, S.J., Hammock, B.D., & Xu, Y. (2017). Nanobody-based enzyme immunoassay for ochratoxin A in cereal with high resistance to matrix interference. *Talanta*, *164*, 154-158.

Love, J. C., Estroff, L. A., Kriebel, J. K., Nuzzo, R. G., & Whitesides, G. M. (2005). Self-assembled monolayers of thiolates on metals as a form of nanotechnology. *Chemical Reviews*, *105*(4), 1103-1170.

Malhotra, S., Pandey, A. K., Rajput, Y. S., & Sharma, R. (2014). Selection of aptamers for aflatoxin M1 and their characterization. *Journal of Molecular Recognition*, *27*(8), 493-500.

Mejri Omrani, N., Hayat, A., Korri-Youssoufi, H., & Marty, J. L. (2016). Electrochemical biosensors for food security: Mycotoxins detection. *Biosensors for Security and Bioterrorism Applications* (pp. 469-490). Springer.

Meneely, J. P., & Elliott, C. T. (2014). Rapid surface plasmon resonance immunoassays for the determination of mycotoxins in cereals and cereal-based food products. *World Mycotoxin Journal*, *7*(4), 491-506.

Molinelli, A., Grossalber, K., Führer, M., Baumgartner, S., Sulyok, M., & Krska, R. (2008). Development of qualitative and semiquantitative immunoassay-based rapid strip tests for the detection of T-2 toxin in wheat and oat. *Journal of Agricultural and Food Chemistry*, *56*(8), 2589-2594.

Muyldermans, S. (2013). Nanobodies: natural single-domain antibodies. *Annual Review of Biochemistry, 82*(1), 775-797.

Nakhjavan, B., Ahmed, N. S., & Khosravifard, M. (2020). Development of an improved method of sample extraction and quantitation of multi-mycotoxin in feed by LC-MS/MS. *Toxins*, *12*(7), 462.

Nji, Q. N., & Mwanza, M. (2024). Three-year multi-mycotoxin analysis of South African commercial maize from three provinces. *Frontiers in Fungal Biology*, *5*, 1426782.

Nguyen, H. H., Lee, S. H., Lee, U. J., Fermin, C. D., & Kim, M. (2019). Immobilized enzymes in biosensor applications. *Materials*, *12*(1), 121.

Nolan, P. (2019). *Development of a multiplex biosensor for the detection of mycotoxins* [Master of Philosophy dissertation, Queen's University Belfast]. Faculty of Medicine, Health and Life Sciences.

Ong, C. C., Sangu, S. S., Illias, N. M., Gopinath, S. C. B., & Saheed, M. S. M. (2020). Iron nanoflorets on 3D-graphene-nickel: A ‘Dandelion’nanostructure for selective deoxynivalenol detection. *Biosensors and Bioelectronics*, *154*, 112088.

Oplatowska-Stachowiak, M., Reiring, C., Sajic, N., Haasnoot, W., Brabet, C., Campbell, K., Elliott, C.T., & Salden, M. (2018). Development and in-house validation of a rapid and simple to use ELISA for the detection and measurement of the mycotoxin sterigmatocystin. *Analytical and Bioanalytical Chemistry*, *410*(12), 3017-3023.

Oplatowska-Stachowiak, M., Sajic, N., Xu, Y., Haughey, S. A., Mooney, M. H., Gong, Y. Y., Varheijen, R., & Elliott, C. T. (2016). Fast and sensitive aflatoxin B1 and total aflatoxins ELISAs for analysis of peanuts, maize and feed ingredients. *Food Control*, *63*, 239-245.

Pires, R. D. A., Alves e Silva, T., Borowsky, A. M., Cortinhas, C. S., Carvalho, V. V. D., & Corassin, C. H. (2025). Evaluation of the occurrence of multi-mycotoxins in the diet of beef cattle feedlots in Brazil. *Ruminants, 5*(2), 12.

Rouah-Martin, E., Mehta, J., Van Dorst, B., De Saeger, S., Dubruel, P., Maes, B. U., Lemiere, F., Goormaghtigh, E., Daems, D., & Robbens, J. (2012). Aptamer-based molecular recognition of lysergamine, metergoline and small ergot alkaloids. *International Journal of Molecular Sciences*, *13*(12), 17138-17159.

Roushani, M., Farokhi, S., & Rahmati, Z. (2022). Development of a dual-recognition strategy for the aflatoxin B1 detection based on a hybrid of aptamer-MIP using a Cu_2O NCs/GCE. *Microchemical Journal*, *178*, 107328.

Said, N. A. M., Awaludin, N., Ismail, M. R., Razali, H., Masdek, E. M., Zulkepli, S. A., & Ramli, S. N. M. (2025). Polyclonal antibodies against Zearalenone: Production, characterisation, and application in food safety biosensors. *Pertanika Proceedings 1*(7), 133 – 137.

Said, N. A. M., Jusoh, N. S., Awaludin, N., Ismail, M. R., Bakri, N. F. M., Sojak, L. S. M., & Salam, F. (2023). Immunosensing of Aflatoxin B1 and Ochratoxin A on a portable device as point-of-care. In *Aspergillus and Aspergillosis-Advances in Genomics, Drug Development, Diagnosis and Treatment*. IntechOpen.

Shim, W. B., Kim, M. J., Mun, H., & Kim, M. G. (2014). An aptamer-based dipstick assay for the rapid and simple detection of aflatoxin B1. *Biosensors and Bioelectronics*, *62*, 288-294.

Stroka, J., & Maragos, C. M. (2016). Challenges in the analysis of multiple mycotoxins. *World Mycotoxin Journal, 9*(5), 847-862.

Sulyok, M., Stadler, D., Steiner, D., & Krska, R. (2020). Validation of an LC-MS/MS-based dilute-and-shoot approach for the quantification of > 500 mycotoxins and other secondary metabolites in food crops: Challenges and solutions. *Analytical and Bioanalytical Chemistry*, *412*(11), 2607-2620.

Tang, X., Li, X., Li, P., Zhang, Q., Li, R., Zhang, W., Ding, X., Lei, J., & Zhang, Z. (2014). Development and application of an immunoaffinity column enzyme immunoassay for mycotoxin zearalenone in complicated samples. *PloS one*, *9*(1), e85606.

Trilling, A. K., Beekwilder, J., & Zuilhof, H. (2013). Antibody orientation on biosensor surfaces: a minireview. *Analyst*, *138*(6), 1619-1627.

Turner, A. P. (2013). Biosensors: Sense and sensibility. *Chemical Society Reviews*, *42*(8), 3184-3196.

Turner, N. W., Subrahmanyam, S., & Piletsky, S. A. (2009). Analytical methods for determination of mycotoxins: A review. *Analytica Chimica Acta*, *632*(2), 168-180.

Valera, E., Ramón-Azcón, J., Rodríguez, Á., Castañer, L. M., Sánchez, F. J., & Marco, M. P. (2007). Impedimetric immunosensor for atrazine detection using interdigitated μ-electrodes (IDμE's). *Sensors and Actuators B: Chemical*, *125*(2), 526-537.

Vidal, J. C., Bonel, L., Ezquerra, A., Hernández, S., Bertolín, J. R., Cubel, C., & Castillo, J. R. (2013). Electrochemical affinity biosensors for detection of mycotoxins: A review. *Biosensors and Bioelectronics*, *49*, 146-158.

Wagner, C. (2015). Critical practicalities in sampling for mycotoxins in feed. *Journal of AOAC INTERNATIONAL*, *98*(2), 301-308.

Wan Syahidah, H., Suhaimi, D., Hartini, Y., & Nurulhayati, A.B. (2021). *Screening of fungal contamination of Aspergillus and Fusarium Mycotoxin in grain corn from Peninsular Malaysia*. In Proc. 40th MSAP Ann. Conf (pp. 157-159). Malaysia Society of Animal Production.

Wang, Y., Wang, X., Wang, S., Fotina, H., & Wang, Z. (2022). A novel lateral flow immunochromatographic assay for rapid and simultaneous detection of aflatoxin b1 and zearalenone in food and feed samples based on highly sensitive and specific monoclonal antibodies. *Toxins*, *14*(9), 615.

Wang, Y., Wei, D., Wang, Y., Wang, M., & Zhai, W. (2024). Recent progress of molecularly imprinted technique for the detection of mycotoxins in food. *Foods*, *13*(24), 4125.

Wang, Y., Zhang, C., Wang, J., & Knopp, D. (2022). Recent progress in rapid determination of mycotoxins based on emerging biorecognition molecules: A review. *Toxins, 14*(2), 73.

Wang, C., Zhao, X., Huang, X., Xu, F., Gu, C., Yu, S., Zhang, X., & Qian, J. (2024). Simultaneous detection of multiple mycotoxins using MXene-based electrochemical aptasensor array and a self-developed multi-channel portable device. *Talanta*, *278*, 126450.

Welch, N. G., Scoble, J. A., Muir, B. W., & Pigram, P. J. (2017). Orientation and characterization of immobilized antibodies for improved immunoassays. *Biointerphases*, *12*(2).

Wen, X., Huang, Q., Nie, D., Zhao, X., Cao, H., Wu, W., & Han, Z. (2021). A multifunctional n-doped cu–mofs (N–Cu–MOF) nanomaterial-driven electrochemical aptasensor for sensitive detection of deoxynivalenol. *Molecules*, *26*(8), 2243.

Whitesides, G. M., Ostuni, E., Takayama, S., Jiang, X., & Ingber, D. E. (2001). Soft lithography in biology and biochemistry. *Annual Review of Biomedical Engineering*, *3*(1), 335-373.

Wu, S., Duan, N., Zhang, W., Zhao, S., & Wang, Z. (2016). Screening and development of DNA aptamers as capture probes for colorimetric detection of patulin. *Analytical Biochemistry*, *508*, 58-64.

Xing, K. Y., Peng, J., Shan, S., Liu, D. F., Huang, Y. N., & Lai, W. H. (2020). Green enzyme-linked immunosorbent assay based on the single-stranded binding protein-assisted aptamer for the detection of mycotoxin. *Analytical Chemistry, 92*(12), 8422-8426.

Yang, Y. F., Feng, Y. J., Hu, X. P., Gu, H. W., Yan, X. F., & Yin, X. L. (2025). Smartphone-based visual sensing platform based on molecular imprinted polymers and aptamers synergistic recognition for simultaneous detection of aflatoxin B1 and ochratoxin A. *Microchemical Journal*, *212*, 113361.

Yazid, S. N. E., Ng, W. J., Selamat, J., Ismail, S. I., & Samsudin, N. I. P. (2021). Diversity and toxigenicity of Mycobiota in grain corn: A case study at pioneer grain corn plantations in Terengganu, Malaysia. *Agriculture, 11*(3), 237

Yue, S., Jie, X., Wei, L., Bin, C., Dou Dou, W., Yi, Y., QingXia, L., JianLin, L., & TieSong, Z. (2014). Simultaneous detection of Ochratoxin A and fumonisin B1 in cereal samples using an aptamer–photonic crystal encoded suspension Array. *Analytical Chemistry*, *86*(23), 11797-11802.

Yamashita, A., Yoshizawa, T., Aiura, Y., Sanchez, P. C., Dizon, E. I., Arim, R. H., & Sardjono. (1995). *Fusarium* mycotoxins (fumonisins, nivalenol, and zearalenone) and aflatoxins in corn from Southeast Asia. *Bioscience, Biotechnology, and Biochemistry*, *59*(9), 1804-1807.

Yoo, S. M., & Lee, S. Y. (2016). Optical biosensors for the detection of pathogenic microorganisms. *Trends in Biotechnology*, *34*(1), 7-25.

Zentai, A., Jóźwiak, Á., Süth, M., & Farkas, Z. (2023). Carry-over of aflatoxin B1 from feed to cow milk—A review. *Toxins*, *15*(3), 195.

Zhang, W., Tang, S., Jin, Y., Yang, C., He, L., Wang, J., & Chen, Y. (2020). Multiplex SERS-based lateral flow immunosensor for the detection of major mycotoxins in maize utilizing dual Raman labels and triple test lines. *Journal of Hazardous Materials*, *393*, 122348.

Zhang, X., Wang, Z., Fang, Y., Sun, R., Cao, T., Paudyal, N., Fang, W., & Song, H. (2018). Antibody microarray immunoassay for simultaneous quantification of multiple mycotoxins in corn samples. *Toxins*, *10*(10), 415.

Zhang, C., Zhang, Q., Tang, X., Zhang, W., & Li, P. (2019). Development of an anti-idiotypic VHH antibody and toxin-free enzyme immunoassay for ochratoxin A in cereals. *Toxins, 11*(5), 280.

Zou, Y., Liu, L., Qiu, Y., Kong, F., Hao, N., & Zhou, N. (2025). A portable visual/electrochemical dual-mode biosensing platform for simultaneous detection of two mycotoxins. *Journal of Hazardous Materials*, *495*, 138954.