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Development of a Biosensor for Rapid Detection of Aflatoxin Contamination in Stored Grain

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ABSTRACT

Background: *Aflatoxins, secondary metabolites of Aspergillus flavus and A. parasiticus, remain among the most significant chemical hazards in stored maize, groundnut, and other grains, with aflatoxin B1 classified as a Group 1 human carcinogen . Conventional confirmatory methods (HPLC, LC-MS/MS) are accurate but slow, costly, and unsuited to on-site screening.* ***Objective:*** *This review synthesises published evidence on biosensor platforms developed for rapid aflatoxin detection, evaluating recognition chemistries, transduction principles, and field-deployment suitability.* ***Methods:*** *A narrative review of published studies on biosensor platforms for aflatoxin detection, including immunoassay-based, aptamer-based, and electrochemical/optical transduction approaches, using PubMed, Google Scholar, and Crossref searches (2007-2025), individually verifying each source.* ***Findings:*** *Antibody-based lateral-flow immunoassays deliver limits of detection near 1 µg/L, adequate for regulatory screening ; aptamer-based electrochemical and photoelectrochemical sensors report sub-ng/L to pg/mL sensitivities ; nanoparticle-enhanced colorimetric and portable surface-plasmon-resonance sensors achieve ppb-level detection directly in grain matrices within minutes .* ***Conclusions:*** *Biosensors now match or exceed regulatory sensitivity requirements for aflatoxin B1, but reproducibility across real matrices, aptamer batch consistency, and field-hardware cost remain barriers to widespread adoption.*

Keywords: *aflatoxin B1; biosensor; aptasensor; immunoassay; grain safety*

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Abstract

Background: Aflatoxins, secondary metabolites of *Aspergillus flavus* and *A. parasiticus*, remain among the most significant chemical hazards in stored maize, groundnut, and other grains, with aflatoxin B1 classified as a Group 1 human carcinogen [1,2]. Conventional confirmatory methods (HPLC, LC-MS/MS) are accurate but slow, costly, and unsuited to on-site screening. **Objective:** This review synthesises published evidence on biosensor platforms developed for rapid aflatoxin detection, evaluating recognition chemistries, transduction principles, and field-deployment suitability. **Methods:** A narrative review of published studies on biosensor platforms for aflatoxin detection, including immunoassay-based, aptamer-based, and electrochemical/optical transduction approaches, using PubMed, Google Scholar, and Crossref searches (2007-2025), individually verifying each source. **Findings:** Antibody-based lateral-flow immunoassays deliver limits of detection near 1 µg/L, adequate for regulatory screening [3,4]; aptamer-based electrochemical and photoelectrochemical sensors report sub-ng/L to pg/mL sensitivities [5,6,7]; nanoparticle-enhanced colorimetric and portable surface-plasmon-resonance sensors achieve ppb-level detection directly in grain matrices within minutes [8,9,10]. **Conclusions:** Biosensors now match or exceed regulatory sensitivity requirements for aflatoxin B1, but reproducibility across real matrices, aptamer batch consistency, and field-hardware cost remain barriers to widespread adoption.

Keywords: aflatoxin B1; biosensor; aptasensor; immunoassay; grain safety

Introduction

Aflatoxins are difuranocoumarin mycotoxins produced predominantly by *Aspergillus flavus* and *A. parasiticus* during pre-harvest crop stress and post-harvest storage under warm, humid conditions [2,11]. Aflatoxin B1 (AFB1) is hepatotoxic and genotoxic, classified by IARC as a Group 1 carcinogen, and chronic dietary exposure in maize- and groundnut-dependent regions is associated with hepatocellular carcinoma and childhood growth faltering [1]. Regulatory maxima for AFB1 and total aflatoxins in

maize and other grains vary by jurisdiction, generally in the low single-digit to twenty micrograms-per-kilogram range, creating demand for screening tools sensitive and specific enough to support real-time acceptance or rejection decisions at silos, mills, and ports [11,2]. Chromatographic reference methods (HPLC-fluorescence, LC-MS/MS) remain the analytical gold standard but require sample cleanup, trained personnel, and laboratory infrastructure unavailable at most grain-handling sites. Over the past two decades, a substantial biosensor literature has emerged around three complementary recognition strategies - polyclonal/monoclonal antibodies, synthetic DNA/RNA aptamers, and molecularly imprinted polymers - coupled to electrochemical, optical, piezoelectric, or lateral-flow transduction [12,13]. Interest in these platforms is driven by three converging pressures: the labour and cost of routine chromatographic testing at high sample throughput, the need for decentralised decision-making by farmers, cooperatives, and traders who lack laboratory access, and growing recognition that aflatoxin contamination is unevenly distributed within a grain lot, so many rapid, low-cost tests at the point of storage can outperform a single centralised assay. This review synthesises that literature, focusing specifically on platforms validated against grain or grain-derived matrices, to characterise current analytical performance and the practical obstacles to field deployment.

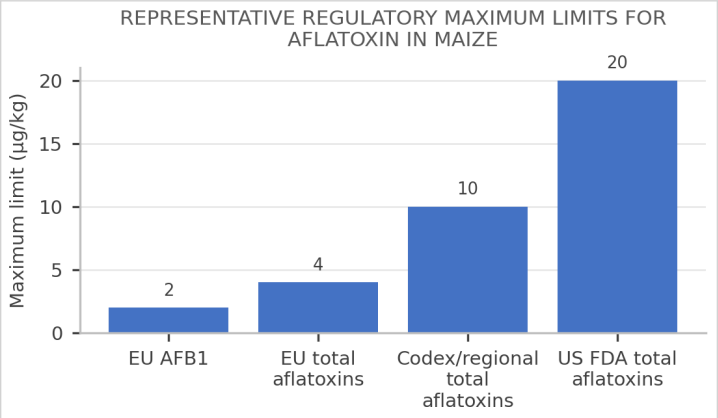
Methods

This is a narrative review, not a primary experimental study. Relevant literature was identified through structured searches of PubMed, Google Scholar, and Crossref (final search September 2026) using combinations of the terms "aflatoxin," "biosensor," "aptasensor," "immunosensor," "lateral flow," "electrochemical," "surface plasmon resonance," and "grain" or "maize." Candidate studies were prioritised where they reported a quantitative limit of detection (LOD) for aflatoxin B1 or M1 in a food, feed, or grain matrix, and each reference retained in the final list was individually verified for author list, journal, year, and DOI/PMID before inclusion. Reviews, primary sensor-development papers, and foundational toxicology/regulatory sources were included; sources whose bibliographic details could not be independently confirmed were excluded rather than cited provisionally.

Aflatoxin Toxicology, Occurrence, and Regulatory Limits in Stored Grain

Aflatoxin contamination of stored maize and groundnut is driven by fungal proliferation under elevated moisture and temperature, and by insect or mechanical kernel damage that facilitates fungal ingress during storage [11,2]. AFB1 undergoes hepatic bioactivation to a reactive 8,9-epoxide that forms DNA and protein adducts; chronic low-dose exposure is a recognised co-factor in hepatocellular carcinoma, particularly alongside hepatitis B infection, and has been associated with growth faltering in aflatoxin-exposed infants in West Africa [1]. Because AFM1, the hydroxylated hepatic metabolite excreted in milk, carries its own regulatory limits, detection methods validated for both AFB1 in grain and AFM1 in dairy are reviewed together here. Regulatory maxima differ across jurisdictions but are uniformly in the low-ppb range for AFB1 and somewhat higher for total aflatoxins, meaning screening biosensors must reliably resolve concentrations at or below a few micrograms per kilogram to be regulatory-relevant [2,11]. Early immunochemical work establishing high-affinity antibodies against AFB1 and its DNA adducts underpins essentially all subsequent immunoassay-based biosensor formats [14]. Comprehensive reviews of aflatoxin biology and management consistently emphasise that pre-harvest biocontrol and post-harvest drying and hermetic storage reduce, but do not eliminate, contamination risk, so testing at receival and during storage remains a necessary complement to agronomic control [11,2].

Figure 1: Representative regulatory maximum limits for aflatoxin B1 and total aflatoxins in maize across major jurisdictions, illustrating the sensitivity margin biosensors must meet for regulatory-relevant screening [2,11].



Recognition Elements: Antibodies versus Aptamers

Antibody-based recognition, whether polyclonal or monoclonal, remains the dominant approach in commercial lateral-flow devices and colorimetric immunoassays because of its established production pipelines and long shelf-life when lyophilised [3,10]. However, antibody batch-to-batch variability, cold-chain requirements, and cross-reactivity with structurally related mycotoxins motivate interest in synthetic aptamers selected by SELEX, which offer smaller size, chemical stability, reversible denaturation, and straightforward conjugation to electrode or nanomaterial surfaces [5,15]. Aptamer-based electrochemical sensors for AFB1 have reported detection limits spanning picogram-to-femtogram-per-millilitre ranges on glassy-carbon and gold electrode platforms, substantially below antibody-based lateral-flow sensitivity, though often demonstrated in buffer or spiked extracts rather than unprocessed grain [5]. Host-guest recognition strategies using β-cyclodextrin-functionalised aptamers, and regenerable ratiometric aptasensors validated directly in grain digests, illustrate progress toward matrix-robust aptasensing [16,7]. Molecularly imprinted polymers, synthetic recognition elements templated against AFB1 or dummy analogues, offer a third, antibody-free option with demonstrated compatibility with fluorescent quantum-dot transduction [17]. Each recognition chemistry carries trade-offs relevant to grain testing: antibodies are robust to lyophilisation but costly and slow to raise against new analogues; aptamers are cheap to synthesise once selected but can show batch-to-batch secondary-structure variability; and imprinted polymers are the most thermally and chemically stable but typically show broader cross-reactivity than affinity biomolecules, a trade-off summarised in Table 2.

Transduction Platforms and Analytical Performance

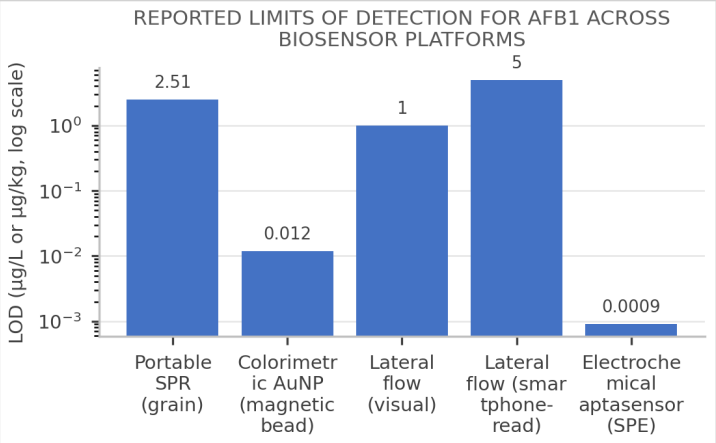
Transduction strategies reported for aflatoxin biosensing include colorimetric/lateral-flow immunoassays, electrochemical (amperometric, impedimetric) aptasensors, optical methods (surface plasmon resonance, fluorescence, surface-enhanced Raman spectroscopy), and piezoelectric quartz crystal

microbalance sensors (Figure 1; Table 1). Portable SPR devices have achieved on-site AFB1 detection directly in rice, peanut, and almond samples at 2.51 µg/kg without laboratory extraction steps [8]. Gold-nanoparticle-based colorimetric and magnetic-bead immunoassays reach limits near 12 ng/L for AFB1 and 0.15 ng/mL across multiple mycotoxins using simple UV-Vis or naked-eye readout [9,10]. Lateral-flow immunochromatographic strips remain the most field-familiar format, with visual and reader-assisted limits around 0.2-1.0 µg/L for AFB1 and validated simultaneous detection of AFB1 and zearalenone [3,4]. Fluorescence- and Raman-based approaches, including handheld fluorescence spectrometers validated on naturally contaminated maize and SERS methods applied directly to maize extracts, extend rapid screening into spectroscopic modalities without antibody consumption [18,19]. Electrochemical aptasensors and quartz-crystal-microbalance immunosensors generally report the lowest LODs but require more sample preparation and instrumentation than lateral-flow formats [7,20,21]. AFM1-specific optical and electrochemical platforms extend these principles to dairy monitoring downstream of AFB1-contaminated feed [22,23,24,25]. Broader reviews of nanomaterial-enabled and electrochemical biosensors for food-safety analytes note that gold, carbon, and quantum-dot nanomaterials are now used almost interchangeably across recognition chemistries to amplify signal, meaning transduction choice and recognition chemistry increasingly vary independently rather than being tied to a single platform generation [13,21].

Table 1: Comparison of principal recognition-element chemistries used in aflatoxin biosensors.

Attribute	Antibodies	Aptamers	Molecularly imprinted polymers
Production	Animal immunisation or hybridoma	In vitro SELEX synthesis	Chemical polymerisation/templating
Stability	Cold-chain generally required	Reversible denaturation, stable dry	High thermal/chemical stability
Specificity	High, established	High, tunable	Moderate, broader cross-reactivity
Typical transduction	Lateral flow, colorimetric	Electrochemical, photoelectrochemical	Fluorescent quantum-dot, electrochemical

Figure 2: Reported limits of detection (LOD) for aflatoxin B1 across representative biosensor platforms, plotted on a logarithmic scale. Values are drawn from individually verified published studies [8,9,3,4,5].



Field Deployability and Point-of-Use Considerations

Analytical sensitivity alone does not determine field utility. Lateral-flow strips and smartphone-coupled colorimetric readers dominate point-of-use deployment because they require no electrical power beyond a phone camera, tolerate ambient temperature, and return results within 5-15 minutes, matching the review's smartphone-based lateral-flow and paper-microfluidic examples [4,26]. Portable SPR and handheld fluorescence instruments narrow the gap between laboratory and field performance while still requiring dedicated, though miniaturised, hardware and periodic calibration [8,18]. Electrochemical aptasensors, despite superior raw sensitivity, generally remain confined to research settings pending demonstrated stability of immobilised aptamers under ambient storage, resistance to matrix interference from grain dust and lipids, and cost-effective mass production of disposable electrodes [5,13]. Across platforms, recurring translational barriers include extraction-free compatibility with whole-grain or flour matrices, multiplexing for co-occurring mycotoxins, and validation against regulatory reference methods across diverse grain types and geographies [12,21]. Cost per test is also decisive for adoption in smallholder and low-resource storage settings: strip-based immunoassays are typically the cheapest to manufacture at scale, whereas aptasensor and SERS platforms currently depend on specialised reagents or instrumentation that limit unit-cost reduction, even though their raw sensitivity is higher [19,15].

Conclusion

Published biosensor platforms for aflatoxin detection now span a wide sensitivity range, from low-ppb portable SPR and colorimetric immunoassays suitable for immediate field screening to sub-ng/L aptamer-based electrochemical sensors suited to confirmatory or research use. Antibody-based lateral-flow formats currently offer the best balance of sensitivity, cost, and deployability for stored-grain surveillance, while aptamer- and nanomaterial-based platforms promise further sensitivity and multiplexing gains once matrix robustness and manufacturing reproducibility are resolved. A recurring gap across the reviewed literature is the scarcity of head-to-head comparisons of competing platforms on the same, naturally contaminated grain lots under field conditions rather than spiked laboratory extracts; most reported limits of detection derive from single-laboratory validation, which likely understates the variability encountered across harvests, storage conditions, and geographic regions. Future work combining aptamer or antibody recognition with low-cost, disposable transduction hardware, alongside inter-laboratory ring trials against chromatographic reference methods, would strengthen confidence that reported laboratory sensitivities translate into reliable accept/reject decisions at grain receival points. Continued matrix-matched validation, in grain rather than buffer, will be essential before any of these platforms can substitute for chromatographic confirmation in routine grain-safety monitoring.

Table 2: Representative biosensor platforms reported for aflatoxin detection in grain and food/feed matrices.

Platform	Recognition element	Matrix validated	Reported LOD
Portable surface plasmon resonance	Antibody (competitive immunoassay)	Rice, peanut, almond	2.51 µg/kg [8]
Magnetic bead colorimetric (AuNP)	Antibody	Maize (spiked)	12 ng/L [9]

Platform	Recognition element	Matrix validated	Reported LOD
Lateral flow immunochromatographic strip	Monoclonal antibody	Food and feed	0.23-1.0 µg/L [3]
Smartphone-read one-dot lateral flow	Antibody	Grain extract	5 µg/kg [4]
Electrochemical aptasensor (screen-printed electrode)	DNA aptamer	Buffer/spiked extract	~0.9 ng/L [5]
Handheld fluorescence spectrometer	Native fluorescence (antibody-free)	Maize kernels	Discriminated 0-1647.8 µg/kg contamination classes [18]

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Balazova P conceived the review, conducted the literature search, and drafted the manuscript. Zupancic R contributed to the literature screening and technical framing and critically revised the manuscript for important intellectual content. Saar R contributed additional context and approved the final version for submission.

Ethics Statement

Not applicable. This is a narrative review of previously published literature and did not involve new experimental data, human participants, or animal subjects.

Data Availability

No new data were generated or analyzed for this review. All sources synthesized are cited in the reference list and are available through their original publishers.

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