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Review

Overview of Various Components of Lateral-Flow Immunochromatography Assay for the Monitoring of Aflatoxin and Limit of Detection in Food Products: A Systematic Review

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Abstract: The detection of aflatoxins is essential for the food industry to ensure the safety and quality of food products before their release to the market. The lateral-flow immunochromatography assay (LFIA) is a simple technique that allows the rapid on-site detection of aflatoxins. The purpose of this review is to evaluate and compare the limits of detection reported in the most recent research articles, published between the years of 2015 and 2023. The limits of detection (LODs) were compared against the particle type and particle size, as well as other variables, to identify trends and correlations among the parameters. A growing interest in the use of different metal and non-metal nanoparticles was observed over the years of 2015–2023. The diameters of the nanoparticles used were reportedly between 1 nm and 100 nm. Most of these particles displayed lower LODs in the range of 0.01 to 1.0 ng/mL. Furthermore, there was a significant level of interest in detecting aflatoxin B1, perhaps due to its high level of toxicity and common appearance in food products. This study also compares the use of metallic and non-metallic nanoparticles in detecting aflatoxins and the dependence of nanoparticles' sizes on the detection range. Overall, the type of particle and particle size used in the development of LFIA strips can affect the sensitivity and LOD; hence, the optimization of these parameters and their modulation with respect to certain requirements can enhance the overall assay performance in terms of the reproducibility of results and commercialization.

Keywords: detection of fungal toxins; point-of-care detection; mycotoxins; lateral-flow assay; biosensing; lateral-flow diagnostics

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1. Introduction

Mycotoxins are a significant concern in ensuring the safety of the global food supply. They are hazardous secondary metabolites produced by certain fungal species, such as *Aspergillus*, *Penicillium*, and *Fusarium* spp. [1]. Mycotoxins are present in commonly consumed cereals, like rice, corn, and wheat. The major types of mycotoxins that pose a risk to human and animal health are aflatoxins (AFs), trichothecenes, ochratoxins (OTs), fumonisins (FMs), and zearalenone (ZEN). The consumption of food contaminated by these toxins can cause liver, kidney, and immune system toxicity, as well as cancer, reproductive system disorders, and Keshan disease. In particular, AFs and FMs can also affect childhood growth and neural tube development. Environmental factors like temperature, humidity, and pests can promote the growth of mycotoxin-producing fungi and alter the type and quantity of mycotoxins produced [2].

According to the Food and Drug Administration (FDA) in the US, mycotoxin contamination in food and feed leads to direct economic losses of as much as USD 932 million each year. Mycotoxins have infected almost 60–80% of crops worldwide [2], which exceeds

the limit set out by the Food and Agriculture Organization (FAO) of the United Nations. Mycotoxin-induced food-safety issues have been particularly severe in developing countries, where they have resulted in widespread fatalities. These harmful compounds are capable of causing birth defects, cancer, and genetic mutations. Cases of mycotoxin contamination causing mass death, have been documented in countries such as England in 1960, Kenya in 2004, and South India in 1995, leading to the deaths of hundreds of people [3]. More than three hundred and thirty-nine species and four subgenera belonging to the genus Aspergillus are responsible for the production of mycotoxins [3]. Aflatoxins are a type of mycotoxin produced by Aspergillus sp. [4]. Aspergillus was reportedly responsible for the production of four major types of aflatoxin: (1) aflatoxin B1, (2) aflatoxin B2, (3) aflatoxin G1, and (4) aflatoxin G2. The derivatives of aflatoxin B1 and aflatoxin B2 are aflatoxin M1 and aflatoxin M2, respectively, which are found in animal milk and urine [5].

Aflatoxins are categorized as furanocoumarin metabolites that can lead to serious and detrimental health effects in both animals and humans by causing aflatoxicosis, immunotoxicity, hepatotoxicity, and teratogenicity [5,6]. Aflatoxin B1 is predominantly associated with aflatoxicosis, as well as acute and chronic toxicity [6]. It can enter the human body via cutaneous, respiratory, or mucous routes of exposure and, as a result, lead to an overactive inflammatory immune response [5]. It causes liver disease in animals and is a powerful carcinogen in humans, as well as having detrimental effects on the renal, nervous, and gastrointestinal systems [4]. Other species that are producers of aflatoxins include *A. nomius*, *A. pseudotamarii*, *A. parvisclerotigenus*, and *A. bombycis* from section Flavi, *A. ochraceoroseus* and *A. rambellii* from section Ochraceorosei, and *Emericella astellata* and *E. venezuelensis* from Nidulatans. Various forms of aflatoxin have been documented, and the fact that they are toxic and can contaminate crops and food products that are crucial to the economy is a significant global concern [1].

The synthesis of aflatoxins in grain and other food and feed items is significantly influenced by various factors, such as commodity and weather conditions, and post-harvest storage conditions. Individuals in sub-Saharan Africa (SSA), are particularly vulnerable to long-term exposure to mycotoxins through their diet since a significant proportion of crops in tropical and subtropical regions are highly prone to mycotoxin pollution [4].

People can be exposed to aflatoxins either by consuming foods that are contaminated with aflatoxins, or by consuming foods from animals that are fed with aflatoxin-contaminated feed, which can lead to the accumulation of aflatoxins in animal products. This can result in adverse health effects [5]. Due to the serious threat of aflatoxins to humans, it is vital to take measures to prevent aflatoxin contamination and ensure that food products are safe for consumption before they reach consumers. However, a significant challenge in achieving this goal is that many food-safety incidents occur suddenly, spread rapidly, and develop on a large scale. This means that the current system must be equipped with rapid and effective on-site detection capabilities to prevent food hazards. However, there are practical challenges that need to be addressed, such as the costs of materials, equipment, and personnel. Another critical challenge to consider is the need to detect low levels of contaminants, sometimes in trace or ultra-trace amounts, in the food matrix, which requires highly sensitive detection methods [6].

To address the issue of aflatoxin contamination in food and feed, regulatory bodies have established certain limits. In 1987, the International Agency for Research on Cancer (IARC) declared aflatoxin B1 and aflatoxin M1 as human Group 1 and Group 2B carcinogens [7,8]. The European Commission has set maximum limits for aflatoxins in food and feedstuff ((EC) No. 165/2010), as well as in vegetable oil, groundnuts (peanuts) and other oilseeds, tree nuts, apricot kernels, and licorice ((EC) No. 178/2010). In the United States, action levels have been established to monitor mycotoxin contamination. However, regulatory limits in SSA (Sub-Saharan Africa) and other developing countries are partially absent or poorly enforced, making the surveillance of mycotoxin contamination a significant challenge, particularly for food intended for local consumption [4].

1.1. Detection of Aflatoxin

Aflatoxins are a major concern to the food industry worldwide as they widely spread in nature and severely contaminate various economically important food supplies and crops, like nuts, wheat, and sweetcorn. Hence, their prevalence makes them one of the most common types of mycotoxin and a global health hazard [9]. Consequently, there is a significant demand for research on aflatoxins to develop effective techniques for their accurate detection, thereby safeguarding the well-being of consumers. Methods such as high-performance liquid chromatography (HPLC), LC–mass spectrometry (MS), and LC–tandem-mass spectrometry (LC-MS/MS) are capable of detecting aflatoxins with high accuracy and precision [5]. They are frequently used for the quantitative and qualitative analysis of food samples. However, these analytical approaches require expensive equipment, well trained personnel, and extensive sample clean-up. Moreover, they are not suitable for rapid on-site detection [7]. The enzyme-linked immunosorbent assay (ELISA) is another rapid technique for detecting aflatoxins. However, it is not considered ideal for on-site detection as it relies on laboratory-based processes that involve several incubations and washing steps. As a consequence of inadequate operation or insufficient sample pre-treatment, false-positive or false-negative results may occur [8]. In addition, these techniques require well-trained personnel and specialized and expensive equipment, and they are time-consuming [10]. The currently expanding food industry demands a technique that enables the rapid on-site detection of aflatoxins in food prior to their distribution to the global market. The swift assessment of the presence of aflatoxins in food shipments can ensure that contaminants are identified and withheld from release, thereby enhancing the quality-control process, mitigating potential health risks, and maintaining consumer trust by delivering safe and aflatoxin-free products to the global market. This has led to the development of cost-effective and rapid detection methods, such as the lateral-flow immunochromatographic assay (LFIA) [11].

1.2. Principle of LFIA

The first LFIAs were developed at the end of the 1960s to monitor serum proteins. In order to find human chronic gonadotropin (hCG) in urine, the first homemade LFIA was conducted in 1976. The principle of this assay was based on the interaction between the antibody and antigen. Since then, it has been widely employed to identify a variety of molecules, including pesticides, microbes, mycotoxins, heavy metals, and cancer indicators [9]. Furthermore, this method is advantageous due to its ability to rapidly yield the desired results, as well as being both inexpensive and reliable.

The LFIA is a simple and user-friendly diagnostic technique that incorporates specific antibodies as capture agents against aflatoxins on a test strip. These captured agents, also known as biorecognition molecules, are positioned on a nitrocellulose membrane to generate test and control lines. The biorecognition components are tagged with signal indicators, like colloidal gold nanoparticles or quantum dots. The assay operates through a capillary action, in which the sample migrates through the membrane and interacts with the strong dipole of the peptide bonds in immobilized antibodies. If aflatoxins are present in the sample, they bind to the labeled capture agents, resulting in an antibody–antigen complex, producing a visible signal to indicate a positive result for contamination present in the sample [12–14].

1.3. Components of LFIA

The nitrocellulose membrane (NC) is the primary component of the LFIA. During the analysis, it offers a platform for the conjugate-pad reactions and the test-line reactions [9]. The powerful dipole of the nitrate group present in the NC membrane interacts with the strong dipole of the peptide bonds in the antibodies. This interaction leads to the development of electrostatic affinity. This electrostatic reaction is the primary factor influencing the adsorption behavior between the protein and the membrane. Furthermore, the surface

properties of the polymer and its capacity to adsorb proteins can also have an impact on the attachment of antibodies to the membrane [10].

The sample pad is typically prepared by using a glass-fiber membrane that has no affinity for proteins, and the cellulose-acetate membrane, which has a low or no affinity for proteins, can also be used. These two materials are typically used to create sample pads. The extra sample is delivered to the absorbent pad via the sample pad. Biorecognition components are labeled in conjugation pads using colloidal carbon, colloidal gold, carbon nanotubes, or quantum dots. The tagged biorecognition components attach to their targets and migrate along a chromatography strip at a controlled rate while the pad absorbs a significant volume of sample. The absorbent pad keeps the liquid flowing across the membrane and prevents sample backflow. These parts are all fastened within a backing card, which provides support to the LFIA strip's components. Different analytes' qualitative and quantitative characteristics can be assessed visually or with the use of portable instruments [9].

The flow rate of a sample over the membrane on an immunochromatographic test dictates the intensity of the signal on the test lines. The "catalog number," according to the manufacturer of NC membranes, indicates the time it takes for a sample front to travel across a membrane at a pace of one second every 4 cm. Due to the different pore sizes of NC membranes, the test lines on various NC membranes have varying widths. Low flow rates and prolonged analysis times are caused by small pore sizes. Different NC membranes with various widths have been tested in attempts to identify one that is ideal for creating LFIAs. The flow rate and signal intensity on the membrane are influenced by the various widths [9].

Furthermore, LFIA strips utilize antibodies as biorecognition molecules in the test and control zones in order to detect the presence of a specific antigen in a given sample. In aflatoxin detection, the antigen is the molecule that is linked to aflatoxins and can bind to specific antibodies. Antibodies are key components of LFIA strips, as they allow the detection of the target antigen. Hence, they are also referred to as detection agents. Antibodies are proteins produced by the immune system in response to the presence of an antigen or a foreign substance in the body. They are highly specific to their target antigen and can identify and attach to that antigen with accuracy and precision. In LFIA strips, antibodies are immobilized on nitrocellulose membranes. When a sample is applied to the sample well, the sample flows across the membrane with the aid of the sample pad and encounters the immobilized antibodies. The presence of the target antigen in the sample prompts the binding of the target antigen to the immobilized antibodies, resulting in an antibody–antigen complex [11].

On the conjugate pad, antibodies are conjugated to a fluorescent or colored label, as in gold nanoparticles. These labeled antibodies are designed to attach to a different epitope on the antigen from the immobilized antibodies. This enables them to attach to the antigen–antibody complex and generate a visible signal indicating the presence of the antigen or analyte of interest. As the sample continues to migrate across the nitrocellulose membrane through capillary action, it encounters the detection zone, which is where the immobilized antibodies are situated. If the matching antigen is present in the sample, the binding of the labeled antibodies with the antigen–antibody complex occurs, forming a visible line, which indicates a positive result. Furthermore, in the detection zone, many strips also contain a control line that holds immobilized [11] antibodies that can attach to the labeled antibodies. The control line acts as a positive control to show that the test is operating adequately. It also indicates that the labeled antibodies are capable of binding to the immobilized antibodies.

Antibodies form immunocomplexes with their specific antigens or target analytes via immunochemical interactions. A primary antibody is one that precisely binds to its specific antigen, while a secondary antibody is one that attaches to an antigen-containing antibody or another antibody. Primary and secondary antibodies are usually coupled in lateral-flow tests. Primary and secondary antibodies are contained in the test line and

control line, respectively. There are two forms of LFIAs based on antibodies: sandwich and competitive formats [9,11].

1.4. Role of Signal Indicators and Antibody Conjugation in Nanoparticle-Based LFIA

Signal indicators are critical components of LFIA strips. They have to be specific, sensitive, and easily detectable. Nanomaterials that have color and absorb light are commonly employed in detection agents. They can easily be observed with the naked eye and are often used as signal labels for the colorimetric detection of mycotoxins in LFIA. The most frequently used colored nanomaterials are colloidal gold nanoparticles (AuNPs) and colloidal silver nanoparticles (AgNPs). They are popular due to their bright colors and exceptional chemical stability. Various shapes and types of nanoparticles, including spherical AuNPs, irregularly shaped gold nanoflowers (AuNFs), and gold nanorods have been synthesized and used for the detection of mycotoxins. In order to generate a visible signal output in the test (T)-line area of the strip, sufficient accumulation of these nanoparticles is required, in which the number of accumulated nanoparticles reaches a colorimetric threshold that can be easily recognized by the naked eye.

However, these nanoparticle sensors are relatively small, exhibit a low molar absorption coefficient, and have insufficient colorimetric brightness. Consequently, some tests are not sufficiently sensitive for the on-site detection of analytes. To improve the sensitivity of AuNPs/AgNP-based LFIA nanosensors, researchers suggest increasing the accumulation of these nanoparticles around an analyte in the T-line region of the strip to enlarge the collective molar extinction of AuNPs/AgNPs. To achieve this, several strategies are proposed, including dual AuNP/AgNP conjugation, AuNPs/AgNP-based composite nanomaterials, and AuNP/AgNP aggregates [12].

The most widely used method for AuNP synthesis is the citrate-reduction method, which is also referred to as the Turkevich–Frens method. This conventional method involves boiling an aqueous solution containing sodium citrate and an Au (III) precursor. This process reduces the precursor, and the resulting AuNPs are dispersed in the solution in a stable form, referred to as colloidal gold or gold solution. The citrate ions serve as both protective and reducing agents. The advantages of synthesizing gold nanoparticles using the Turkevich–Frens method include its simplicity, its reproducibility, its applicability to a variety of precursors, and the production of stable AuNPs with controllable sizes. However, AuNPs greater than 30 nm in diameter tend to lose their spherical shape. Furthermore, under suboptimal pH or reagent conditions, the NPs become unstable [13,14]. The HEPES is an example of another reducing agent that can be employed for the synthesis of gold nanoparticles [13,14].

For silver nanoparticle synthesis, one of the most popular methods involves the reduction of silver nitrate using ice-cold sodium borohydride. In order to reduce the ionic silver and produce stable nanoparticles, an excess amount of sodium borohydride is required. The following chemical reaction explains the synthesis of AgNPs by using sodium borohydride:

$$AgNO_3 + NaBH_4 \rightarrow Ag + H_2 + B_2H_6 + NaNO_3$$

Signal labels are generally conjugated with detection agents, like antibodies, for the specific binding to the target analyte. This allows a visible signal to be generated by the recognition elements to indicate the presence of the toxin. The challenges in immobilizing antibodies onto gold nanoparticles are avoiding aggregation and ensuring that the antibodies are orientated correctly to maintain their functionality and the accessibility of their paratopes [15]. In order for signal labels to precisely and accurately detect aflatoxins, they must fulfill a range of criteria, which include high stability, the exhibition of little or no non-specific binding, cost-effectiveness, and the formation of reproducible and efficient conjugates without compromising the functionality and activity of the detection molecule [16].

To effectively use antibody–NP (Ab-NP) bioconjugates for biosensing, it is vital to develop robust and reliable techniques to ensure that the produced biosensor is reproducible, selective, and sensitive. An efficient bioconjugation approach must preserve the colloidal stability of the nanoparticles (NPs) while maintaining the capacity of Ab-NP bioconjugates to identify their target antigen [17]. Nanoparticles can be conjugated by physical adsorption. This is typically the preferred method for LFIA applications, which involves immobilizing detection molecules onto noble metal surfaces through hydrophobic and electrostatic interactions, hydrogen bonds, and Van der Waals forces [18]. The optimization of this process can be achieved by testing different pH values near the isoelectric point of the binding molecule [19].

Changes in the environments of gold nanoparticles often result in the formation of aggregates. The term aggregate is used to refer to individual nanoparticles that interact with each other to form a larger super-structure without altering the shapes or sizes of individual nanoparticles. As maintaining the stability of conjugates in LFIA strips is crucial, it is essential to gain a deeper understanding of nanoparticle conjugates for the effective optimization of their performances in LFIAs. This can be achieved by characterizing standard nanoparticles and conjugates using analytical techniques and measuring different parameters, like size, shape, zeta potential, absorbance, and optical density, to monitor their stability.

The sizes of nanoparticles play a crucial role in the sensitivity of LFIA. If aggregation occurs, the color of gold nanospheres in suspension changes from wine-red to darker shades, affecting the intensity of the lines on the strip [13]. According to the study conducted by Sahoo and Singh (2014), the sizes of nanoparticles can be controlled by adjusting parameters like the concentration of sodium citrate, pH, and temperature. Nanospheres with diameters in the range of 20–40 nm are commonly used in optimizing parameters for LFIA sensitivity, as larger nanoparticles can provide enhanced color observation. However, they are less stable. As aggregation is a challenge during conjugation, the monitoring of the sizes of standard nanoparticles, as well as of conjugates, can aid in determining their aggregation state [20].

A critical aspect of LFIAs is the presence of signal indicators. The adequate accumulation of these signaling molecules on the surfaces of antibodies is required to generate a visible signal output on the test line, where the number of accumulated nanoparticles approaches a colorimetric threshold that is visible to the naked eye [15]. Factors such as the type of nanoparticle, its size, and its morphology contribute to the binding efficiency of antibodies to signaling molecules. Moreover, these parameters play a vital role in influencing the overall performance of the assay, including its sensitivity, the reproducibility of the results, and the limit of detection (LOD). The LOD is defined as the minimum concentration of the target analyte that can be reliably detected and distinguished from false signals. It is a critical parameter that directly reflects the sensitivity of the assay. A low LOD is an indication of high sensitivity, enabling the detection of trace quantities of aflatoxins in samples [16]. This is particularly vital when considering the possible health hazards linked to aflatoxin exposure, because these toxins can be extremely harmful, even at low concentrations. The protection of consumer health is a major priority for the food industry. Employing a sensitive assay with low LOD can add another line of defense against aflatoxin contamination and ensure that contaminated food batches are not accidentally released to the market [21].

This review compiles and compares the LODs reported in 49 research articles in which LFIA strips were developed and that were published between the years of 2015 and 2023. By examining the relationship between the LODs and key factors such as the type of nanoparticle, its size, its shape, and other properties, this review aims to uncover any patterns or correlations that may exist. This analysis may potentially provide valuable insights into the efficacy of various LFIA strip designs and their suitability for detecting aflatoxins. Aflatoxins have stringent regulatory limits and ensuring their accurate detection in food and feed samples is of utmost importance. Therefore, this review can shed light

on the most effective approaches for developing LFIA strips that offer high sensitivity and provide guidance for the food industry on the design and optimization of more efficient LFIA strips. Ultimately, this comprehensive review could potentially drive advancements in the development of accurate and reliable LFIA strips for aflatoxin detection in the food and feed industries.

2. Research Method

The information was gathered by using Science Direct. An advanced search was conducted using keywords such as lateral flow assay, lateral flow immunoassay, lateral flow immunochromatographic strips, rapid antigen biosensing strips, immunoassay-based lateral flow, lateral flow, lateral-flow immuno-dipstick, and aflatoxin or aflatoxins detection, in order to find articles that closely aligned with the research objective. Figure 1 shows a comparison of 49 research publications to ascertain the lowest LOD for total aflatoxins, aflatoxin M1 (AFM1), and aflatoxin B1 (AFB1). Readers should note that some research studies created more than one LFIA strip and employed multiple particle types.

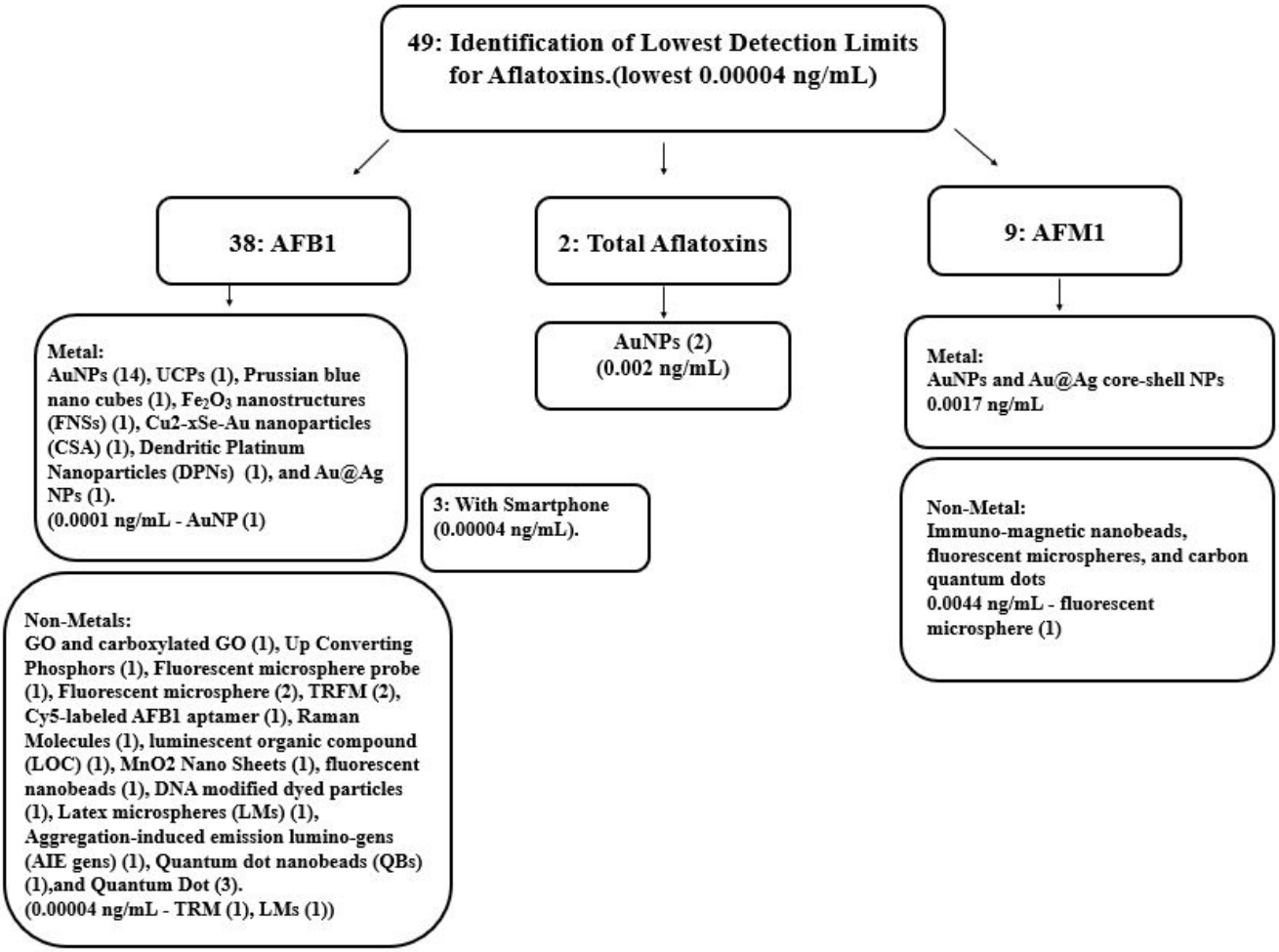

Figure 1. Hierarchy of different research publications dealing with the types of aflatoxin and the types of material associated with their detection. Note: The number in the bracket e.g., (1), show the number of articles from the specific domain considered for this review.

3. Results and Discussion

The presence of the very poisonous carcinogenic Aflatoxin B1 in tainted feed and food items has potential adverse effects on human health. According to research, the carcinogen Aflatoxin B1 is well known for generating hepatocellular carcinoma (HCC), the most frequent primary liver cancer in both animals and humans. Additionally, the International

Agency for Research on Cancer (IARC) reviewed a number of epidemiological studies that revealed a strong association between the consumption of AFB1 and the likelihood of developing cancer [17].

Table 1 presents a breakdown of the information collected from the 49 research publications. It provides a summary of the types of aflatoxin investigated in the published articles, as well as the particle types utilized for developing LFIA strips. Additionally, it provides information on the particle sizes and shapes, the coefficient of variation, the reported limit of detection (LOD), and the quantification technique employed for the further characterization or detection of aflatoxins in the tested samples. Each research paper was thoroughly evaluated and analyzed utilizing data-extraction methods, such as keyword searches and data graphing, in order to discover and extract the data. The data are categorized according to the different aflatoxins, specifically into AFM1, AFB1, and total other aflatoxins, in order to provide a brief overview of the overall techniques and their limits of detection.

Table 1. Summary of particle sizes and shapes, coefficients of variation, and limits of detection in aflatoxin detection in 49 papers.

Type of Aflatoxin	Particle	Size nm	Shape	Year	Detection Method	LOD ng/mL	COV %	Sensitive Toxins	Reference
AFM1	Magnetic	180	-	2015	ELISA	0.02	-	-	[21]
	GNPs	40	-	2016	Lateral-flow assay	0.1	-	-	[22]
	Fluorescent microsphere	-	-	2016	FM-ICTS assay	0.0044	4–14.7	AFM2,AFB1, AFB2, AFG1 and AFG2	[23]
	GNP	24	-	2018	Lateral-flow assay	0.05	3.9–8.5	AFB1	[24]
	GNP	35	-	2019	Lateral-flow assay	0.016	-	-	[25]
	GNP	-	-	2015	ELISA, HPLC	0.50	-	-	[26]
	Fluorescent microspheres (TRFMs)	329	Sphere	2022	Dual ICTS/ UPLC-MS	0.018	2.84–7.48	OTA	[27]
	Au@Ag core-shell NPs	38–98	-	2020	SERS immune-assay	0.0017	11.4–16.7	-	[28]
	Carbon quantum dots	8	Quasi-Sphere	2022	Lateral-flow assay	0.07	-	-	[29]
AFB1	Gold nanoparticle	-	-	2016	ELISA, Lateral-flow assay	1.0	-	-	[30]
	GNP	32	-	2016	Multiplex lateral-flow assay	0.0001–0.00013	<16.7	ZEN, OTA	[31]
	GNP	-	-	2016	Multistage ICTS	0.6	<8	ZEN	[32]
	GNP	17.4	-	2018	LC-MS/MS	0.1	-	-	[33]
	Phosphors (UCPs)	50	-	2016	Lateral-flow assay	0.0001–0.005	1.0–9.4	-	[34]
	Graphene	-	-	2017	Lateral-flow assay	0.3	-	-	[35]
	GNP	75 ± 5	Flower	2015	ICTS	0.00032 ng/mL	4.8	AFG1, AFG2, AFM1, AFB2, ZEN, OTA, DON	[36]
	Fluorescent microsphere	-	-	2015	ICTS/LC-MS	0.0025	-	-	[37]
	GNP	20–60	-	2018	Lateral-flow assay	0.1	-	DON, FB1	[38]
	Multicolour GNP + phone	30/75	Sphere Rose	2019	Lateral-flow assay	1	-	FB	[39]
	Cy5-aptamer	-	-	2018	Dual lateral-flow assay	0.1	>5	AFM1, AFM2 AFG1	[40]
	GNPs	36/120	Sphere/ flower-	2020	ICTS	0.06	<13.0	AFB1, ZEN, OTA	[41]
	Fluorescent microspheresPhone	TRFMs- 200	-	2020	Lateral-flow assay	0.00004	8.7–15.8	ZEN, DON, T-2, FB1	[42]
	Ag GNPs	Au 52 Ag 91	-	2020	SERS–lateral-flow assay	0.00096	9.9–15.6	ZEN; FB1, DON, OTA, T-2 toxin	[43]
	Nanotags GNPs	61.34	Sphere	2023	Lateral-flow assay	0.00024	-	OTA	[44]
	GNPs	-	-	2017	Lateral-flow assay	0.002	-	-	[45]
	Prussian nlue nanocubes (PBNs)	950	Cubic	2021	Lateral-flow assay	0.023	-	AFB2, AFG2	[46]
	GNP + phone	-	-	2020	Multiplex ICTS	0.004	-	FB1, T-2, DON, ZEN	[47]
	Iron	79.5/3	Cubic	2021	ICTS	0.0125	-	FB2, AFG1	[48]
	Luminescent compound (LOC)	-	-	2021	Lateral-flow assay	1.3	-	DON, FB1, T-2 T-2, ZON	[49]
	MnO_2 nanosheets	100/300	Sheet	2022	Enzyme-based LFA	0.015	<9.7	-	[50]
	Fluorescent nanobeads	247	Uniform	2023	Lateral-flow assay	0.05	-	-	[51]

Table 1. *Cont.*

Type of Aflatoxin	Particle	Size nm	Shape	Year	Detection Method	LOD ng/mL	COV %	Sensitive Toxins	Reference
	Cu2-xSe- GNP	46.3 /7.7	Flower	2023	Optical camera, thermal imager	0.00842	5.62	AFB2, AFM1, AFG1, AFG2	[52]
	Dyed particles	-	-	2022	Lateral-flow assay	4.56	-	-	[53]
	Latex microspheres (LMs)	200	Sphere	2022	Lateral-flow assay	0.00004	3.0–5.2	T-2, ZEN	[54]
	Luminogens (AIEgens)	60	Sphere	2021	UPLC-MS/MS	0.003	4.6–6.7	-	[55]
	GNP	30	-	2023	Computational, RPI platform	0.1–0.5	-	-	[56]
	GNP	-	-	2020	Lateral-flow assay	0.05	-	-	[57]
	Dendritic platinum nanoparticles (DPNs)	30	Crystalline	2021	ICTS	0.03		-	[58]
	Quantum-dot nanobeads (QBs)	50–100	Quasi-sphere	2021	LC-MS/MS	1	-	-	[59]
	Magnetic quantum dot (QD)	220	-	2022	ICTS	0.00042	-	OTA, FB1	[60]
	Quantum -dot microsphere (QDM)	164	-	2022	ICTS	0.01	10.4	OTA, ZEN	[61]
	Fluorescent microsphere	-	-	2022	ICTS	0.021	<8	-	[62]
	Fluorescent microsphere	-	-	2022	Lateral-flow assay	0.035	<8	-	[63]
	Fluorescent microsphere	310.8	-	2021	ICTS	0.019	4.91–8.31	-	[64]
	GNP	30/ 15	Sphere Flower	2023	ICTS	0.1	-	-	[65]
	GNP Red-emitting quantum dots	74	Flower	2023	Lateral-flow assay	0.005	15.43	OTA, ZEN	[66]
	-	-	-	2017	Commercial lateral-flow device	<3	<10.7	-	[67]
Total aflatoxins	GNPs	-	-	2018	Lateral = flow assay	0.002	-	-	[68]
(B_1, B_2, G_1, G_2)	GNPs	-	-	2016	Lateral-flow assay	0.002–0.15	-	FA and FB	[69]

Fumonisin B1 (FB1), T-2 toxins (T-2), Deoxynivalenol (DON), Ochratoxin A (OTA), Zearalenone (ZEN), and Coefficient of variation (COV).

Figure 2 shows a comparison of 49 research publications to ascertain the lowest LOD for the total aflatoxins, aflatoxin M1 (AFM1), and aflatoxin B1 (AFB1). Readers should note that some research studies created more than one LFIA strip and employed multiple particle types. Aflatoxin B1 was the subject of 78% of articles, Aflatoxin M1 was the subject of 18%, and the remaining aflatoxins were the subject of 4% of publications. Aflatoxin B1 was the most heavily researched and studied in the past decade, as illustrated in Figure 1. The remaining 4% included other aflatoxins, like B1, B2, G1, and G2. Of the different aflatoxins, Aflatoxin B1 is considered to be the most heavily studied, appearing in 78% of the total research performed, due to its abundance in food materials.

Figure 3 compiles the various materials used in the detection of aflatoxins through lateral-flow-assay strips. Gold nanoparticles and colloidal gold-composed nanoparticles are of particular interest; they are the most popular because of their physicochemical properties, which allow them to be employed in the development of lateral-flow assays. Almost 42% of the reviewed research articles used AuNPs as the bio-probes in lateral biosensing devices, which was followed by the use of quantum dots (8%). The lowest detection limit was achieved by using gold nanoparticles. Among all the materials, gold nanoparticles and colloidal gold-composed materials were employed the most frequently in the detection of aflatoxins. They are preferred due to their ease of synthesis in the desired shape and size, their simple surface functionalization, and their antibody-immobilization capability.

It is noteworthy that the vast majority of the researchers employed AuNPs to create their lateral-flow test strips. These are flexible tools for creating biosensors since they are extremely stable and simple to functionalize with a variety of biomolecules [18]. It is also important to highlight the employment of quantum dots, which made up 8% of the employed particles in lateral-flow assays. Due to their exceptional optical characteristics, such as high quantum yield, photostability, and narrow emission spectra, quan-

tum dots are luminescent semiconductor nanoparticles that are favored for the synthesis of biosensors [19].

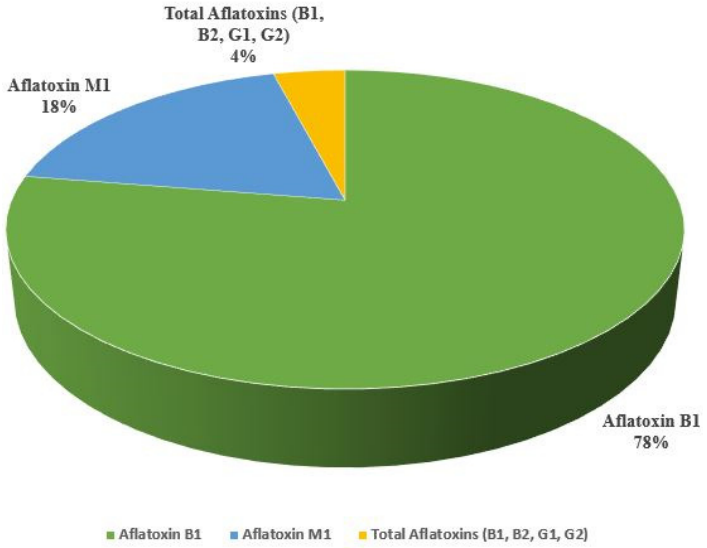

Figure 2. Percentage of research and studies on the types of aflatoxin.

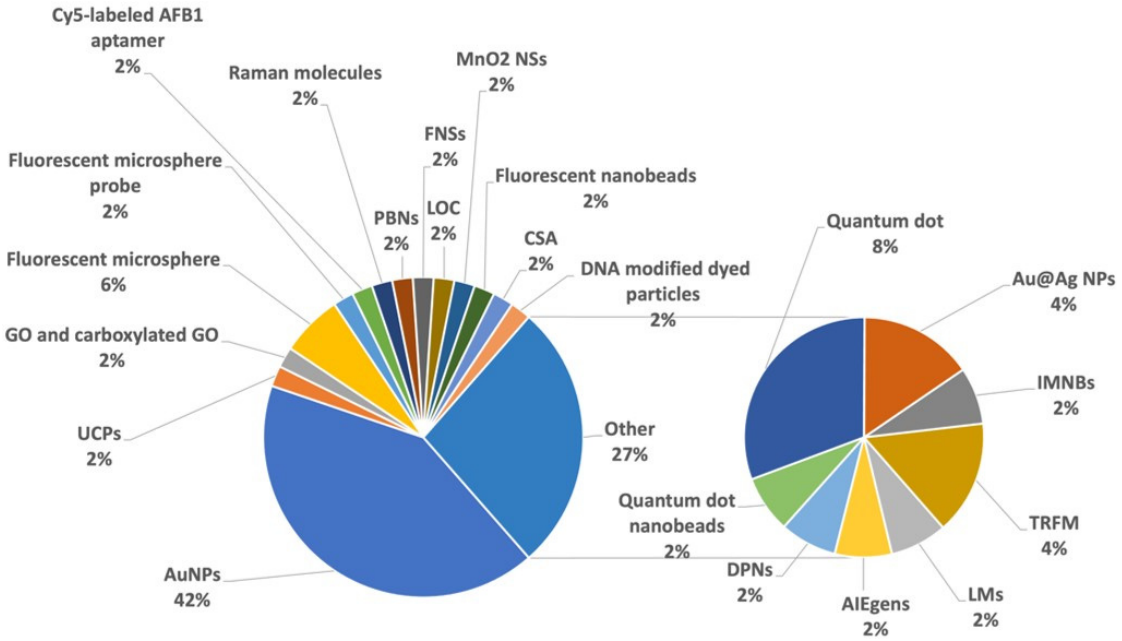

Figure 3. Percentages of various materials used in the detection of aflatoxins using lateral-flow im-immunochromatographic strips. AuNPs—gold nanoparticles, UCPs—upconverting phosphorous, PBNs—Prussian blue nano-cubes, LOC—luminescent organic compounds, FNSs—Fe_2O_3 nanostructures, MnO_2 NSs—MnO_2 nanosheets, CSA—Copper 2 se- Au nanoparticles, LM—latex microspheres, DPNs—dendritic platinum nanoparticles, IMNBs—immunomagnetic nano-beads, AIEgens—aggregation-induced emission immunogens, TRFM—time-resolved fluorescent microspheres.

Figure 4 shows the LOD for aflatoxin B1, aflatoxin M1, and the total aflatoxins, as well as the type of nanoparticle, based on metals or non-metals. It provides valuable insights into the detection sensitivities of the different methods with the different types of nanoparticles employed by the researchers. The graphs demonstrate no trends in the decrease in LOD over time. The majority of the LODs fall within the range of 0.1 to 3 ng/mL, suggesting a common level of detection capability among the methods examined in the articles. The majority of the LOD values in Figure 4 are between 0.1 and 1.3 ng/mL. With

LOD values of 3 and 4.56 ng/mL, respectively, in 2017 and 2022, there are two noticeable peaks in Figure 4a. Note that, as the particle type is unknown, the peak in 2017 is not present in Figure 4b.

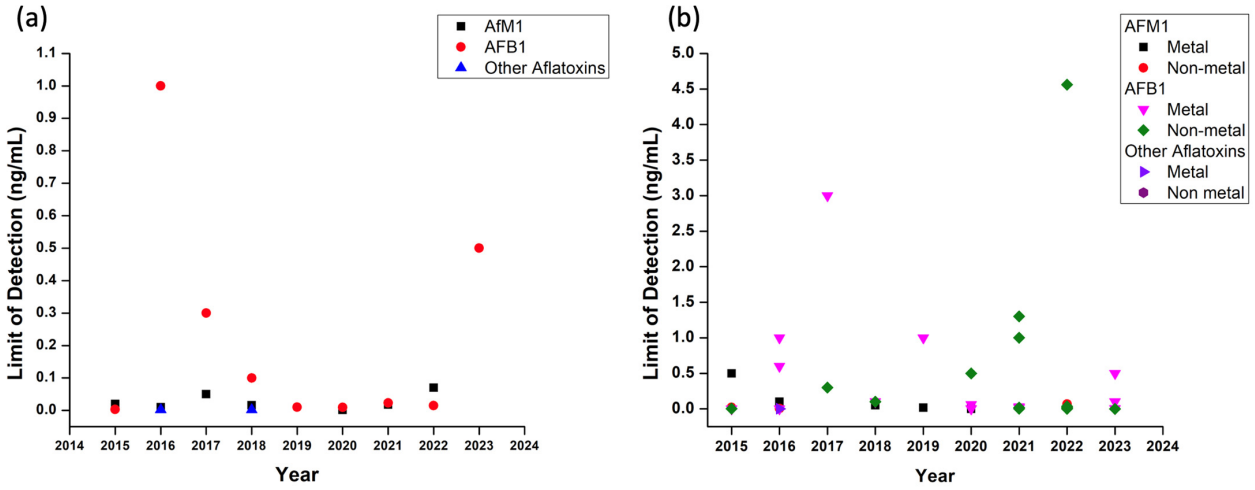

Figure 4. Timeline of the limits of detection of aflatoxins and types of material. (**a**) Different types of aflatoxin (AFM1—black rectangle, AFB1—red circle, and other aflatoxins—blue triangle); (**b**) types of material used in detecting aflatoxins through lateral-flow assays.

Figure 5 represents the dependence of the LOD on the nanoparticle size across 49 papers on aflatoxins. It can be seen that the data are predominantly clustered in the nanoparticle-size range of 1–100 nm, with the detection limits ranging from 0.01 to 1.0 ng/mL. Similarly, in Figure 5b, the size range is the same regardless of whether the particles were metallic or non-metallic in nature. All these LODs are far lower than the EU limits, which shows the potential to use these assays at the commercial level to screen for toxins.

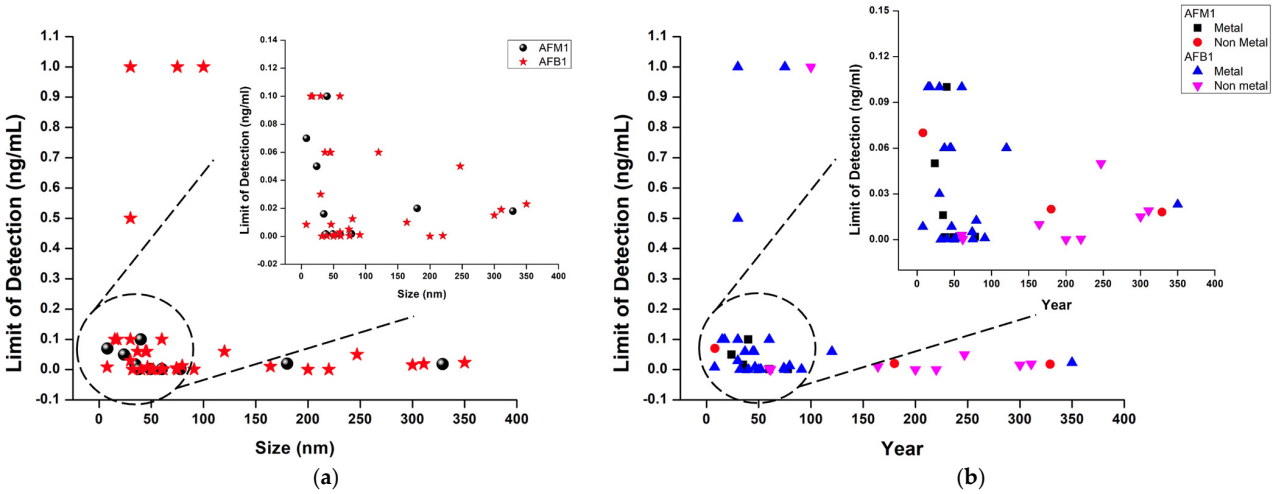

Figure 5. Dependence of limits of detection on the sizes of the nanoparticles. (**a**) LODs of different aflatoxins (AFM1—black circle, AFB1—red star); (**b**) types of nanoparticles, with sizes. Note: Inset of figures (**a**,**b**) shows the zoomed-in view of the graphs.

While non-metallic particles were mostly employed for AFB1 detection, metal-based nanoparticles were predominantly used for AFM1 detection. These findings imply that the sensitivity of the assay for the identification of various aflatoxins is not influenced by the selection of the type of nanoparticle. Nevertheless, there were no clear patterns or inferences in the LOD values over time from 2015 to 2023, and a significant por-

tion of the reported LODs was extremely low, indicating that the LFIA strips generated had high sensitivity.

The relationship between LOD and particle size for the examined aflatoxins throughout the research papers gathered is illustrated in Figure 5a. The LODs' reliance on different particle sizes is shown in Figure 5b, based on the same research articles. The majority of the data are in the 10–350 nm particle size range, with the LODs between 0.1 and 1 ng/mL. The recommended maximum limit for the concentrations of AFM1, AFB1, and total Aflatoxins in foodstuffs in the EC regulations are 0.05, 12, and 15 ng/mL, respectively [20]. It seems promising that the reported values for the LODs of the AFB1 and total aflatoxins are within these limits since they satisfy the requirements set out by the regulations. The regulatory threshold of 0.05 ng/mL is exceeded by the AFM1 LODs. These studies emphasize how crucial it is to tailor LFIAs to certain aflatoxins in order to adhere to regulatory standards and maintain the effectiveness of the biosensing strips in food-safety applications.

Sensitivity is an important element in aflatoxin detection since it has a direct impact on assay accuracy and reliability. Recent research has shown numerous techniques for increasing aflatoxin-detection sensitivity. For example, the aptamer-based lateral-flow assays investigated in reference [10] exhibited enhanced sensitivity, with the ability to detect aflatoxin B1 (AFB1) in milk samples at an incredibly low threshold of 0.01 ng/mL. This degree of sensitivity is advantageous in meeting the regulatory limits set by the FDA and resulted from the use of a monoclonal antibody (mAb) that recognizes a single epitope on AFM1, resulting in a high affinity for the target molecule [10].

Zhao et al. [34] presented the concept of upconverting-phosphor-technology-based lateral-flow assays (AFB1-UPT-LF), which outperform standard techniques by providing remarkable sensitivity, with a detection limit of 0.03 ng/mL for AFB1. The coefficients of variation for the assay were less than 10%, indicating high precision. Importantly, the assay exhibited strong specificity, with no cross-reactivity with various other mycotoxins, except for aflatoxin M1 (AFM1), which does not significantly affect the detection of AFB1 in crop samples [34]. Chen et al. [31] emphasize the critical need to optimize gold-nanoparticle size and test-line location to increase sensitivity and, hence, contribute to the efficacy of lateral-flow immunoassays (LFIAs). They found that larger GNPs (32 nm) led to lower cut-off values, resulting in better assay sensitivity. Additionally, the position of the test line on the lateral-flow immunoassay (LFIA) strip affected the sensitivity, with distal positions from the conjugation pad yielding the best results. This study's findings demonstrate that modifying these parameters can enhance assay sensitivity in the development of competitive LFIAs.

Furthermore, Zhang et al. [23] found that their developed FM-ICTS assay demonstrated higher sensitivity compared to the conventional colloidal gold LFIA. The cut-off value for the FM-ICTS was 100 ng/L, while that for the CG-ICTS was 400 ng/L, indicating that the FM-ICTS assay was four times more sensitive. It was found that a moderate hapten-to-protein-coupling ratio in the coating antigen resulted in enhanced sensitivity. These developments highlight the critical importance of sensitivity in aflatoxin detection, allowing the reliable identification of even the smallest toxin concentrations.

Another critical factor associated with the longevity and efficacy of aflatoxin-detection systems is stability. Various reagents have been used in research studies to address this issue. For example, the authors of the reference [35] investigated the durability of GO-labeled immunochromatographic strips over a 4-month period by preserving them in a sealed bag with desiccants at room temperature. The running buffer and detector reagents were stored at 4 °C. Following this interval, testing on negative and positive samples (0–1 ng/mL AFB1) revealed no significant differences in the color intensities of the test and control lines. This highlights the GO-labeled strip test's stability for at least four months, with consistent findings provided throughout the controlled storage conditions. This approach guarantees that the assay's reliability remains preserved even after storage, ensuring consistent results over time.

Another essential aspect is reproducibility, which has a significant influence on the credibility of aflatoxin tests. Recent research efforts have focused on investigating the repeatability of detection methods in order to ensure dependable and accurate findings. In studies conducted by Zhao et al. [34], for example, the AFB1-UPT-LF test demonstrated outstanding repeatability, with coefficients of variation (COV) of less than 10% over a range of AFB1 values. This high level of accuracy verifies the assay's dependability. Furthermore, Wang et al. [28] investigated the reproducibility of four batches of strips developed using anti-AFM1 monoclonal antibodies conjugated with Au (core)@Ag (shell) nanoparticles as SERS nanoprobes. The results indicated that the assay variations between the four measurements were lower than 15%, demonstrating acceptable reproducibility. The GO-labeled immunochromatographic test described by Yu et al. [35] similarly excels in terms of repeatability, yielding consistent findings for a variety of spiked levels, with a few deviations seen around the visual limit of detection. These latest research aims highlight the critical relevance of repeatability in ensuring that aflatoxin-detection technologies provide consistent and trustworthy results, even when subjected to varying conditions or frequent testing scenarios.

4. Conclusions

This review provides an overview of the lateral-flow-assay platform for the detection of mycotoxins, particularly aflatoxins. Among all the types of materials used in detection, nanoparticles show great potential and performance in terms of both sensitivity and selectivity. Both metallic and non-metallic nanoparticles provide qualitative results, along with a clear visual interpretation of T and C lines on the strip with the naked eye. Gold nanoparticles are the most common colored nanomaterials, with excellent chemical stability, vivid colors, and ease of functionalization, employed in lateral-flow assays. Compared to other techniques that demand high sample volumes, trained individuals, highly technical equipment, and time-consuming processes, lateral flow assays are much more efficient, feasible, and swift. Current research on lateral-flow assays is focused on increasing their sensitivity, multianalyte-detection capability, and reproducibility.

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