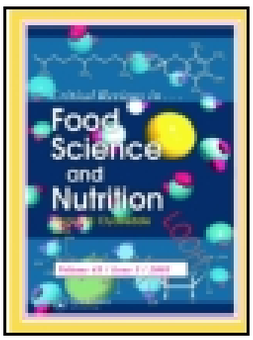

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Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

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To cite this article: Pranav Tripathi, Neha Upadhyay & Seema Nara (2017): Recent advancements in lateral flow immunoassays: A journey for toxin detection in food, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2016.1276048

To link to this article: http://dx.doi.org/10.1080/10408398.2016.1276048

Accepted author version posted online: 10 Jan 2017.
Published online: 10 Jan 2017.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2016.1276048

Recent advancements in lateral flow immunoassays: A journey for toxin detection in food

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ABSTRACT

Biotechnology embraces various physical and chemical phenomena toward advancement of health diagnostics. Toward such advancement, detection of toxins plays an important role. Toxins produce severe health impacts on consumption with high mortality associated in acute cases. The most prominent route of infection and intoxication is through food matrices. Therefore, rapid detection of toxins at low concentrations is the need of modern diagnostics. Lateral flow immunoassays are one of the emergent and popularly used rapid detection technology developed for detecting various kinds of analytes. This review thus focuses on recent advancements in lateral flow immunoassays for detecting different toxins in agricultural food. Appropriate emphasis was given on how the labels, recognition elements, or detection strategy has laid an impact on improvement in immunochromatographic assays for toxins. The paper also discusses the gradual change in sensitivities and specificities of assays in accordance with the method of food processing used. The review concludes with the major challenges faced by this technology and provides an outlook and insight of ideas to improve it in the future.

Abbreviations: CA

KEYWORDS

Diagnostics; toxins; immunoassay; LOD; commercial LFIA

Introduction

Food matrices on contaminated with toxins, whole cell microbial entities, and spores are responsible for the onset of food-borne diseases. Contamination of food and water with toxins results into intoxication, while microbes and spores give rise to infection upon ingestion (Shames, 2010). Toxins are lethal secondary metabolites produced by living organisms as a fraction of survival strategy as stated (Food and Drug Law Institute, 2008). These survival strategies are studied for metabolic adaptability leading to production of biologically active compounds such as enzymes, carcinogens, and antibiotics. On the basis of their origin, toxins can be of various types such as bacterial toxins, fungal toxins, or other miscellaneous toxins. Bacterial toxins are the toxins released from bacterial origin, generally exogenous and endogenous. These toxins cause damage either at the site of application or in a distance-directed form. Bacterial toxins act non-invasively and propagate in a limited manner. A typical bacterial toxin is generally a two-domain system, one of which is an effector, while the other is binding moiety. To detect bacterial toxin, any of the domains can be utilized but the binding domain is more preferred due to its constitutive nature and ease to synthesize in recombinant form (Barth et al. 2004). On the other hand fungal toxins are secondary metabolites released in agricultural commodities under favorable conditions. A wide range of similarities in fungal toxins makes them less susceptible to be detected in-vitro due to increase in cross reactivity (Pittet, 1995). Apart from bacterial and fungal toxins, there are phyto-toxins, dinoflagellate toxins, and organophosphorus toxins, which are grouped under miscellaneous toxins. These toxins prevail as either secondary metabolites or due to use of insecticides or weedicides (Graniti et al. 1992). The harmful effects of toxins in food matrices result in severe health complications and give rise to necessity for their detection according to permissible limits. The presence of live bacterial cells in the food while consumption is not always necessary for food poisoning to occur, rather ingestion of pre-formed bacterial toxins present in the food may also result in food poisoning due to intoxication and can prove fatal. It is highly crucial to detect toxin contagion in food products to prevent the occurrence of disease. It justifies the need for brisk, responsive, and explicit multi-analyte screening assays to detect simultaneously different toxins in a sample. The food industry is in the requirement of rapid and sensitive techniques which can detect preformed toxins at extremely low levels with enough specificity (Vunrcrzant and Pllustoesser, 1987).

Toxins can be reliably analyzed in any matrix, such as food or water sample, using physical techniques such as high performance liquid chromatography (HPLC), gas chromatography (GC), and immunoassays such as enzyme-linked immunosorbent assay (ELISA), fluorescence polarization immunoassay, and immunochromatographic assay (Krska et al. 2005, Ngundi et al. 2006, Ngom et al. 2010, Posthuma-Trumpie et al., 2009). With the advent of new technologies, detection of toxins is feasible with more accurate and sensitive assay platforms, which

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include polymerase chain reaction (PCR; Tighe et al. 2015, hybridization assays, and microarrays. However, limitations to these assays are their high cost, dependency on expensive equipments, and requirement of highly skilled technicians.

A simple, cost-effective, rapid, and less sophisticated solution is to use membrane-based immunoassays for screening. Use of membranes as solid supports have been promising setups, and permit execution of congruent assays in the same sample, thus offering advantages for multi-analyte tests (Gabaldon et al., 2003). The lateral-flow immunochromatographic assay (LFIA) is one of the superior membrane based assay formats with high efficiency and precision. Movement of analyte to binding moiety immobilized on the membrane surface is the basis of one-step lateral-flow method by incorporating labeled antibodies (Warsinke 2009). LFIA aids in the rapid point-of-care (POC) diagnosis, and efficiently meets the affordable, sensitive, specific, user-friendly, rapid/robust, equipment-free, and deliverable (ASSURED) criteria of end users, thereby attracting the interest of researchers (Anfossi et al. 2013; Li and Macdonald, 2016). Conventionally used formats of the lateral flow assay systems rely upon the employment of gold nanoparticles (GNPs) as reporting labels. However, low analytical sensitivity for a number of target analytes is one of its major deteriorating factors. Various approaches have been pragmatically utilized to enhance the sensitivity of these formats such as using of different nanoparticles, e.g. liposomes, core-shell Ag–Au nanoparticles, magnetic, and fluorescent nanoparticles, as reviewed by the reports (Shukla et al., 2011, Liao et al. 2010, Xu et al. 2009, Bamrungsap et al. 2014).

The place of LFIA in rapid testing systems and its suitability in analyzing the safety of food matrixes inspire us to evaluate the current scenario of LFIAs for testing toxins, particularly in contaminated food and water. A number of reviews have assessed the status of assays for detecting other food contaminants such as microbial pathogen/pesticides or chemical contaminants, but a comprehensive status of LFIA-based detection of toxins that spoil food and water is still needed. Therefore, this review is written to bring together all the relevant information available in this context and to evaluate advancements made in the LFIA system toward toxin detection. The search criterion is restricted to reviewing literature of the last decade in order to follow the latest progress in this field. The present contribution reviews the evolution of lateral flow biosensors over the last decade in the context of bacterial, fungal, and other miscellaneous toxin detection in agricultural food matrices. The decade long advances seen in lateral flow immunoassays for toxin detection with respect to recognition elements, labels, and detection strategies are discussed systematically. Need for multi-analyte analysis and the present scenario of multiplex lateral flow assays for toxin detection in food matrices are also presented in the review. Further, it discusses the challenges faced and the future outlook of this technique toward toxin detection.

Lateral flow immunoassay

A general lateral flow device comprises four components: sample pad, conjugate pad, nitrocellulose membrane, and absorbent pad, assembled to form a continuous flowing channel based on capillary forces (Paek et al. 2000). LFIA is generally constructed in either competitive or sandwich format. Competitive LFIA is commonly developed for low molecular weight analytes or those possessing single epitope. As shown in Figure 1, the test zone is coated with an analyte–protein conjugate, which captures a labeled anti-analyte monoclonal antibody complex, allowing the concentration of nanoparticles to increase in a specific area of test line. Another antibody is immobilized on the control line, allowing the capturing of antibody complex present in excess. Control zone always produces a visible color band irrespective of target, thereby confirming

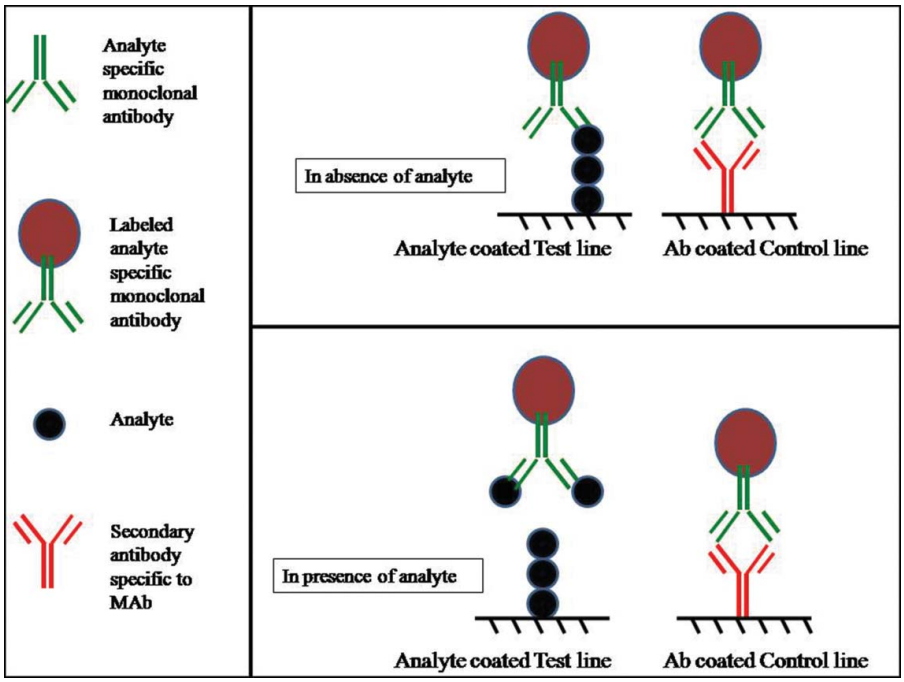

Figure 1. Competitive ICA depicting the number of bands formed in the presence and absence of analyte.

the validity of test strip (Wang et al. 2007). On the contrary, a sample devoid of target analyte will show colored band on both test and control lines. Sandwich type of format is different from competitive type of format (Qian and Bau, 2004).

As depicted in Figure 2, in sandwich type of format, target analytes comprise more than one antigenic determinants or epitopes. The test zone on the nitrocellulose membrane is coated with a non-labeled anti-analyte polyclonal antibody (Peng et al. 2008). The analyte after dropping on the sample pad flows across the reservoir matrix containing analyte-specific monoclonal antibodies conjugated to labels, i.e., either colloidal gold or other entities such as fluorescent molecules. The polyclonal antibodies capture the labeled monoclonal antibody–analyte, allowing the concentration of nanoparticles to increase in a specific area of test line. The control line is coated with anti-species antibody, which allows the capturing of antibody complex present in excess. Colored line in control zone in every run confirms the validity of test strip just similar to the competitive format. On the contrary, a sample devoid of target analyte will show colored band on control line only (Peng et al. 2007).

Nevertheless, LFIAs also have some flaws, such as the response obtained with bare eyes is simply qualitative, although with the help of certain reading devices it can be converted to semi-quantitative. Another drawback is the availability of sample in liquid state, with optimum viscosity to flow across along with obstruction caused by different interferants rendering unspecific adsorptions. Hence, to reduce the level of interference, sample pretreatment is a requisite. In order to obviate the limitations of LFIA and enhance its performance, various strategies have been adopted, which are discussed systematically ahead.

Recognition elements

The main principle of any immunoassay is the selective and specific interaction of target analytes by receptor molecule. Antibodies are glycoproteins in which the variable region consists of a hypervariable region, which corresponds to the antigen binding site or "paratope." Antibodies are employed as biorecognition receptors in immunoassays, and till now several antibodies have been introduced for development of immunosensors for detecting various bacterial toxins (Byrne et al. 2009). Specific antibodies are always needed for a sensitive and specific immunoassay, and various strategies are being used to generate specific antibodies. It is difficult to generate antibodies against toxin substance as the tolerance capacity of animal is not defined. Recently, a new technique was developed on utilization of half antibody fragments generated via reducing it with tris(2-carboxyethyl) phosphine for development of more sensitive immunosensor (Sharma and Mutharasan, 2013). On the other hand, molecular biology techniques are used to develop single-chain variable fragment (ScFv) attached to a short peptide by employing heavy and light chain variable regions of parent antibody (Pleckaityte et al. 2011). Compared with the traditional antibodies, antibodies found in camelids have a unique structural property, i.e., they are characterized by having only heavy chain (hcABs; Muyldermans, 2013). Similar fragments of heavy chain antibody, designated as "single domain antibody," have already been applied for the detection of bacterial toxins (Slocik et al., 2010). The use of these engineered antibodies or fragments has greatly improved the sensitivity and specificity of immunosensor (Wang et al. 2013).

The affinity of antibody and antigen toward each other is a concentration-dependent factor, since an effective immune response is elicited between them at a concentration range of 10^7 to 10^{10} M^{-1}. Therefore, the concentration of the target molecule is also a critical factor in deciding the potential of antibodies as a recognition element. In spite of being the backbone of immunoassays, antibodies do have certain limitations, such as the production of antibodies is a tedious and costly process. Lot-to-lot variations may hinder the assay reproducibility, and

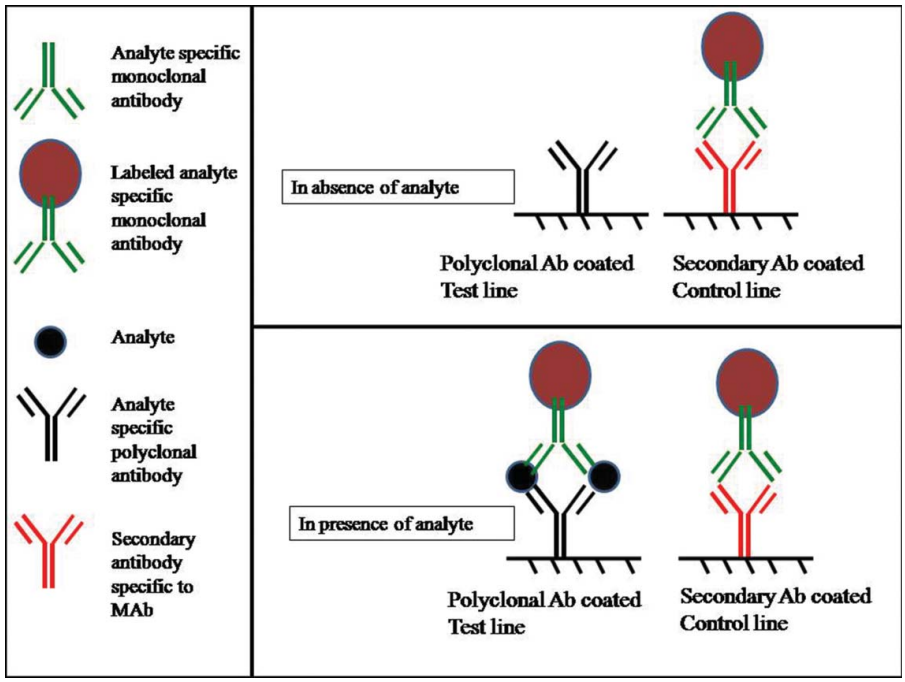

Figure 2. Sandwich ICA depicting the number of bands formed in the presence and absence of analyte.

dependency on animals for production and susceptible to denaturation in harsh assay conditions are other limitations. An LFIA strip is required to be used in field conditions, which, if not appropriate, may deteriorate the quality of immobilized antibodies. Hence, a quest for alternate recognition elements that overcome the limitations of antibodies and retain their advantages is always there.

The advanced formats of lateral flow immunoassays capitalize on using aptamers as molecular recognition elements. Aptamers are short, single stranded three-dimensional nucleic acid molecules selected by a process called systematic evolution of ligands by exponential enrichment (SELEX; Klug and Famulok, 1994; Gopinath, 2007; Zhou et al. 2014). They are also known as synthetic antibodies with specific three-dimensional structures and are extremely specific for target analytes such as proteins, peptides, ions, toxins, and pathogens (Ellington and Szostak, 1990; Tuerk and Gold, 1990; Mayer, 2009; Radom et al. 2013). In spite of the complexities associated with aptamer screening strategies, a significant hike in the number of aptamers screened against pathogens or their toxins is witnessed (Duan et al. 2013; Dwivedi et al. 2013; Han and Lee, 2013). In comparison to antibodies, aptamers are smaller in size, highly stable, non-immunogenic, and easy to synthesize and modify, therefore adapted as a promising substitute for conventional antibodies (Zuo et al. 2013). Recently published articles emphasized on the adaptation of aptamers in food security assessments (Yadav et al., 2010; Kärkkäinen et al. 2011; McKeague et al., 2011; Mehta et al., 2011; Campàs et al., 2012; Dong et al., 2014; Rhouati et al., 2013). Various lateral flow assays are also developed by using aptamer–nanoparticle conjugate, and limit of detection (LOD) has been also improved in some reported assays by replacing antibodies with aptamers. Wang et al. (2011a) have also used aptamer–GNPs conjugation system to detect ochratoxin in a competitive LFIA. This aptamer-based strip assay proves to be more sensitive than the conventional antibody-based strips, and an LOD of 1 ng/mL is achieved by visual analysis and 0.18 ng/mL in semi-quantitative mode using scanning reader. Wang et al. (2011b) further used aptamer–quantum dots (QD) for detection of OTA in a chromatographic strip assay to achieve an LOD of 1.9 ng/mL. However, LOD for QD-based chromatographic strip is low than GNPs based strip (Wang et al. 2011b). Later, Shim et al. (2014) have employed biotinylated aptamers for aflatoxin (AFL) to develop a competitive fluorescent LFIA for detection of AFL B1 (AFB1). The dipstick assay involves pre-incubation of the target molecule with the recognition element, which overcomes the hybridization deficiency problem of competitive LFIAs where aptamer–label conjugates detache from the target and hybridize with cDNA immobilized at nitrocellulose membrane. The assay gives an LOD of 0.1 ng/mL with AFB1 in the buffer (Shim et al. 2014). In sandwich format, it is often difficult to obtain a strong control line due to less hybridization time, and to address this issue, a dual-labeled aptamer coupled with QD-based LFIA is reported by Bruno (2014) for detection of food-borne pathogens. The utilization of peptide-based aptamers is also reported in numerous studies for detecting food-borne pathogens (Arcidiacono et al. 2008; Kulagina et al. 2005; Mannoor et al. 2010; Yonekita et al. 2013). Various formats of aptamer-based LFIAs are presented in a review by Sharma et al. (2015).

Labels

Over the years, number of labels, such as GNPs, silver nanoparticles, selenium nanoparticles (SNPs), quantum dots, upconverting phosphor nanoparticles (UCNPs), etc., have been utilized for developing LFIAs. The size of these substances can be tuned, resulting into varying optical properties that can be utilized to develop an LFIA. An ideal label for an LFIA should be able to increase assay sensitivity, amenable to surface modification, stable, and should not display high background signal or noise. In order to develop an efficient LFIA for toxin detection, various labels have been employed. This section traverses the journey of labels through LFIAs for toxin detection in food/water matrices.

Carbon nanoparticles (CNPs)

Carbon nanoparticles, or colloidal carbon, are comparatively less expensive label, and its manufacturing can be easily scaled up. These are characterized as black color nanoparticles (NPs) and can be detected effortlessly with high sensitivity. CNPs are also functionalized with various biomolecules and are used in detection of several toxins (Blažková et al. 2009). CNPs have displayed 10 times lower LOD in comparison to GNPs or latex beads in a study (Linares et al. 2012). CNPs are highly stable nanoparticles, which exhibit a higher contrast than GNPs and facilitate in enhancing sensitivity and LOD. As a substitute label, CNPs suffice for properties such as good stability, non-toxicity, easy conjugation, and no need for activation (Van Amerongen et al., 1993; Posthuma-Trumpie et al., 2012). Several reports are available on the successful utilization of CNPs in LFIAs for detecting pathogens, toxins, or other contaminants in food environmental monitoring (Aldus et al. 2003; Blažková et al., 2010; Holubová-Mičková et al. 2010). The main drawback associated with CNPs is the presence of larger biomolecules and nonspecific adsorption of proteins. However, if the way of analysis of core physicists is taken into consideration, such a limitation could arise because of loopholes in synthesis part as CNPs are prepared in a less accomplished manner.

Gold nanoparticles

Gold nanoparticles are the most popularly used labels in rapid tests (Seydack et al., 2005) mainly because of their high degree of stability, easy synthesis, and surface functionalization with molecules such as DNA, protein, polymers, sugars, etc. Antibodies when attached to these GNPs with vast strength and when coupled successfully offer a high degree of long-term stability (Sperling and Parak, 2010; Zhang et al., 2013). The principle behind the absorption-dependent colorimetric detection entails aggregation-induced inter-particle surface plasmon coupling of GNPs. The distinctive optical property of GNPs enhances the sensitivity of detection in LFIA (Reybroeck et al. 2014; Urusov et al. 2014; Gholamzad et al. 2015; Jawaid et al. 2014; Ching et al. 2015; Majdinasab et al. 2015a). Ching et al. (2015) analyzed shiga toxin by using a GNP-based LFIA with an LOD of 0.1 μg/L in less than 10 min. Multi-analyte antibody–GNPs-

based LFIA is also reported for simultaneous detection of shiga toxin (Stx1 and Stx2) and E. coli O157:H7. The assay is highly specific for shiga toxin and reached to an LOD of 10^5 CFU/mL for E. coli O157 in fortified ground beef samples (Wang et al. 2016).

Signal amplification techniques. The advantage of GNP labels is marred to some extent by the limit imposed on LFIA sensitivity because of their inherent optical surface resonance.

The issue of limited sensitivity of GNPs is addressed to some extent, first by modifying their shape, size, or making GNP composites with other metals, and second by employing silver enhancement. Tang et al. (2009) used modified GNP, wherein GNP-shelled nano-Fe_2O_3 microspheres are employed as label in an LFIA for qualitative detection of AFL B2 in food within 15 min. Ji et al. (2015) developed an LFIA using gold nanoflowers (GNFs) and reported that GNFs have high detection sensitivity with an inhibitory concentration (IC) of 4.17 pg/mL. The LOD for AFB1 is achieved at 0.32 pg/mL in rice extract. Another modification in GNP-based LFIA is reported with utilization of urchin-like gold nanoparticles (UGNs). Ren et al. (2015) used UGNs for sensitive detection of fumonisins (FUM) in grains, and the sensitivity reached up to 5 ng/mL, which rendered a four-fold enhancement in sensitivity over conventional strip. Various other groups have utilized silver-based and enzyme-based enhancements, as per the reports available (Horton et al. 1991; Yang et al. 2011; Wang et al. 2013; Kuang et al. 2013; Parolo et al. 2013). Brunt et al. (2010) utilized horseradish peroxidase (HRP) in a streptavidin-biotin-based LFIA to detect botulinum toxin (BTo) in skimmed milk and fruit juice samples. The assay shows an LOD of 0.5 ng/mL but has compromised specificity and time of detection.

Fluorescent dyes

Fluorescent dyes have been used as labels in lateral flow immunoassay but their use in LFIA for toxin detection is comparatively less. At present several fluorescent dyes, viz. Alexa Fluor, PromoFlor, DyLight Fluor, ATTO dyes, and HilyteFluor, are available with enhanced fluorescent characteristics and more stability. Zhang et al. (2015) developed an LFIA using colloidal gold and fluorescent microspherical entities (FMs) for detection of T-2 toxins, which are type A trichothecene. However, stability of these dyes and their limited signal time restrict their use as labels.

Quantum dots

Quantum dots (QDs) are an inorganic semiconductor nanostructures that show great promise as label in immunoassays because of their size-dependent luminescence, symmetric photoluminescence spectra and strong photo-stability (Algar et al. 2010). These properties make QDs a preferred choice over traditional fluorescent dyes in developing highly sensitive multiplex LFIAs for simultaneously detecting more than one analyte. Reports are available that QDs are employed for detecting toxins, pathogens, or proteins in immunoassays (Berlina et al., 2013; Wang et al. 2011). Taranova et al. (2015) have used multicolored QDs, what is referred to as "traffic light" competitive lateral flow immunoassay, for simultaneously detecting and quantifying antibiotics in milk. LODs for antibiotics are 80–200 times superior to previously reported ELISA.

There are also some drawbacks associated with QDs, which include water insolubility, toxicity, and lack of functional groups on QDs for bio-conjugation. These problems are circumvented to certain extent by encapsulating QDs in silica nanoshells or by modifying their surface with functional groups such as mercaptoacetic acid (Bruchez et al. 1998; Chan et al. 1998). In this regard, new strategies have been developed by doping or encapsulation of QDs on or into nanobeads to enhance detectable signal via enriching QDs in every binding. Ren et al. synthesized QDs beads by encapsulating CdSe/ZnS particles and uses it first time in a LFIA for detecting AFLs in maize extract. The LOD of this assay was 0.42 pg mL^{-1}, which is two-fold better than GNPs-based LFIA and conventional ELISA methods (Ren et al. 2014). Further, competitive LFIAs are also developed by using QD nanobeads for detection of Zearalenone (ZEL) in standard solution and real corn sample with a sensitivity of 0.0625 μg/L and 3.6 mg/kg respectively (Duan et al. 2015). Recently, Nardo et al. (2016) have reported an immunochromatographic assay for detection of FUM with an LOD of 2.8 μg/mL in an assay time of 22 min. While Foubert et al. (2016) detected four mycotoxins simultaneously in an assay time of 15 min with 5% reduction in false negative rates.

Selenium nanoparticles

Selenium nanoparticles are also known as colloidal form of selenium characterized as rust colored particles, which can be easily detected. In comparison to GNPs, SNPs are easy to synthesize, are inexpensive, and have been used as alternative labels in LFIAs; however, their use in LFIA for toxin detection is relatively scarce. SNPs were first used as labels in lateral flow strip for detection of hCG in urine (Osikowicz et al., 1990), followed by an LFIA for detection of lipoproteins in plasma (Lou et al. 1993). Recently, Wang et al. (2014) have reported the use of colloidal selenium in LFIA for detecting melamine (MA) in milk powder, liquid milk, and animal feed with an LOD of 150, 1000, and 800 ng/g respectively. So far no studies have been published to compare sensitivity, stability, reproducibility, and accuracy of LFIAs based on SNPs and other labels. Therefore, the scenario for SNPs used as labels remains uncertain.

Up-converting phosphor nanoparticles

Up-converting phosphor nanoparticles comprise an advanced category of luminescent nanoparticles, which emit visible or ultraviolet (UV) spectrum upon exposure with low energy radiation (van de Rijke et al. 2001). UCNPs possess several unique properties as compared to other nanoparticles, including high chemical and photo stability, less toxicity, narrow bandwidth, and low fluorescence (Tu et al. 2014; Wang et al. 2010). UCNPs were used in immunosensing approach for the first time by Niedbala et al. (2001). Recently, Zhao et al. (2016) developed a competitive LFIA based on up-converting phosphor technology for detection of AFL B1 in crops. The assay developed a detection sensitivity 0.03 ng mL^{-1} for standard AFL B1 solutions. The assay showed good tolerance to various crop samples, with LOD ranging from 0.1 to 5 ng g^{-1}.

Use of LFIA readers for quantization

Increase in sensitivity up to the level of picogram or femtogram is seen with the use of new labels such as fluorescent nanoparticles in conjunction with portable assay-reading devices. There are many companies, such as Detekt Biomedical, Axxin Inc, Qiagen Lake Constance, etc., which are the leading manufacturers of lateral flow assay readers, and LOD of LFIAs is greatly improved with these devices. Uses of these portable readers have also made the quantization of LFIAs feasible. LFIA-based qualitative, semi-quantitative, and quantitative test kits are also available commercially. Figure 3 depicts various types of LFIA readers available in the market.

Kim and Park (2004) proposed an idea of recording the optical density of signals by using LFIA strip readers, which converts the intensity of color at test/control zone into readable values. This assists in the quantitative assessment of analyte by determining the optical density of test zone (Zhao et al., 2008; Gonzalez et al., 2011). Nevertheless, the optical density of test and control lines depends on many factors, including, concentration of target compounds, immunoreactions time, operation temperature, variation in signals between different strip runs, and nature of sample matrices (Huang et al. 2013). However, ratio of absorbance of test and control lines can significantly equalize the effect of these factors (Ahn et al. 2003; Anfossi et al. 2010). Europium (III) nanoparticles-tagged antibodies are the most novel types of labels used for the detection of ochratoxins in rice samples as shown by Majdinasab et al. (2015b). The time-resolved fluorescent lateral flow assay had an LOD of 1 μg/kg with 100% specificity in 8 min of assay time using TRFICA strip reader.

Another advancement in the arena of quantitative and semi-quantitative analysis is by the application of smart phones. Smart phones are highly sensitive devices that are capable of detecting low light intensities also. Hence, a promising digital platform to suffice POC diagnostics relies on smart phones because of their high resolution camera, a powerful processor, and high storage capacity along with wireless connectivity. Powerful software platforms compatible with smart phones, personal computers, etc. to analyze images are being marketed by various companies such as Skannex and Novarum. This allows the use of smart phone as a reading device. Lee et al. (2013) have reported a sensitive LFIA for analyzing AFLs in maize. Gold-labeled antibodies as label sufficed an LOD of 5 μg/kg with 100% specificity. Fast and accurate point of care diagnosis is accompanied with a reading system consisting of smart phone and reader. Peak and area values as determined with smart phone-based readers allow segregation of blank samples from contaminated ones. In-depth and broader information about point of care reading devices for global health monitoring can be retrieved from a recent review of Rasooly et al. (2016).

Multiplexed LFIAs

Today, the diagnostic market is giving stress on the need of multiplex rapid detection methods because these assays heavily cut down the cost and time by analyzing more than one sample in a single test. A multiplex lateral flow immunoassay is developed to detect multiple target analytes by printing test lines on the strip for each specific analyte. Multiplex LFIAs have been reported as well as reviewed for detection of mycotoxins in various studies (Kolosova et al. 2007; Shim and Eremin, 2009; Lattanzio et al. 2012; Huang et al. 2012; Li et al. 2013; Song et al. 2014; Xu et al. 2016). Huang et al. (2012) have developed a competitive LFIA for simultaneously detecting deoxynivalenol (DOX) and ZEL in maize and wheat samples with LOD

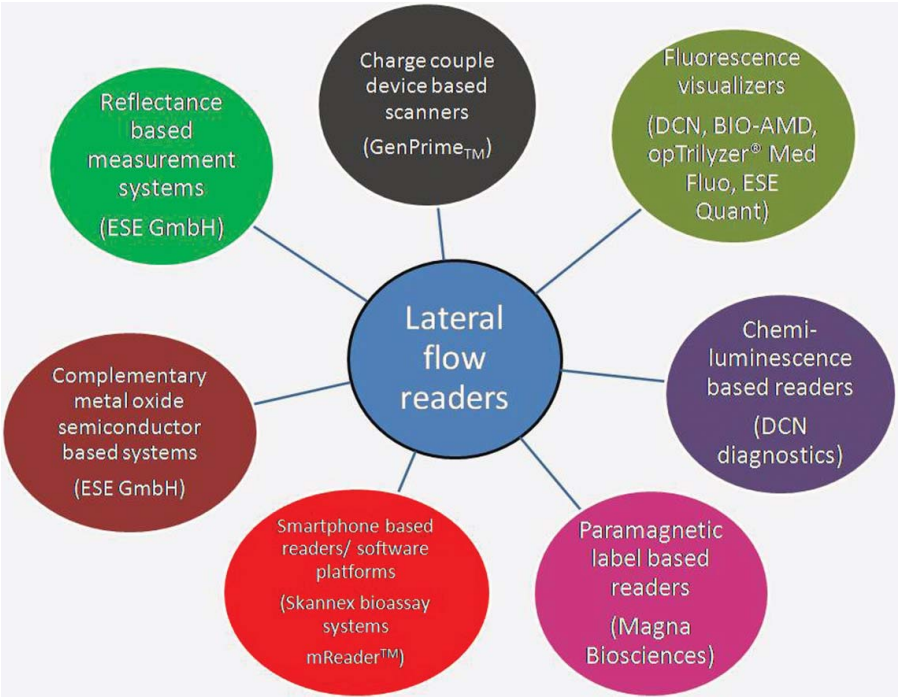

Figure 3. Various types of LFIA readers available in the market.

values of 100 ng/mL and 6 ng/mL for DOX and ZEL respectively. Another multiplex dipstick immunoassay has been reported for semi-quantitative determination of ZEL, DOX, T-2, and HT-2 toxins, and FUM in oats, wheat, and maize in a single assay (Lattanzio et al., 2012). A multi-component LFIA is also reported for simultaneously analyzing AFL, ochratoxin, and ZEL in agro-based foods. The LOD values of this assay were 0.5, 0.25, and 1 ng/mL for ochratoxin, AFL, and ZEL, respectively, without using any reading device (Li et al., 2013). Zangheri et al. (2014) developed a chemi-luminescent LFIA combined with charge-coupled device (CCD) camera for quantitative estimation of FUM and AFL. Furthermore, Song et al. (2014) detected AFL, ZEL, DOX, and their analogues in cereals in multiplexed LFIA. Another multiplex LFIA is reported for the estimation of three mycotoxins simultaneously in rice, corn, and peanut matrices. A visual LOD values of 10, 50, and 15 lg/kg were achieved for AFL B1, ZEL and ochratoxin A, respectively, and the quantified LOD values ranged from 0.10–0.13, 0.42–0.46, and 0.19–0.24 lg/kg for AFL B1, ZEL, and ochratoxin A, respectively, which meet the regulatory limits of European Commission (Chen et al., 2016). Multiplex LFIA have been also reported for detecting chemical contaminants, including testosterone, lead, microcystins, chloramphenicol, and chlorothalonil, simultaneously in drinking water (Xing et al., 2015).

Lateral flow immunoassays for toxins in food

Bacterial toxins

Till 1995, there was no proper nomenclature for bacterial toxins. Granum et al. (1995) for the first time classified food poisoning into infection and intoxication-type. They further illustrated that bacterial toxins can either be purely exogenous or enterotoxin-type but in some special cases production of enterotoxin is dependent upon interaction of bacteria with host. Bacterial toxins have been detected using various formats of lateral flow assays as listed in Table 1.

Botulinum toxin

The neurotoxin is produced as polypeptide consisting of two chains that can be cleaved into heavy (~100 kDa) and light chains (~50 kDa). The heavy chain assists access of toxin across neuronal membrane by endocytosis, while the light chain cleaves SNAREs, thereby causing inhibition of acetylcholine release, subsequently resulting in flaccid muscle paralysis.

GE Homeland Protection Inc. Newark, CA (US) has disclosed a lateral flow assay for detecting botulinum toxoid up to 1-ng level. The Raman-active tags, which are immuno-functionalized with anti-BTo antibodies, are employed to recognize the target botulinum toxoid. The zone of detection comprises immobilized anti-botulinum toxoid antibodies retaining the Raman-active complex at the zone of detection with a small amount of nonspecific signal US patent (US 11/223,353).

Stanker et al. (2010) developed two novel detection antibodies in amalgamation with two mAbs for developing a lateral flow assay. Using these antibodies, Ching et al. (2012) developed an LFIA proficient in differentiating type A and B neurotoxins as distinct colorimetric lines on a single strip, with a sensitivity of ~10 ng/mL and ~10–500 ng/mL for pure toxins and fortified beverages respectively. This data established the ability of monoclonal antibodies for space synchronized detection of both A and B toxins on a simple test strip. Chiao et al. (2004) developed a lateral flow device using silver enhancement technique for detection of BTo type A, having sensitivity of 50 pg/mL with 100% specificity with an assay time of 10 min. Attrée et al. (2007) have designed an LFIA for quickly analyzing BTo A in milk with a sensitivity of 0.018 picometer (pM)/mL by using GNP-stagged antibodies as label. The main advantage of this assay is its high sensitivity of 0.09 pM/mL when used with human serum. Sangho Bok et al. (2013) developed an ultrasensitive lateral flow assay for detecting BTo in orange juice by utilizing ultra bright fluorescent suprananoparticles. The assay appears to be the most sensitive assay with an LOD of 145.8 femtograms (fg)/mL with small reduction time and comparatively low photobleaching, although specificity was not discussed by the authors. The special part of their report was their membrane which had a pore size of <5 nm, resulting into the nullification of Rayleigh scattering, causing dispersion of bands. Chiao et al. (2008) elaborated on devising an immunochromatographic strip by using gold-tagged antibodies as label in cooked meat matrices. An LOD of 50 ng/mL was achieved with 100% specificity, which was further enhanced to 1 ng/mL by silver enhancement within a time limit of 10 min. The sensitivity of these assays was in the range of picometer (pM) or femtogram, and was excellent for LFIAs. Such high sensitivity is achieved by either using fluorescent suprananoparticles or silver enhancement, or good quality gold antibody conjugates. Brunt et al. (2010) utilized HRP-streptavidin and biotin-labeled antibodies to detect BTo in fruit juice and skimmed in an immunochromatographic column assay. The devised assay although had good detection limit of 0.5 ng/mL but compromised for specificity and assay time. Detection time of 24–48 h was achieved with simultaneous detection of *E. coli*. In the assay, workers used columns with immobilized detection antibody, which was used to capture analyte–antibody complex pre-tagged with biotin. After washing steps, biotin-specific HRP-tagged antibody was used to detect the presence of analyte using Tetramethylbenzidine (TMB) as substrate. Klewitz et al. (2006) developed a non-competitive LFIA for efficiently detecting BTo type D as low as 50 pg/mL. Here variable membrane systems of varying pore size diameters and capillary flow rates were employed. Thus, the properties of LFIA strip components can strongly influence assay sensitivity and specificity, and hence a careful selection is required before developing an LFIA. The described noncompetitive lateral flow assay shows false-positive results. These signals are considered background signals, as none of their tested parameters could reveal accurate reason for false-positive signals. The intensity of signal from the blank sample amplified with an increasing amount of gold-labeled antibody flowing across the test zone. The shelf life of this assay is up to three months.

Staphylococcal enterotoxin

Staphylococcal enterotoxins have low molecular weight and are produced by Staphylococcus aureus strains. These toxins bring shock-like syndromes and are the cause of food poisoning and various autoimmune diseases. Khreich et al. (2008) utilized

Table 1. Immunochromatographic assays for bacterial toxins.

S. No.	Toxin and source	Label used	Sample	Sensitivity	Specificity	Remarks	References
1.	Botulinum neurotoxin (*Clostridium botulinum*)	Gold nanoparticle-tagged antibodies	Milk, apple juice	5 ng/mL of purified bont/A and 10 ng/mL of bont/B in 2% and 1% milk respectively. In undiluted apple juice, 25 ng/mL of bont/A and 10 ng/mL of bont/B	No cross reactivity with B serotype of toxin	10–15 min of assay time, milk was processed by removing fatty content, and orange juice was processed by treatment with phosphate buffer	Ching et al., 2012
		Gold nanoparticle-tagged antibodies	Serum and urine	50 pg/mL	100%	10-min assay time, including silver enhancement	Chiao et al., 2004
		Gold nanoparticle-tagged antibodies	Milk and serum	2 pg/mL	100%	Lower LOD and 20-fold safety margin than LFIAs	Attrée et al., 2007
		Ultra-bright fluorescent suprananoparticles	Orange juice	145.8 fg/mL	—	High sensitivity, small reduction time, and low photo-bleaching	Bok et al., 2013
		Gold nanoparticles-tagged antibodies	Cooked meat broth	50 ng/mL	100%	Less than 10 min of assay time with enhanced LOD of 1 ng/mL after silver enhancement	Chiao et al., 2008
		HRP-labeled streptavidin and biotin-labeled antibodies	Skimmed milk and fruit juice	0.5 ng/mL	Less than 100%	Immunochromatography column requires 24 to 48 h of assay time and simultaneously detects *E. coli* also	Brunt et al., 2010
		Raman active tag-labeled antibodies	—	1 ng effective concentration	Less than 100%	—	US patent (US 11/223,353)
2.	Staphylococcal eneterotoxin (*Staphylococcus aureus*)	Fluorescent immunoliposomes	Cheese extract, ham extract, and surface water	20 pg/mL	100%	Food matrices were processed in analysis buffer with a run time of 30 min	Khreich et al., 2008
		Gold nanoparticle-tagged antibodies	Purified toxin	1 ng/mL	100%	25 min of run time	Rong-Hwa et al., 2010
3.	Cholera toxin (*Vibrio cholerae*)	Gold-tagged antibodies	Standard or spiked horse fecal sample	50 pg/mL	—	3.5 h of assay time is accompanied with relative error of 7%	Yamasaki et al., 2013
		Ganglioside liposomes	Clam, shrimp, and salmon	80 fg/mL, 180 fg/mL, and 3 pg/mL respectively	100%	20 min of assay time was elapsed and proper correspondence with crystallographic studies	Ahn and Durst, 2008
4.	Shiga toxin (*E. coli*)	Gold nanoparticle-tagged antibodies	Spiked food matrices	0.1 ng/mL		5 min of assay time preceded by processing of sample by adding PBS and further taking the supernatant after centrifugation at 12,000 g	Ching et al., 2015

fluorescent immunoliposomes for detection of staphylococcal toxins in cheese extract, ham extract, and water samples in a lateral flow assay format. Food matrices were processed for analysis in an assay time of 30 min, and 20 pg/mL of sensitivity was achieved with 100% specificity. Rong-Hwa et al. (2010) reported an LFIA kit for detecting *staphylococcal* enterotoxins by using gold-labeled antibodies as label and achieved an LOD of 1 ng/mL with no cross reactivity in an assay time of 25 min. Cow's milk was chosen as the matrix of choice, which was processed by diluting in 2% w/v phosphate buffer solution (PBS).

Cholera toxin

Cholera toxin (CT) is a key toxin for the intoxications induced by *Vibrio cholerae* infection by their ADP ribosylation activity, marking it critical for detection. Ahn and Durst (2008) reported the most sensitive assay for cholera toxin by using ganglioside liposomes as label. The assay achieved very fine sensitivities of 80 fg/mL, 180 fg/mL, and 3 pg/mL for clam, shrimp, and salmon samples. No cross reactivity is reported along with proper correspondence with crystallographic studies in an assay time of 20 min. Yamasaki et al. (2013) presented a study in which an LFIA for detection of cholera toxin was constructed. To enhance sensitivity, polyclonal antibodies against both A and B subunits were used. Test strip could detect toxins with minor mutations as the specificity of polyclonal antibodies was against multiple epitopes. The LOD was found to be 10 ng/mL for purified recombinant toxin. In terms of specificity, the assay could discriminate toxin from heat-labile toxin. The reported LOD was 100 folds higher than the earlier reports; the only constraint was the usual observation of biased specificity toward the recombinant toxin.

Shiga toxin

Infection of *E. coli* producing Shiga toxin (STEC) generally causes hemorrhagic colitis, fever, and bloody diarrhea, resulting in acute kidney disease (Tarr et al., 2005). Shiga toxins are generally detected by detection of STEC, contrary to the fact that Ching et al. (2015) have reported an LFIA for detecting toxins by utilizing gold-labeled antibodies in fortified food products. Food matrix incorporating lettuce, beef, and milk was processed by adding PBS and subsequent centrifugation. An LOD of 0.1 ng/mL was reached within an assay time of 5 min. The only constraint was the absence of any cross-reactivity data.

Mycotoxins

According to a Joint Expert Committee on Food Additives (JECFA), the scientific advisory committee to the Codex Alimentarius Committee on Food Additives and Contaminants (CCFAC), mycotoxins, such as ochratoxin A, ZEL, AFL, and FUM, led the priority list of risk assessment. Various researchers have summarized immunodiagnostic assays for detection of mycotoxins in an efficient manner (Krska and Molinelli, 2009; Anfossi et al., 2013; Sajid et al. 2015). Fungal toxins detected using various formats of lateral flow assays are listed in Table 2.

Aflatoxins

Being classified as group I carcinogens by International Agency for Research on Cancer (IARC), AFL B1 (AFB1) is the most toxic AFLs (Galvano et al. 2005). Tang et al. (2009) utilizes magnetic nanogold microspheres for detection of AFL in pistachia, hazelnuts, peanuts, and almonds. The method has an LOD of 0.9 ng/mL in 15 min of assay with 25% cross reactivity. The assay results are in agreement with HPLC. Anfossi et al. (2011) developed an immunochromatographic strip assay by using gold-labeled antibodies for detection of AFL in maize. An LOD of 0.1 μg/L with 73% specificity is achieved within 10 min of assay. Lee et al. (2013) also developed a sensitive LFIA for analyzing AFLs in maize. Gold-labeled antibodies as label suffice an LOD of 5 μg/kg with 100% specificity. Fast and accurate point of care diagnosis is accompanied with a reading system consisting of smartphone and reader. Anfossi et al. (2013) devised another lateral flow strip for milk samples, where sample is processed using surfactant. Here 17 min of assay time produced a sensitivity of 20 ng/L and 65% of specificity by using gold-labeled antibodies.

The RIDA® QUICK AFL assay devised by r-biopharm detects AFL with an LOD of 4 μg/kg in grains such as wheat, barley, corn, oats, rye, millet, rice, nuts, and canola with 99% specificity in an incubation time of 5 min (r-biopharm (http://www.rbiopharm.com), accessed on September 10, 2016).

Reybroeck et al. (2014) developed the most sensitive assay of the decade by using gold-labeled antibodies only. An LOD of 15 ng/L was achieved with 80% specificity in 15 min for commingled milk samples without any dilution step in processing.

Deoxynivalenol

Fusarium fungi produce DOX, which contaminates a wide range of cereal grains. Kolosova et al. (2008) utilized gold-labeled monoclonal antibodies for detection of DOX in maize in a lateral flow assay format. In just 10 min of assay time, an LOD of 250 μg/kg was achieved with 84% specificity. Xu et al. (2010) designed a brisk LFIA for DOX detection in maize and wheat. The test qualitatively estimated contamination of DOX on a 10-min time scale, with a visual LOD of 50 ng m/L. The sensitivity of the assay was satisfactory enough to detect DOX within its maximum permissible limit of 1 mg/kg as advised by their regional legislature. Yu et al. (2015) detected DOX using a gold nanoparticle-based LFIA in maize with a sensitivity of 800 μg/kg within 6 min. Further, silver enhancement technique was employed to lower LOD to 320 μg/kg.

Fumonisins

Fumonisins are causative toxics for disparities such as irreversible tissue damage, and thus produce carcinogenic, pro-oxidative, pro-inflammatory, and immunosuppressive effects.

Anfossi et al. (2010) analyzed FUM in maize samples with an LFIA employing gold-labeled antibodies as label. Maize samples were prepared by using PBS and polyethylene glycol (PEG), which enabled them to achieve sensitivity of 120 μg/L in 10 min of assay time. Wang et al. (2013) also utilized gold nanoparticles-labeled mAb for detecting FUM and ZEL in corn and wheat samples in a multiplex assay. An LOD of 313.8 μg/kg was achieved in an assay time of 30 min but no discussion on specificity was presented.

Venkataramana et al. (2014) used LFIA to detect FUM levels in cereals. Their study initially focused on blocking reagent used to reduce nonspecific interactions between toxin-ovalbumin

Table 2. Immunochromatographic assays for mycotoxins.

Sl No.	Toxin and source	Label used	Sample	Sensitivity	Specificity	Remarks	References
1.	Aflatoxin M1 (*Aspergillus flavus*)	Gold-labeled antibodies	Milk	20 ng/L	65%	17 min of assay line with 2 min of sample processing using surfectant	Anfossi et al., 2013
		Gold-labeled antibodies	Commingled Milk	15 ng/L	80%	15 min of assay time without any sample dilution step	Reybroeck et al., 2014
		Gold-labeled antibodies	Maize	0.1 μg/L	73%	12, 97, and 2% cross reactivity along with 10 min of assay time	Anfossi et al., 2011
		Gold-labeled antibodies	Maize	5 μg/kg	100%	Fast and accurate point of cure diagnosis with a reading system consisting of smartphone and reader	Lee et al., 2013
		Magnetic nanogold microspheres	Peanuts, hazel nuts, pistacia, and almonds	0.9 ng/mL	75%	15 min of assay time without any false negative result in correspondence to HPLC	Tang et al., 2009
		—	Grains and nuts	4 μg/kg	100%	4–16 min of assay time	r-biopharm
2.	Deoxynivalenol (*Fusarium spp.*)	Gold-labeled antibodies	Wheat and maize	50 ng/mL	Cross reactivity with toxin derivatives	10 min of assay time preceded by mechanical crushing and filtering	Xu et al., 2010
		Gold-labeled monoclonal antibodies	Wheat	250 μg/kg	84%	10 min of assay time	Kolosova et al., 2008
		Gold-labeled monoclonal antibodies	Maize	40 ng/mL	—	6 min of silver enhancement resulted in 2.5-fold increase in sensitivity	Yu et al., 2015
3.	Fumonisin B1 (*Fusarium and Aspergillus spp.*)	Gold-labeled polyclonal antibodies	Cereal samples	5 ng/mL	—	3 min of assay time preceded with toxin extraction in a competitive format	Venkataramana et al., 2014
		Gold-labeled monoclonal antibodies	Corn and wheat	313.8 μg/kg	—	30 min of assay time in multiplex format for zearalenone detection also	Wang et al., 2013
		Gold-labeled antibodies	Maize	120 μg/L	—	10 min of assay time with maize sample processed by using PBS and PEG	Anfossi et al., 2010
		—	Corn	0.8 mg/kg	100%	5 min of assay time	r-biopharm
4.	Ochratoxin A (*Aspergillus ochraceus* and *Penicillium Verrucosum*)	Gold-labeled antibodies	Coffee samples	5 ng/mL	—	10 min of assay time preceded by sample dilution in a competitive format	Liu et al., 2008
		Gold-labeled aptamer	Red wine	1 ng/mL	100%	10 min of assay time and LOD was 0.18 ng/mL after using scanning reader	Wang et al., 2011
		Europium (III) nanoparticles-tagged antibodies	Rice	1 μg/kg	100%	Time-resolved fluorescent ICA with 8 min of assay time using TRFICA strip reader	Majdinasab et al., 2015
		Gold-labeled antibodies	Maize, wheat, and durum wheat	1.5 μg/kg	—	Use of PEG in extraction of toxin from matrix with an assay time of 20 min	Anfossi et al., 2011
		Gold-tagged antibodies	Wine and grape must	0.1 μg/L	93.6%	5 min of assay time along with sample processing using 0.15 M $NaHCO_3$ and 4% PEG	Anfossi et al., 2012
		Gold-labeled antibodies	Wine and grape must	0.9 μg/L	77.8%	20 min of assay time with silver enhancement	Anfossi et al, 2013
5.	Zearalenone (*Fusarium spp.*)	Gold-labeled antibodies	Spiked wheat	100 μg/kg	Cross reactive with alcoholic derivatives of Zearalenone	10 min assay time accomplished without the need for any equipment and fast sample preparation by methanolic extraction	Kolosova et al., 2007
		Gold-labeled antibodies	Wheat and maize	6 ng/mL	100%	5 min of assay time along with effective multiplexing for deoxynivalenol	Xu et al., 2010
		Antibody-labeled quantum dot submicrobeads	Corn	3.6 μg/kg	Negligible cross reactivity	10 min of assay time with 2800 times brighter luminescence than corresponding QDs	Duan et al., 2015
		Antibodies labeled with colored particles	Bivalve shellfish	160 μg/kg	Cross reactive with dinophysistoxin	Assay time of 40 min but this assay does not corresponds to results of LC-MS	Vale et al., 2009
		—	Grains and nuts	75 μg/kg	100%	5 min of assay time	r-biopharm

conjugate and colloidal gold. The competitive format discussed the immobilization of fumonisin–ovalbumin conjugate on nitrocellulose membrane, and here also antibody played the role of capturing agent. Advantageously, LOD was found to be 5 ng/mL within 3 min. The RIDA® QUICK fumonisin assay devised by r-biopharm detected fumonisin with an LOD of 0.8 mg/kg in corn with 99% specificity in an incubation time of 5 min (r-biopharm, accessed on September 10, 2016).

Ochratoxin A

Ochratoxin demonstrates strong hepatotoxicity, nephrotoxicity, teratogeny, mutagenicity, and immunosuppressive effects after absorption from gastrointestinal mucosa (Leszkowicz et al. 2007). Liu et al. (2008) raised mAb against ochratoxin and used it for developing LFIA for ochratoxin detection in coffee. Although the visual LOD of 5.0 ng/mL was less than that of cd-ELISA (0.32 ng/mL) the nature and trustworthy point of care nature provided significant results. The strip was rapid, visual as well as quantitative within a short time interval of 10 min. Gold-labeled aptamer is innovatively used by Wang et al. (2011) for detecting ochratoxin in red wine. Sensitivity of 1 ng/mL with 100% specificity was achieved in 10 min of assay time. The limit of detection was further increased to 0.18 ng/mL with an assay reader. Anfossi et al. (2011) also reported an LFIA for ochratoxin detection in wheat, maize, and durum samples with an LOD of 1.5 μg/kg. Polyethylene glycol was used for processing of samples and the total assay time was 20 min with gold-labeled antibodies as label. Anfossi et al. (2011) again targeted ochratoxin but this time with a different food matrix of wine and grape must. An LOD of 0.1 μg/L was achieved with 93.6% specificity, and a 5 min of assay along with sample processing using 0.15 M $NaHCO_3$ and 4% PEG was accompanied by gold-labeled antibodies as label. Anfossi et al. (2013) also used silver enhancement technique for wine and grape must samples and reported enhanced sensitivity and specificity of 100 and 94.1% with slight increment in assay time. Europium (III) nanoparticles-tagged antibodies are most novel types of labels used for the detection of ochratoxins in rice samples as shown by Majdinasab et al. (2015b). The time-resolved fluorescent lateral flow assay has an LOD of 1 μg/kg with 100% specificity within 8 min of assay time using TRFICA strip reader.

Zearalenone

Zearalenone remains intact in the food chain and causes damage to reproductive system of mammals as per report (Abbes et al., 2007). Shim and Eremin (2009) and Li et al. (2013) utilized GNPs as label for determination of ZEL with a visual LOD of 2.5 ng/mL and 1.0 ng/mL respectively. Wang et al. (2013) also used GNPs for quantification of ZEL and presented a better LOD of 0.35 ng/mL and 21 mg/kg respectively. The RIDA® QUICK ZEL assay devised by r-biopharm detected ZEL with an LOD of 75 μg/kg in corn with 99% specificity in an incubation time of 5 min (r-biopharm, accessed on September 10, 2016).

Duan et al. (2015) developed an ultrasensitive method to determine the levels of ZEL in corn samples. Luminescent quantum beads were used as detection strategy component in competitive format where labels present 2800-fold high brightness than reported QDs. An LOD of 62.5 pg/mL stands 5.6 folds higher than previously reported assays. Acceptable precision, accuracy, and significant correlation to ELISA defined its superiority.

Miscellaneous toxins

Apart from bacterial and fungal toxins, various toxins of animal and plant origin as well as man-made toxins prevail as food contaminants causing serious health complications. Such toxins have been detected using various formats of lateral flow assays as listed in Table 3.

Abrin-a

Abrin-a is the most toxic isoforms of abrin and comprises two polypeptide chains linked through disulfide bond. Weia et al. (2011) developed a silver enhancement strategy in a colloidal gold-based lateral flow assay to detect Abrin-a in both water and soybean milk. Silver enhancement schemes have shown a 100-fold improvement in sensitivity, which minimized from 10 to 0.1 ng/mL. Without complex handling, the optimized assay was completed in 20 min, rendering simplicity. Noteworthy, cross reactivity with homologous toxins was also not reported. They also possessed significant storage of all assay materials for 12 weeks.

Brevetoxins

Zhou et al. (2009) designed an introductory semi-quantitative assay for detecting the remaining toxin concentration above or below the LOD. Visualization of results was in accordance with ELISA data. The reported assay showed easy, rapid, convenient, and unsophisticated handling within 10-min process time. Recently, Zhang et al. (2014) designed an immuno-dipstick assay to detect brevetoxin by using hollow nanogold microspheres as labels. They reported a sensitivity of 0.1 ng/mL with 72.2% specificity for spiked fish samples. Hollow gold nanospheres caused 20-fold increase in sensitivity in comparison to colloidal GNPs.

Carbofuran and triazophos

Carbofuran is a carbamate pesticide incorporated to control insects of crops such as potatoes, corn, and soybeans (Jin et al., 2006). Triazophos belongs to organophosphorous class and is used for primary controlling of insects in cotton, rice, oil, and seeds (Gui et al. 2008). Guo et al. (2009) detected triazophos and carbofuran in two distinct LFIA strips. In one strip, monoclonal antibodies for both carbofuran and triazophos were used for preparation of probe that limited detection up to 8 μg/L and 64 μg/L for triazophos and carbofuran respectively. In the second strip, two GNP-labeled antibodies against carbofuran and triazophos were placed separately on conjugate pads and cut-off levels of 32 and 4 μg/L for carbofuran and triazophos, respectively, were achieved. No cross reactivity for other structurally related compounds was reported.

Chlorpyrifos

Chlorpyrifos is an insecticide belonging to the class of organophosphorus type of insecticides. Kima et al. (2011) devised a competitive lateral flow assay in an antigen-coated format to

Table 3. Immunochromatographic assays for miscellaneous toxins.

Sl No.	Toxin and source	Label used	Sample	Sensitivity	Specificity	Remarks	References
1.	Abrin-a (beans of *Abrus precatorius*)	Gold-labeled antibodies	Water or soybean milk sample	10 ng/mL	100%	20 min of assay time with 100-fold increase in sensitivity on silver enhancement	Yang, 2011
2.	Brevetoxins (planktonic red tide dinoflagellate *Karenia brevis*)	Gold-labeled antibody	Fishery product samples	20 ng/mL	100%	10 min of assay time is preceded by sample disruption by DMSO and filtering in a competitive format	Zhou et al., 2009
		Nanogold microsphere	Spiked fish samples	0.1 ng/mL	72.2%	The hollow nanogold microspheres provided 20-fold increase in sensitivity that colloidal gold particles in an assay time of 10 min	Zhang et al., 2014
3.	Carbofuran and triazophos	Gold-tagged antibodies	Water samples	32 and 4 μg/L respectively	100%	8–10 min of assay time accompanied with sample processing by spiking of water samples in a competitive format	Guo, 2009
4.	Chlorpyrifos	Gold-tagged antibodies	Agricultural samples	10 and 50 ng/mL	87% in presence of methyl derivative	10 min of assay time but sensitivity was challenged by five folds when spiked samples were assayed	Kim et al., 2011
5.	Microcystin (cyanobacteria)	Gold-labeled antibodies	Tap water and lake water	1 ng/mL	High cross reactivity	5 min of assay time showing cross reactivity with four analogs	Lu et al., 2012
		Fluorescent-tagged antibodies	Tap water	95.38 pg/mL	Slight cross reactive	12 min of assay time is accompanied by loss of point of care	Kim et al., 2003
		Gold-tagged antibodies	Water samples	5 μg/L	—	15 min of assay time accompanied by high cost affectivity and storage over 115 days	Tippkötter et al., 2009
		Gold-labeled recombinant antibodies	Water samples	0.5 μg/L	Less reactivity for methylated microcystin -LRr	35 min of assay time with microcystins containing arginine being slightly less sensitive	Lawton et al., 2010
6.	Okadaic acid (Dinoflagellates)	Lateral flow ICA	Shellfish products	10–50 ng/mL	Cross reactive with dinophysistoxin at 20 ng/mL	10 min of assay time is preceded with sample preparation by spiking and methanolic extraction	Lu et al., 2011
		Antibodies labeled with colored particles	Bivalve shellfish	160 μg/kg	Cross reactive with dinophysistoxin	Assay time of 40 min but this assay does not corresponds to results of LC-MS.	Vale et al., 2009

detect chlorpyrifos. Competition was observed between mobile and immobilized analyte for limited binding sites at test zone. Relative migration speed of analyte and conjugate was observed to be critically important for the sensitivity of assay. The assay can detect as low as 10 ng/mL of chlorpyrifos in less than 10 min and could be employed for its sensitive on-site testing (Kima et al., 2011).

Microcystins

Microcystins are cyclic peptide toxins within a molecular weight range of 900 and 1200. Kim et al. (2003) used fluorescent-tagged antibodies to detect the presence of microcystins in tap water matrices through lateral flow assay. A very high sensitivity of 95.38 pg/mL was reported in 12 min of assay time but specificity was slightly compromised along with loss of point of care. Gold-tagged antibodies were efficiently used by Tippkötter et al. (2009) to detect levels of microcystins in water samples; 5 μg/L of sensitivity was achieved in 15 min of assay accompanied with high cost-effectivity and storage for 115 days. Lawton et al. (2010) utilized gold-labeled recombinant antibodies for detection of microcystins in drinking water. An LOD of 0.5 μg/L was achieved in 35 min of assay with microcystins containing arginine being slightly less sensitive for the test. Specificity was also compromised for methylated microcystins. Liu et al. (2008) developed an amalgam of biosensors, which were rapid and reproducible for detection of microcystins. The detection time, being 10 min, renders usage for field detection of microcystins. The group developed a straightforward method for the synthesis of microcystin conjugate, which was used to produce monoclonal antibodies. In addition to the developed biosensor system, they developed an immunochromatographic sensor for semi-quantification of microcystins with LOD equivalent to 1 ng/mL.

Okadaic acid (ODA)

Okadaic acid, an analogue of dinophysistoxin, fish poisoning toxins accumulated in mollusks was produced by dinoflagellates. Vale et al. (2009) developed an immunochromatographic strip using antibodies labeled with colored particles to detect ODA in bivalve shellfish matrices. An LOD of 160 μg/kg was achieved within 40 min of assay time but specificity was compromised by cross reactivity with dinophysistoxin. Moreover, the assay did not correspond to results of LC-MS.

Lu et al. (2011) developed a competitive LFIA using gold-labeled mAb for detecting ODA in shellfish-based sea foods. The sensitivity of the assay was 150 ng/kg, which was close to the secure permissible limit of 160 ng/kg. The test strip fulfilled the parameters of point of care and numerous samples could be screened within 10 min, making the assay convenient and efficient.

Additional parameters influencing LFIA performance

Properties of LFIA components

The LFIA strip is assembled using four components overlapped over each other, viz. sample application pad, reservoir matrix or conjugate pad, a membrane, and an absorbent pad or wick to maintain a steady and continuous liquid flow through capillary forces. All the components are laminated onto a plastic backing sheet (Wong and Tse, 2008; Xu et al., 2009). The chemical and physical nature of these components influence various properties of LFIA such as flow characteristics, amount of biomolecules absorbed on membrane, release of labeled molecules, and sample requirements, which in turn affect the LFIA sensitivity and specificity. Elaborate discussions on making a wise choice for selection of these strip components has been presented in various reviews or product letters of different LFIA strip manufacturing companies (Millipore Corporation, 2002; Dzantiev et al., 2014). A detailed discussion on this aspect is, therefore, not a part of this review; however, readers may refer the suggested reviews before selecting the lateral flow assay components.

Method of processing food matrix

Processing of food matrix becomes pivotal when toxins are to be detected from food matrix. To detect any toxin, the focus is on its availability to detection system, and to ensure such availability, toxins are to be extracted from the food matrix. Extraction of toxins depends on sampling and preparation method employed and is affected by the following parameters: (1) Nature of toxin to be detected; (2) distribution of toxin in matrix; (3) physical attributes; (4) toxin accessibility from matrix; (5) sample size, and (6) sampling procedure. The proximity and interaction of food with toxin and the type of toxin govern the choice of processing method (Kataoka, 2003). When we discuss extraction of toxins from food matrices, we make sure to note down the place of localization within the cell and within the plant/animal part. The localization of toxin is decided by the range and diversity of surfaces in a food matrix. To ensure proper extraction, matrices are subjected to decompartmentalization, such as spiced chicken sandwich with vegetables and cheese, and also include processing of fats, particulate matter, and inorganic food components along with presence of non-target microbes (Brehm-Stecher et al., 2009). In this context, to prepare spiked matrix for shiga toxins detection, Ching et al. (2015) mixed PBS, and centrifuged at 12,000 g for 12 min at 4°C.

When LFIAs are focused for dairy products, such as milk, it is greatly affected by fat–water balance resulting in partitioning within the matrix. One of the major issues when labels are introduced to matrices is the interaction of label to food ingredients, phenolic compounds, and divalent cations, which can act as inhibitors. In context of food matrices used, milk stands as a matrix with the longest assay time (Ching et al., 2015). Various assays show milk as not competing colloid for the detection of bacterial systems, but Reybroeck et al. (2014) used undiluted milk for the detection of aflatoxin, and recorded an efficient limit of detection. So, apart from juices, broths, clams, shrimps, and salmons, milk needs to be analyzed for its interactions inside detection systems. If processing strategies are kept in consideration, then we could critically say that removal of fatty content of milk can decrease the assay time as evident through the reports. Anfossi et al. (2013) simply centrifuged milk for 2 min and supernatant was mixed with surfactant-like tween-20 before application to lateral flow strip.

For food commodities that are available in granular forms, grinding becomes the foremost processing technique followed by buffer dissolution and surfactant addition (Xu et al., 2010). Extraction of toxins may also require use of advanced techniques such as solvent extraction and solid phase extraction methods. As far as animal products are concerned, cell lysis and homogenization become vital foregrounds. Figure 4 depicts various methods such as solid phase extraction, supercritical fluid extraction, membrane-base extraction, solid phase microextraction, and liquid phase microextraction for analyte extraction from solid and liquid food matrices.

Matrix processing helps in achieving a homogenous environment that does not interfere with analytical reaction. In a general schema, matrices are homogenized or lyophilized to disrupt cell membranes, and finally contaminants are extracted into fractions (Guo et al., 2010). Generally, pesticides, mycotoxins, heavy metals, and various low molecular weight organic compounds are extracted in organic solvents such as methanol, acetone, acetonitrile, or n-hexane. However, the most important fact that has to be considered is the effect of organic solvents on the properties of the antibody further impeding the immune recognition. As a preventive measure, liquid fraction can be diluted with salt solutions (aq.; Beloglazova et al., 2012), but this also sometimes compromise sensitivity. Further, as an alternate option, organic solvents can be eliminated by evaporation and residual entities are to be solubilized in other solvent (Wang et al., 2013). Thus, the process becomes complicated at every step. One more technique to ease down the matrix-processing step is the use of microfluidic technology to simplify surface modification of components, removal of interfering agents by application of novel fabrication technique, and minimizing the consumption of immunoreagents (Li et al., 2012). So, on the basis of all these reports we can say that before designing any immunochromatographic detection system, one need to keep in mind that: (1) sample loss should be minimum and analyte recovery should be maximum; (2) sample preparation should remove all the coexisting components to minimize interference; (3) matrix processing should be a quick and convenient process, and (4) matrix processing should always be a cost-effective process with minimal requirement of expert handling.

As per the reports of Lu et al. (2012), after homogenization, okadaic acid-spiked tissues are subjected to 80% aqueous methanol, defatted using n-hexane, and extracted by chloroform. Accu Bond II solid phase extraction (SPE) silica column extractor is utilized and eluents are dissolved in 80% methanol (Lu et al., 2012). Zhou et al. (2009) also reported the use of dimethyl sulfoxide (DMSO; 50% w/v) and further centrifugation at 1500 g for 15 min for detection of shell fish toxins. The filtered supernatant is incorporated with toxins for spiking at various concentrations using vortexing. In 2010, Anfossi et al. (2010) discussed the detection of FUM, in which simple processing of sample was done using PBS and PEG. Venkatramana et al. (2014) detected FUM in cereal samples, in which extraction was done with 100-mL methanol/water (3:1 v/v) for 3 min. After centrifugation at 1500 g for 10 min, mixture was filtered, and 5 mL of extract was applied to solid phase extraction cartridge (Bond-Elut, Varian, Harbor City, CA, USA) containing 500-ng sorbent at a flow rate of less than 2 mL/min. Further elution of FUM was done using 1% acetic acid in methanol (10 mL) under gravity. If these two methods are compared in aspect of their limits of detection, then we could justify the need of sample processing, as the later has enhanced it by 24,000 folds by utilizing solid phase extraction technique (Venkatramana et. al., 2014). In addition, LFIA strip components may also contribute in minimizing the matrix interference. For example, sample pad may absorb various interfering substances, partial separation of compounds may take place when the sample moves along the strip or the pre-soaking of sample pad also reduces matrix interference (Anfossi et al., 2012; Posthuma-Trumpie et al., 2012).

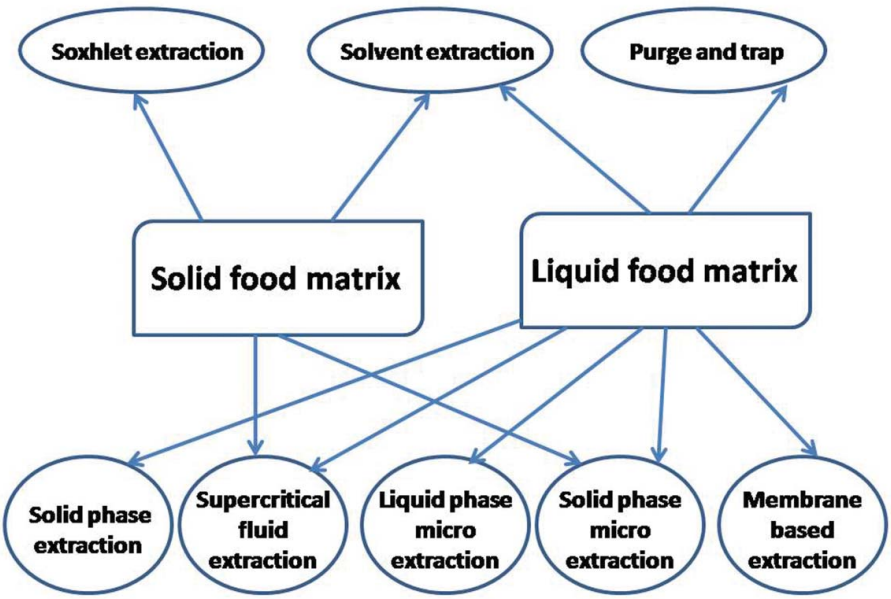

Figure 4. Various methods for analyte extraction from solid and liquid food matrices.

Table 4. List of commercial LFIA kits available for detection of toxins.

S. No.	Toxins	Matrix	Test kit	Manufacturer
1.	Deoxynivalenol	Wheat, triticale, maize	RIDA Quick DON	r-Biopharm GmbH, Darmstadt, Germany
		Wheat, barley	ROSA DON P N-1	Charm Sciences Inc., Andover Street Lawrence, MA, USA
		Barley, corn, malted barley, milled rice, oats, rough rice, sorghum, wheat, wheat flour, wheat midds	ROSA DON Q	Charm Sciences Inc., Andover Street Lawrence, MA, USA
		Wheat (GIPSA)	Reveal DON	Neogen Corporation, Lansing, MI, USA
		Not specified	AgraStrip DON	Romer Labs, Newark, DE, USA
		Corn, wheat, barley	QuickTox DON	Enviro Logix, Portland, ME, USA
		Corn, wheat, barley	DONCHECK	Vicam
		Grain and feed	MycoCheck	Strategic Diagnostic Inc., Pencader Drive, Newark, Delaware
		Wheat (Q-body)	DON-V kit	VICAM, Milford, MA, USA
2.	T2, Ht-2	Barley, corn, corn gluten meal, oats, soybean meal, wheat, wheat flour	ROSA T2-HT2	Charm Sciences Inc., Andover Street Lawrence, MA, USA
3.	Zearalenone	Barley, corn, flaking corn grits, milled rice, oats, rough rice, sorghum, wheat, wheat flour	ROSA Zearalenone	Charm Sciences Inc., Andover Street Lawrence, MA, USA
4.	Fumonisin B	Corn	RIDA Quick Fumonisins	r-Biopharm GmbH, Darmstadt, Germany
		Corn	ROSA DON Q	Charm Sciences Inc., Andover Street Lawrence, MA, USA
		Not specified	Reveal Fumonisins	Neogen Corporation
		Cereals and other matrices (not specified)	AgraStrip Fumonisins	Romer Labs, Newark, DE, USA
		Corn	QuickTox Fumonisins	Enviro Logix, Portland, ME, USA
5.	Zearalenone, deoxynivalenol, T2/Ht2, Fumonisin B	Wheat, oats, maize	4MycoSensor	Unisensor, Ougrée, Belgium
6.	Botulinum neurotoxins	Food samples (ice cream, milk, and honey)	Bot-Tox-BTA kit BoNT/E kit	Alexeter Technologies; NMRC, Wheeling, IL, USA Tetracore
7.	Staphylococcal enterotoxin B	Milk and milk products	Tetracore	Gaithersburg, MD, USA
8.	T-2 toxin	Maize	T-2 toxin lateral flow device test kit	AC Diagnostics, Fayetteville, AR, USA
		Maize	Visual T-2 Toxin Rapid Test	Quicking Biotech, Shanghai, China
9.	Okadaic acid	Shellfish samples	Jellett Rapid Testing kit	Nova Scotia, Canada
10.	Aflatoxin M1	Milk samples	MRLAFMQ test Kit	Charm Sciences

Conclusions

Over the last few years, lateral flow immunoassays have gained enormous interest among researchers for detecting clinical and non-clinical analytes. The present review discussed the recent advancements made toward the development of lateral flow immunoassays for detecting toxins in processed and unprocessed food materials. The review discussed advancements made in various aspects starting from recognition element, labels, detection system, and multiplexing with respect to food-associated toxins in aspect to enhance sensitivity and reduce detection time. The utilization of nanoparticles in LFIA enhances the sensitivity of immunoassay by increasing signal to noise ratio, decreasing reaction time, and employment of simultaneous detection of multiple analytes. When reviewing LFIA with different types of labels, the majority of studies involved the application of GNPs-conjugated LFIA; however, other labels have also been extensively worked upon toward the enhancements of sensitivities against toxins posing major threat to food safety, military, and homeland security. The paper presented the LFIAs reported for various kinds of bacterial, fungal, and miscellaneous toxins affecting quality of agricultural products. A common limitation associated with LFIAs is their low sensitivity when the test is read with naked eyes, but during this review it was keenly observed that most of the assays achieved sensitivity in microgram/nanogram levels. This improvement in sensitivity was observed by using very good quality antibodies or aptamers as recognition elements. Further, increase in sensitivity up to pictogram or femtogram level was seen with the use of new labels, such as fluorescent nanoparticles, in conjunction with a portable assay reading device. Use of these portable readers has also made the quantization of LFIAs feasible. Specificity is the main concern with fungal toxins because fungal toxins show high cross reactivity than other toxins. Therefore, it is required to either raise more specific antibodies or to screen and test other recognition molecules such as aptamers or any specific toxin-binding protein to overcome this drawback. One more limitation associated with the detection of any toxin in agricultural food samples is the inherent complex nature of various food matrices, which makes direct analysis of the toxins in these matrices a difficult task. New, simple, and fast extraction/processing methods are required to overcome this drawback and maintain the point of care nature of these tests. Extraction procedure must be selected by considering its efficiency for specific toxin and by analyzing solvent compatibility with biomolecules like antibody. LFIA-based qualitative, semi-quantitative, and quantitative test kits are also available commercially as enlisted in Table 4. However, reproducibility, applicability with different food matrices, and sensitivity are some limitations which need to be fully addressed to. In recent advancements, it has been seen that sensitivity-based researches are less prone to stop, as the permissible limits of toxins are not changing. Advancement in sciences has made us learn that every component of life system can pose as toxins on the basis of dosage-based studies. The need to address challenges of diagnostic industry requires more dwelling into the toxin-

type and interaction studies for developing more sensitive and specific LFIA that show POC characteristics.

Declaration of interest

It is hereby declared that authors have no conflicts of interest.

Acknowledgments

The authors thank Ministry of Human Resource and Development (MHRD) and Council of Scientific and Industrial Research (CSIR) for their valuable support.

Funding

The financial support received form DST-SERB, India (SB/YS/LS-261/2013) to develop multiplex lateral flow immunoassay for bacterial toxins is gratefully acknowledged. The review is written while carrying out this project work.

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