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REVIEW

Lateral-flow immunoassays for mycotoxins and phycotoxins: a review

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Abstract Natural toxin (for example mycotoxin and phycotoxin) contamination of food is of safety and economic concern, so much effort is devoted to the development of screening methods which enable the toxins to be continuously and widely monitored in food and feed. More generally speaking, rapid and non-instrumental assays for detection of a variety of food contaminants are generating ever-increasing scientific and technological interest because they enable high-throughput, economical, on-site monitoring of such contaminants. Among rapid methods for first-level screening of food contaminants, lateral-flow immunoassay (LFIA), also named immunochromatographic assay or immune-gold colloid immunoassay, has recently attracted scientific and industrial interest because of its attractive property of enabling very rapid, one-step, in-situ analysis. This review focuses on new aspects of the development and optimization of lateral-flow devices for mycotoxin and phycotoxin detection, including strategies for management of matrix interference and, particularly, for investigation of the improvements achieved by signal-enhancing strategies or by application of non-gold nanoparticle signal reporters.

Keywords Rapid methods · Food analysis · Immunogold colloidal immunoassay

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Abbreviations

AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFM1	Aflatoxin M1
AFs	Aflatoxins
ASP	Amnesic shellfish poisoning
BSA	Bovine serum albumin
DA	Domoic acid
DON	Deoxynivalenol
DSP	Diarrhetic shellfish poisoning
FMB1	Fumonisin B1
FMB2	Fumonisin B2
FMs	Total fumonisins
GNP	Gold nanoparticle
LFD	Lateral-flow device
LFIA	Lateral-flow immunoassay
LOD	Limit of detection
MC	Microcystin
OA	Okadaic acid
OTA	Ochratoxin A
PbTx	Brevitoxin
PEG	Poly(ethylene glycol)
PSP	Paralytic shellfish poisoning
PVA	Poly(vinyl alcohol)
PVP	Poly(vinyl pyrrolidone)
STX	Saxitoxin
T2	T-2 toxin
ZEA	Zearalenone

Introduction

Mycotoxins and phycotoxins are natural metabolites, produced by fungi and microalgae, respectively, which can

affect countless varieties of food. The most common mycotoxins are produced by fungi of the genera Aspergillus (aflatoxins, ochratoxins, patulin), Fusarium (fumonisins, trichothecenes, zearalenone), and Penicillium (ochratoxins, patulin). A non-exhaustive list of commodities potentially affected by these fungi includes cereals (especially corn, wheat, rice), nuts, peanuts, spices, coffee, tea, apples, grapes, cottonseed, and soybeans. Crops can be infected before, during, and after harvest. Because of the relative stability of fungal toxins to thermal and chemical stress, they are found on commodities despite elimination of the mould, after long periods of storage, and also after transformation of raw materials; therefore, the presence of mycotoxin contaminations has been detected in commodities such as composite feed, flour, bakery products, roasted coffee, roasted peanuts, raisins, beer, wine, and apple juice. Moreover, products of animal metabolism of mycotoxins can retain toxicity; for example, AFB1 is metabolized into AFM1 which can then occur in milk. Meat, milk, and derived products are, consequently, also implicated in the spread of toxins. The chronic toxicity of mycotoxins covers a wide range of adverse effects, for example carcinogenicity (AFB1, AFM1), genotoxicity (T2), mutagenicity (OTA), teratogenicity (DON), immune-suppression and/or toxicity (OTA, DON, T2), nephrotoxicity (FMs), hepatotoxicity (OTA, FMs), and endocrine disruption (ZEA). Acute toxicity has also been demonstrated for patulin (gastrointestinal lesions), DON (vomiting, feed refusal), and T2 (vomiting, diarrhoea, haemorrhage). Regulations defining maximum admissible levels for major mycotoxins in numerous commodities exist throughout the world; these vary in the μg-to-mg kg^{-1} range, except for limits imposed by the European Union for AFM1 in milk which are in the ng kg^{-1} range [1].

Phycotoxins, more properly called "marine and freshwater toxins", belong to many different groups of small or medium-sized compounds (ca 300–3000 Da). Besides the chemical variability of the classes, each class group includes several compounds characterized by similar structures, which are either produced by algae or are secondary products of the primary algal toxin produced during metabolism by fish and shellfish. The algae responsible for phycotoxin production and excretion belong to the cyanobacterium (STX, MCs, nodularins) and dinoflagellate (STX, OA, PbTx, DA) groups. The risk posed by these contaminants is increased by accumulation and concentration of excreted toxins by different aquatic organisms, including feed-filtering bivalves (mussels, clams, oysters), shellfish, and herbivorous fish; these subsequently enter the food chain and cause toxicosis in consumers, for example predatory fish, marine mammals, birds, and humans. Human poisoning as a result of ingestion of seafood contaminated by phycotoxins has frequently occurred; each group of compounds has a distinct poisoning action. STX is one of the best known neurotoxins (in fact it is also listed as a chemical weapon) and is responsible for paralytic shellfish poisoning (PSP); adverse neurological effects, collectively known as neurotoxic shellfish poisoning (NSP), are also caused by PbTxs. The adverse effects of OA affect the gastrointestinal tract (diarrhetic shellfish poisoning, DSP) and intake of DA can lead to neurological symptoms known as amnesic shellfish poisoning (ASP). Given the strong acute toxicity of phycotoxins, chronic diseases have not been yet demonstrated. As is true for mycotoxins, cooking and transformation of food are inadequate for destroying phycotoxins, so maximum admissible levels for these contaminants in seafood and water bodies have been set to prevent severe risk to human health associated with consumption of contaminated seafood [2, 3].

In addition to safety issues, natural toxin contamination of food is of great economic concern, so much effort is devoted to the development of rapid, inexpensive, and simple screening methods, and to optimization of accurate, sensitive, multi-residue instrumental methods. Several validated sampling and analytical methods are available for measurement of mycotoxins [4–8]; the development of analytical methods for phycotoxins is a more recent objective. However, screening and instrumental methods for their assessment in water and seafood have been published and recently reviewed [9–11]. However, affordable monitoring of mycotoxins and phycotoxins to ensure food safety requires high-throughput, and economical methods of detection. In addition to these priorities, little or no sample treatment, user-friendliness, use of non-hazardous chemicals, and in-situ applicability would be welcome attributes. With regard to mycotoxin analysis, additional requirements include low detection limits (especially for aflatoxins and ochratoxin A) and adaptability to very different commodities. Conversely, low detection limits are not of great concern in phycotoxin analysis, the principal requirement of which is class-selectivity rather than compound-selectivity, i.e. analytical methods should be capable of measuring numerous structurally different compounds at the same time.

Among rapid methods for first-level screening of food contaminants lateral-flow immunoassay (LFIA) (also named immunochromatographic assay or immune-gold colloid immunoassay, IGC) has recently generated scientific and industrial interest, because of the attractive property of enabling very rapid, one-step, in-situ analysis. In the early 2000s, scientific papers and commercial devices directed at the measurement of natural toxins in food and feed started to appear (a list of commercial LFDs for mycotoxin detection validated by the USDA-GIPSA is given in Ref. [12]), and, in the last few years, the literature on this subject has included comprehensive and critical reviews [13, 14]. Although new applications appear daily in the literature, little

innovation and few real breakthroughs in materials, procedures, or signalling have been described and discussed. The research is still application-driven; it is also driven by the demand for rapid devices, so the strategy applied is exploitation of well-established practices, and effort has been focussed on the development of good antibodies. This review will focus on new perspectives and alternative routes that could be investigated in:

1. the development and optimization of lateral-flow devices (LFDs), including discussion of established procedures for preparing components of the LFD;
2. the management of matrix interference caused by food components; and
3. the investigation of alternative signalling by use of signal-enhancing strategies or by application of non-gold nanoparticle reporters.

Competitive lateral-flow immunoassays for mycotoxins and phycotoxins

Because mycotoxins and phycotoxins are low-molecular-mass compounds, immunoassays in a competitive format should be used to measure them. The same principles and reagents as in microwell-type immunoassays could be applied, except that separation of bound and unbound antibody sites is achieved by means of lateral flow on a suitable support (the membrane). A liquid flow transports immunoreagents along the membrane where they encounter their counterparts and immunoreactions occur in a spatially confined zone of the membrane itself. With few exceptions, the indirect competitive format (in which the antigen is coated on the membrane and the antibody is labelled, Fig. 1) is strongly preferred to the direct format (in which the antigen is labelled and the antibody is coated on the membrane, Fig. 2), although no experimental data support the first approach over the second. On the contrary, when the two formats have been compared, sometimes the direct format was preferred, sometimes the indirect format [15, 16]. The principles of the indirect competitive immunochromatographic assay have been widely described and are shown schematically in Fig. 1. Briefly, a labelled specific antibody is suspended in a liquid sample and flows through the membrane where it first encounters the coated antigen (test line, T-line). In the absence of the target compound in the sample (negative sample, Fig. 1a), labelled antibodies bind to the coated antigen and are focused on the T-line, so that a visible (detectable) line is formed. When the target is present in the sample above the lower detectable concentration (positive sample, Fig. 1b), labelled antibody sites are saturated and cannot bind to the coated antigen, resulting in a non-visible (undetectable) T-line. Usually, a second control line (C-line) follows and is constituted by secondary anti-species antibodies which capture any excess of specific antibodies. The appearance of a C-line can be regarded simply as confirmation of the correct development of the assay (integrity of reagents and materials) or can be exploited to calculate the T/C signal ratio with the objective of normalizing strip-to-strip variations [17]. It can also be

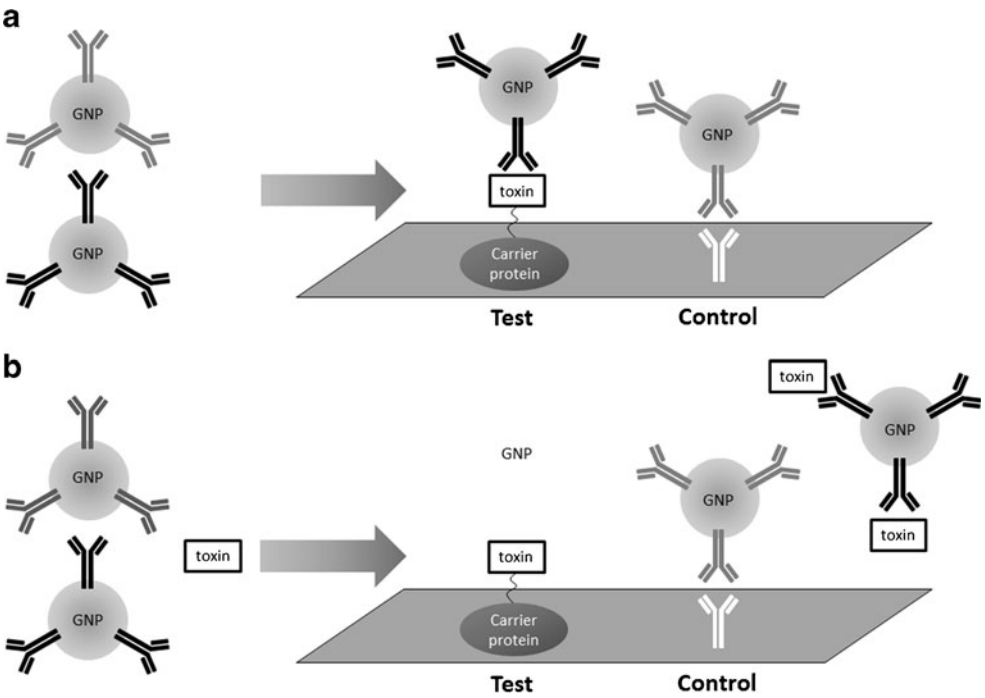

Fig. 1 Schematic diagram of the indirect format of a competitive lateral-flow immunoassay. The test zone is formed by adsorbing a conjugate of the target compound (*toxin*). The control zone is formed by anti-species antibodies (*white*), reporters are specific (anti-toxin antibodies, *black*), and non-specific antibodies (*grey*) labelled with GNP. Focussing of GNP-labelled antibodies and colour appearance on both the test and control lines occurs in the absence of the target compound (**a**), whereas only the control line appears when the target compound is present, because saturation of specific antibodies prevents their binding in the test zone (**b**)

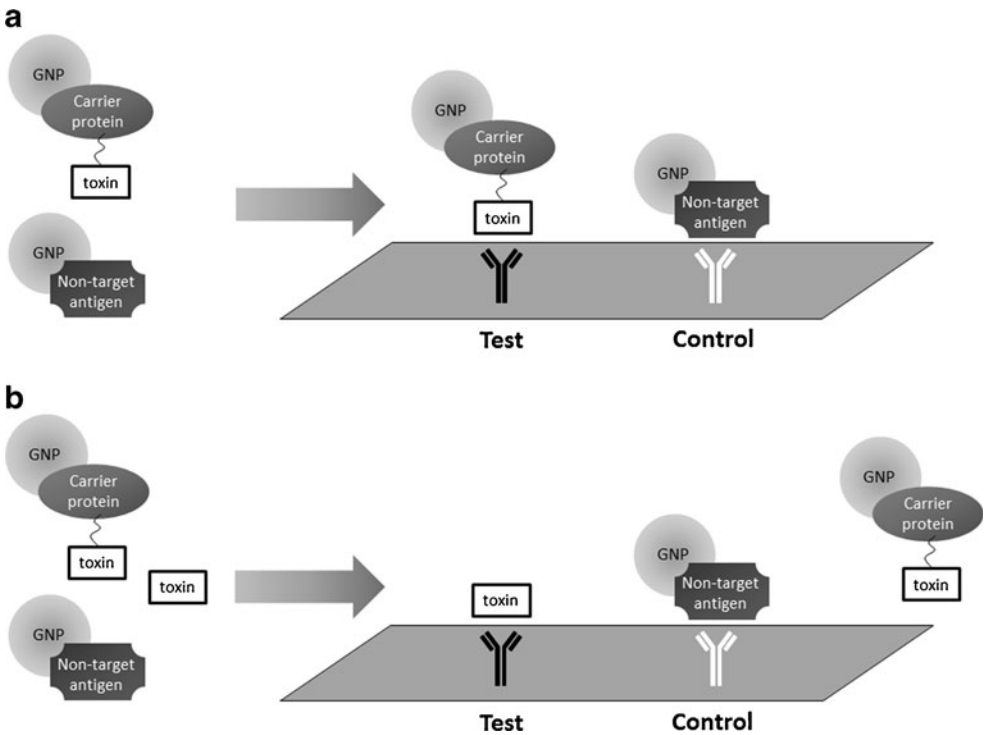

Fig. 2 Schematic diagram of the direct format of a competitive lateral-flow immunoassay. The test zone is formed by adsorbing the antibody specific (*black*) for the target compound (*toxin*). The control zone is formed by a second ligand, for example an antibody directed toward a non-target antigen (*white*) or streptavidin. Part of the GNPs are functionalized with a conjugate of the target compound, the other part with the partner of the second ligand (e.g. a non-target antigen or biotin). The biotin–streptavidin pair ensures colouring of the control zone irrespective of what happens in the *Test* zone. In the absence of the target compound, GNPs functionalized with the target conjugate are captured and colour appears in the test zone (**a**). The target compound, if present, competes with functionalized GNPs for binding in the test zone and thus inhibits colouring of the line (**b**)

regarded as an internal standard to which the intensity of the T-line is compared to determine positivity and/or negativity [18, 19].

Materials

In addition to the porous membrane (almost exclusively nitrocellulose) which ensures lateral flow, LFDs usually include an absorbent pad positioned at the top of the membrane to increase the volume of the flowing liquid, a sample pad to ensure contact between the liquid sample and the membrane, and a rigid backing. The simplest LFD is a dipstick, which is dipped directly into the sample solution. Labelled antibodies can be added to the sample as a concentrated suspension or provided in a lyophilized form to be re-suspended by the sample itself. Alternatively, the labelled antibody can be pre-adsorbed on a releasing pad (usually a glass fibre pad, though more materials are available commercially) which partially overlaps the membrane. The liquid sample flow itself causes re-suspension of the adsorbed labelled antibodies when the assay is performed. A further sample pad, usually made of cellulose and sometimes soaked with proteins and/or surfactants, may be added with the objective of reducing matrix interference in such a way that it overlaps the membrane or the releasing pad [16, 20]. Besides the most popular dipstick format, some authors have described LFDs in which the strip is inserted into a rigid plastic cassette provided with a sample well and a reading window. The main advantage of these housings is the guarantee of reproducible compression of all components in the overlapping zones, which ensures faster and more reproducible flows.

Types and quality of materials are generally regarded as well established, and optimization in this field is limited to variation of the porosity of the nitrocellulose membrane to modify the flow rate [21–23], although when membranes from different suppliers were compared significantly better performance was obtained by use of AE99 and Prima 40 (Whatman) rather than Immunopore FP, Sartorius CN140, and Hi-flow135 (Millipore) membranes [24]. Furthermore, membrane pore size is not the only. nor the predominant. factor affecting flow rates, especially in analysis of food samples. Many contrasting effects affect capillary flow, including but not limited to:

- the viscosity of the liquid;
- the volume of the sample;
- the length and type of adsorbent and sample pads;

- the presence of micro-dispersed insoluble matter in the liquid;
- the amount and nature of organic solvents; and
- addition of surfactants.

Strong flow-rate conditioning is required as a result of membrane treatment conducted by some authors with the intention of saturating nitrocellulose binding of proteins after line deposition. Membrane saturation has been demonstrated to be particularly effective in limiting matrix interference in the analysis of mycotoxins in cereals [17, 20–22, 25–29], and has also been reported to improve the determination of brevitoxins in molluscs [30]. For this purpose, the nitrocellulose membrane is soaked with buffers which contain proteins (mostly BSA [17, 20–22, 25, 26, 30], but also casein [23], ovalbumin [27] and skimmed milk) and one of a variety of blocking agents (PVA, PVP, dextran, PEG [23, 24]). Membrane saturation strongly affects the capillary flow, which becomes substantially independent of pore size and hardly compatible with acceptable run times. The joint use of surfactants as flow modifiers is mandatory, especially with casein, to aid liquid flow. SDS has been reported to serve this purpose by Xu et al. [30]. Nevertheless, Tween 20 in significant amounts (>0.1 %) is frequently preferred and can be added to the membrane-blocking solution directly, or to the solutions used for subsequent washing of the membrane itself, or to the labelled antibody solution, or, last, to the sample. By use of such flow modifiers run times are reduced to 10–15 min, which is the upper limit for methods regarded as a truly rapid. Remarkable exceptions are some LFIAs for phycotoxins which require more than 30 min to enable definitive judgment of results [18, 19, 31, 32]; this is substantial, indeed excessive time for a so-called "rapid analysis".

As previously observed, with a few noticeable exceptions which will be discussed below, colloidal gold is the signal reporter of choice for labelling antibodies. Gold nanoparticles (GNPs) of approximately 40 nm mean diameter have good properties in terms of handling during conjugation to antibodies, stability, and, above all, line detectability. In addition, the availability of commercial GNP suspensions aside, preparation of GNPs of predictable dimensions by the methods of Frens and Turkevich [33] is relatively economical, easy, and rapid. Conjugation of GNPs with antibodies also follows established procedures, including:

1. determination of the saturation quantity of antibodies, according to Horisberg and Rosset [34];
2. incubation of antibodies and GNPs under mild alkaline conditions for a time which varies from a few tens of minutes to some hours;
3. overcoating of the potentially free GNP surface with excess BSA; and
4. washing of unbound antibodies by repeated centrifugation and re-suspension of the pellet.

Finally, GNP-labelled antibodies (GNP-Ab) are typically re-suspended in buffered solutions supplemented with high concentrations of proteins and sucrose for long-term storage. Occasionally, one of the listed steps is subject to verification and optimization, as, for example, in the work of Xiulan et al. [25] and Tippkotter et al. [35], in which the optimum pH for the GNP-Ab conjugation was observed to depend on antibody p*I*. Tippkotter et al. also studied the course of the gold–antibody conjugation reaction and found that 30–40 min incubation at room temperature is sufficient to completely saturate the GNP surface when working with an excess of antibodies and at pH>p*I*.

Development and optimization

Development of an LFD implies identification of balanced conditions between the amounts of the following reagents:

- the coated antigen of the T-line;
- the antibody conjugate to GNPs; and, less importantly
- the secondary antibodies of the C-line.

Checkerboard titrations are conducted to achieve the best sensitivity and good detectability of signals, similarly to the procedure used for microwell-based immunoassays. Guidelines for selecting the best conditions, which would specially apply to visual devices, were recently stressed in a critical review by Krska and Molinelli; they can be summarised as:

- the presence of an intense and reproducible C-line;
- the complete disappearance of the T-line at and above the designated cut-off level; and
- the absence of background colouring of the membrane [13].

The recent tendency to evaluate results semi-quantitatively by means of an instrumental reader enables these criteria to be less stringent—background subtraction and normalization of colour intensity are sufficient. Moreover, in place of judging the cut-off level with the naked eye, definition of a detection limit in a manner similar to that for microwell-based immunoassays is feasible. The major advantage is improved detectability, mostly when the slope of the inhibition curve is limited. Reduction of the uncertainty in attribution of samples near the cut-off level and of the number of false positives and/or false negatives could, hopefully, be also achieved. The requirement of a reproducible C-line has been addressed by some authors by introducing a second pair of affinity reagents, completely independent of the antibody–antigen interaction which occurs in the test zone. Kim et al, for example, coated streptavidin to form the C-line and mixed a labelled biotin with the immunoreagents in such a way that the intensity of

the C-line was completely unaffected by the immunoreaction and, therefore, by the presence and amount of the target compound in the sample [16].

Surprisingly, some factors which are recognized as crucial for determining sensitivity in competitive microwell-based immunoassays are rarely evaluated when developing LFIAs. One example is the nature of the coated antigen (number of antigen moieties per molecule of carrier protein and use of heterologous antigen). In this regard, Xu et al. compared the performances of three protein conjugates of DON as the coated antigen of the T-line and demonstrated that use of a cationised BSA as the carrier protein improved sensitivity, probably because of different reactivity toward the derivatised DON used to synthesize the antigen [24]. Liu et al. showed better sensitivity for an LFD in which an ovalbumin instead of a polylysine conjugate of OTA was used to form the T-line [36]. A slight sensitivity improvement in the detection of microcystins and nodularins was also reported by Kreich et al. when a heterologous conjugate rather than the immunogen itself was used to form the T-line [37]. In contrast, Kolosova et al. observed better performance when the T-line was constituted by a homologous rather than heterologous conjugate compared with the immunogen [29].

Likewise, studies aimed at demonstrating the convenience of the practice of saturating GNPs with the specific antibody rather than working with a limited specific antibody concentration are still lacking. The work of Laycock et al., although unfortunately not very detailed, suggests that reducing the amounts of antibodies in GNP–antibody preparations would strongly positively affect the sensitivity of the resulting LFDs [18].

Papers dealing with additional critical points in the optimization of LFDs (which have recently been emphasized in some critical reviews [14, 38, 39]) have also been published, for example:

1. addressing problems of quality control and achieving correct attribution of positivity and/or negativity;
2. evaluating cross-reactivity with other toxins; and
3. extending applicability by counteracting matrix interference.

The last, being of major concern, is discussed separately. For validation and quality-control purposes, the accuracy of commercial LFDs for detecting STX and DA, meaning the ability to correctly identify positive and negative samples, was investigated, together with the agreement of results from several operators [40, 41]. As expected, the number of incorrect attributions depended on the level of sample contamination. In particular, no false negatives (n=77) were observed when STX contamination was higher than twice the cut-off level, whereas 3 % of false negatives (n=135) was recorded when STX concentrations were between the cut-off and twice the cut-off level. A value as high as 18 % (n=335) of false positives was also assessed. Interestingly, whereas judgment of the colour intensity of the T-line only by use of the naked eye resulted in some discrepancy, strong agreement among the eight participants in the trials was achieved in interpretation of results, because of the use of the C-line intensity as a colour reference. Results on the DA system reflect the same qualitative behaviour. Definition of a cut-off level is intrinsically a source of uncertainty, as first pointed out by Kolosova et al., who proposed the definition of an indicator range of analyte concentrations within which the colour of the T-line gradually faded, rather than a cut-off level [23]. Most frequently the positivity is assigned to samples that cause complete disappearance of the T-line at the expense of detectability. Reduction of uncertainty and misinterpretation of signals in qualitative LFDs has been pursued by some authors by provision of some kind of colour reference. Tsao et al. proposed use of a control strip to judge the result of measurement of DA in mussel extract by use of a qualitative dipstick [42]. Laycock et al. assumed the colour of the C-line as the reference intensity for a negative sample and defined the T-line colour as being half the C-line colour or weaker as the indication of positivity [31]. The same approach was used by Komano et al. in evaluation of a commercial LFD for measuring PSP toxins [19]. The availability of portable readers which enable objective measurement and numerical comparison of line colours must be regarded in this context not only as a step towards (semi-)quantitative measurements, but primarily as achievement of simpler positive–negative discrimination and greater sensitivity [20, 35, 43].

Cross-reactivity toward other mycotoxins in comparison with reactivity to the target compound has also started to be evaluated. Wang et al. showed no interference of DON, ZEA, and FMB1 in the determination of OTA [28]; Shim et al. reported no interference of OTA, ZEA, citrinine, patulin, and T2 in the determination of aflatoxins [44]; and Molinelli et al. observed negligible cross-reactivity of ZEA and several trichothecenes in measurement of FMs [22]. Moreover, reported development of multi-residue LFDs also contributes to regarding interference from other toxins as, presumably, no problem. The same does not apply to the cross-reactivity towards toxin derivatives which results from reaction of the target toxin with matrix components (proteins, starch, …). The recognition of such derivatives by antibodies directed towards the toxin has been demonstrated to enable detection of hidden mycotoxins in an ELISA for measuring Fms, and has been suggested as a fascinating explanation of the general over-estimation of immunoassay techniques compared with chromatographic methods for quantification of mycotoxins [45]. The capability of LFIA of measuring masked mycotoxins deserves more investigation and could also account for

deviations when LFD quantification is compared with HPLC results.

On the other hand, cross-reactivity studies in the development of LFIAs for phycotoxins deserves a separate discussion. Assays in this field should be oriented toward determination of a class of related compounds rather than of a specific target, because the relevant information is the potential toxicity of the sample and this is associated with numerous compounds, although strongly variable for the individual compounds. For instance, Laycock et al. revealed that a commercial LFD developed for measurement of STX had broad selectivity which enabled detection of some mildly toxic STX parent compounds whereas highly toxic members of the PSP family were poorly detected [18]. This aspect is partially counterbalanced by the fact that tolerable limits of phycotoxins in waters and food are rarely a constraint compared with typical sensitivities of LFIAs, which enable the detection of low cross-reacting compounds. Excessive sensitivity to low-toxicity parent compounds accounted for the high incidence of false-positive results observed for some LFDs which measure phycotoxins [40].

In addition to the aforementioned concerns, two other aspects should attract more attention in the development of lateral flow-based immunoassays—stability (for the separate components and for the complete ready-to-use device) and the ruggedness of the methods of analysis. Ruggedness of LFDs and of the analytical methods should be evaluated primarily because the devices are claimed to serve as point-of-use tests (without environmental constraints, for example, temperature and humidity) and are intended to be used by untrained personnel [46]. The effect of environmental variations (in particular ambient temperature) was studied during the optimization of LFDs for FMs, AFs, and OTA, and reproducible results were obtained for temperatures varying from 22 to 30 °C [17, 26, 27]. As part of ruggedness evaluation, authors have emphasized the great inconsistency of results obtained as a consequence of small variations in the sample matrix, even, simply, the grain size of cereal samples [22] or changing from drinking water to river water [16]. When evaluated, the stability achieved turned out to be limited. Storage of GNP–antibody conjugates (with glycerol added) at pH 9 has been monitored by Tipptokker et al. at 4 and −18 °C; stability was maintained for 120 days [35]. Nevertheless, dipsticks for MC developed by the same authors were shown to be stable at room temperature for no more than 20 days. Similar short stability at room temperature was verified by Shim et al. for dipstick-format strips intended for measurement of OTA and ZEA in corn [44]. Wang et al. also reported one-month stability (at 4 °C) of their devices for measuring OTA by use of an aptamer–quantum dot approach [47]. Only Molinelli et al. reported longer storage stability (four months at room temperature for their LFD for T2 toxin [21] and one year at 4 °C for the components of a device intended for detection of FMs [22]). In these cases, however, the signal reporter (GNP-antibody) was stored apart from the strip and as a solution rather than in lyophilized form or adsorbed on a releasing pad.

Application of LFDs in food analysis

The major concern in the development of LFDs for phycotoxins, and primarily for mycotoxins, is unpredictable effects because of food components co-extracted from the sample beyond the target and which affect not only the antigen–antibody interaction on which the immunoassay is based but also the mechanics of the device itself. From this general observation the difficulty arises in defining appropriate standards for calibration and the fact that individual foods require distinct devices to be developed for them (which means not only that a different calibration of the same device is required, but even that several devices, each characterized by its own materials and/or treatments, ought to be devoted to different food materials). In addition, some authors experienced the apparently inexplicable failure of recovery experiments conducted on fortified materials and the incongruity of results obtained for artificially and naturally contaminated samples, which makes the definition of calibrators disappointingly arduous. Therefore, the group of Molinelli pointed out the need for matrix-matched calibrations [21, 22], as also experienced by our group [17, 26, 27] and recommended the use of naturally contaminated samples, blended in different proportions with blank samples, as calibrators to overcome both matrix interference and non-matching between fortified and naturally contaminated materials. Similar dependence of results on sample characteristics has also been observed in the application of LFDs to the analysis of MCs in water [16, 35] and STXs in shellfish and phytoplankton; matrix-matched calibrators in the form of fortified samples were, therefore, used in this work also [18].

Nevertheless, it should be noted that most authors reported calibration of newly developed LFDs by use of standard toxins diluted with buffers (to which methanol is often added in different proportions). The observation is valid for both mycotoxin and phycotoxin determination and, with the above discussed exceptions, interference from matrix components is generally regarded as insignificant, given limited dilution (1:2–1:10) of sample extracts. In part, the same LFD materials help to abate interferences (by filtering particulate matter and adsorbing various co-extracted components); second, counteracting strategies can be implemented by pre-soaking pads with suitable matrix modifiers (buffer salts, surfactants, proteins, …). The ultimate objective is minimization and simplification of sample processing to render LFDs suitable for effective on-site usage. Thus, typically, liquid samples are directly

analysed (or simply diluted before analysis), whereas solid samples are extracted with aqueous methanol. Several papers agree that methanol in proportions below 30–35 % does not affect assay performance; established procedures for extracting target toxins from food, which typically involve use of methanol–water mixtures, can, therefore, be safely used. Appropriate dilution of methanolic extracts suffices to achieve conditions suitable for direct submission of diluted extracts for analysis (see, for example, Refs, [42] and [29] for phycotoxins and mycotoxins, respectively).

Mycotoxins

Rapid and affordable analytical methods for monitoring major mycotoxins in food and feed at virtually every stage of production, an preferably at the place of production or processing, are being actively pursued. The non-uniform distribution of such contaminants in commodities should further prompt an increase of the number of controlled samples, given acceptable cost and time investment. Therefore, lateral flow technology has been promptly exploited to develop disposable devices for qualitative assessment of the presence of mycotoxins in a variety of commodities. Indeed, industrial rather than scientific research on this topic came first and remains the major force behind new and forthcoming developments.

An extensive list of papers reporting devices for measurement of mycotoxins in food and feed is given in Table 1 (validated LFIA methods) and Table 2. The prominence of AFs in monitoring of mycotoxins is also apparent from the prevalence of LFDs developed to detect these contaminants [20, 25, 44, 48–50]. Major aflatoxins have been detected in grain and feed, typically after methanol–water extraction followed by dilution to reduce the proportion of the organic solvent. Visual devices have been developed which enable aflatoxin detection at levels complying with the legislation in force. An aqueous extracting medium associated with an LFD with instrumental detection has also been proved to enable total aflatoxin quantification in maize samples [26].

Very recently, Wang et al. first described an LFD for detection of AFM1 [51]. The cut-off level (0.5–1 μg L^{-1}) is just above the value specified by US legislation [52] and far beyond the more severe limits imposed by the European Union for this contaminant [1]. However, it is a thoroughly sensitive and rapid assay, and the whole analytical procedure can be completed in 10 min, because no sample treatment is required. Validation of a commercial device for quantitative measurement of AFM1 in milk has also been described [53]. As the result of an interlaboratory trial which involved 21 participants, the ROSA Charm Aflatoxin M1 was verified at four levels above and two below the declared LOD (0.4 μg L^{-1}). Fewer than 5 % of false negative results (n=83) and no false positive results below 300 ng L^{-1} were obtained. For contamination between 350 and 450 ng L^{-1} false positivity increased from 21 to 93 %. Some LFDs based on non-GNP reporters have also been proposed; these use aflatoxins as system models (vide infra) [54–56].

Besides aflatoxins, rapid detection of OTA has attracted much attention because of its toxicity and the widespread presence of this contaminant in different types of food [57]. The applicability of LFDs exploiting GNP reporters has been demonstrated for a variety of cereals [27, 28, 58, 59], beverages [28], and coffee [28, 60]. Moreover, instrumental recording enabled high sensitivity to be achieved by use of the device developed by Urusov et al. [43] and in a quantitative assay developed by our group (LOD 1.5 μg kg^{-1} in maize and wheat) [27].

A fluorescent dye was used as the label in the work of Wang et al. [47], who also replaced use of a specific

Table 1 Overview of the literature on validated lateral-flow immunoassays for measurement of mycotoxins

Target	Detection	Signal reporter	Cut-off/LOD	Commodities	Ref.
AFB_1	Visual	GNP	5 μg kg^{-1}	Pig feed	48
AFs	Instrumental	GNP	1 μg kg^{-1}	Maize	26
AFM_1	Instrumental	GNP	0.4 μg L^{-1}	Milk	53
AFM_1	Visual	GNP	0.5–1 μg L^{-1}	Milk	51
AFB_1, OTA	Visual	GNP	AFB_1 10 μg kg^{-1}, OTA 50 μg kg^{-1}	Feed	62
DON	Visual	GNP	250 μg kg^{-1}	Wheat, pig feed	23
DON, ZEA	Visual	GNP	DON 1500 μg kg^{-1}, ZEA 100 μg kg^{-1}	Wheat	29
FMBs	Instrumental	GNP	200 μg kg^{-1}	Maize	22
FMB_1	Instrumental	GNP	120 μg kg^{-1}	Maize	17
FMB_1	Instrumental	Peroxidase	25 μg kg^{-1}	Maize	73
OTA	Instrumental	GNP	1.5 μg kg^{-1}	Maize, wheat	27
OTA, ZEA	Visual	GNP	OTA 5 μg kg^{-1}, ZEA 10 μg kg^{-1}	Corn	63
T-2	Visual	GNP	100 μg kg^{-1}	Wheat, oats	21

Table 2 Overview of the literature concerning LFIA developed for measuring mycotoxins. Cut-off and/or limit of detection are provided as evaluated in buffered solutions. Applicability in food samples, if preliminary evaluated, is highlighted

Target	Detection	Signal reporter	Cut-off / LOD	Commodities	Ref
AFB_1	instrumental	dye-encapsulating liposomes	18 ng	-	54
AFB_1	visual	GNP	2.5 μg L^{-1}	-	25
AFB_1	visual / instrumental	GNP	2.5 μg L^{-1} / 0.1 μg L^{-1}	rice, corn, wheat flour	20
AFB_1	visual	GNP	0.5 μg L^{-1}	grain, feed	44
AFB_1	visual	GNP	0.5 μg L^{-1}	rice, barley, feed	50
AFB_2	visual	magnetic nanogold microspheres	0.9 μg kg^{-1}	peanuts, hazelnuts, pistachio, almonds	55
AFB_1	visual	silver-gold nanoparticles	0.1 μg L^{-1}	cereals, nuts	56
AFB_1	visual	GNP	1 μg L^{-1} (fortified sample extract)	peanuts, oil, feedstuffs	49
DON	visual	GNP	50 μg L^{-1}	wheat, maize	24
FMB_1	visual	GNP	1 μg L^{-1}	corn, barley, peanuts, oats, rice, sorghum	64
FMB_1	visual	GNP	1-5 μg L^{-1}	maize	65
OTA	visual	GNP	500 μg L^{-1}	-	58
OTA	visual	GNP	1 μg L^{-1}	cereals, raisins, beer, coffee	28
OTA	visual	GNP	5-10 μg L^{-1}	coffee	60
OTA	visual	GNP	10 μg L^{-1}	cereals, soybean	59
OTA	visual / instrumental	Fluorescent QDs	5 μg L^{-1} / 2 μg L^{-1}	red wines	47
OTA	visual / instrumental	GNP	50 μg L^{-1} / 5 μg L^{-1}	maize, barley	43
ZEA	visual	GNP	2.5 μg L^{-1}	corn	63

antibody with that of an aptamer capable of selective recognition of the target toxin. The feasibility of using the developed test to assess OTA contamination of red wines was established. An interesting approach has been proposed by Lai et al., who profited from the fact that a peptide mimicking OTA had been previously described [61]. LF strips prepared by spraying the mimotope peptide or an OTA–BSA conjugate on to the membrane performed similarly in terms of detection limit (10 ng mL^{-1} with visual evaluation), reproducibility, and agreement with a classic microwell-based immunoassay [59]. As mimotope peptides exist for other mycotoxins (Ref. [61] and references cited therein) this approach could be extended with the advantage of avoiding handling of large amounts of the toxic compounds typically involved in the synthesis of toxin conjugates. OTA has also been determined in association with other mycotoxins (AFB1 [62] and ZEA [63]) in multi-analyte devices.

LFDs for detection of FMs were among the first to be described [64], and a variety of concerns in their development have been accurately discussed by Molinelli et al. [22]. Recent papers have reported an improved sensitivity by exploiting LFDs with both visual [65] and instrumental [17] detection.

Some examples of lateral-flow immunoassays for detection of the most relevant tricothecenes (DON in Refs. [23] and [24]; T-2 in Ref. [21]) and one applying to detection of ZEA [66] can also be found in the literature.

Phycotoxins

Lateral-flow immunoassays for principal phycotoxins started to be published from 2003 when Kim et al. first reported a quantitative assay for measuring MC in water exploiting a fluorescent reporter [16]. Subsequent work by Pyo et al. [67] and Kreich et al. [37] also investigated the use of fluorescent labels, sulforhodamine B encapsulated in liposome and quantum dots, respectively, for development of LFDs for MCs. In contrast, Tipptokker et al. exploited a more classical GNP reporter for the same purpose. In this work, the authors widely studied the interaction between antibodies and gold nanoparticles to determine the effect of time, antibody concentration, and pH on achievement of optimum GNP stabilization [35]. The optimized LFD had a visual cut-off of 5 μg L^{-1} in water, and measurement of line intensities by means of a CCD camera enabled fivefold improvement in sensitivity. Responses of the LFD differed depending on which matrix was tested (buffered solution, drinking water, salt water), although, surprisingly, the cut-off level remained the same. Moreover, interpretation of the results could be rendered independent of matrix interference by using the C-line signal as a reference, because the matrix affected both lines equally. Use of the LFD to measure MCs in mussel extracts, after a simple filtration of sample extracts, was also demonstrated.

Only one LFD for the detection of brevitoxin has been described so far [30]. This is a qualitative assay, which used GNPs as the signal reporter, and was applied to toxin detection in molluscs. The visual cut-off was set at 10 ng mL^{-1} in buffered solutions and at 20 ng mL^{-1} in food extracts. To extract PbTx, molluscs were whisked in a DMSO–water mixture, which was then centrifuged and filtered before being placed in contact with the strips. The chromatographic run took 10 min. The authors also evaluated strip stability (six weeks at 4 °C) and cross-reactivity towards other marine toxins. The system had good recognition properties toward most NSP and no interference from phycotoxins belonging to different classes (MCs, DSP, and ASP). Moreover, LFD performance agreed well with results obtained by use of a parallel ELISA method.

Commercial systems for measurement of PSP, DSP, and ASP have been available since the early 2000s, and have been the objects of several evaluations [19, 31, 32, 40, 41]. The accuracy of the devices was assessed by comparison with instrumental methods of analysis; agreement between results provided by different operators was also assessed and enabled the authors to confirm the reliability of the assays. Comparisons with other screening methods were also performed. For example in the work of Laycock et al. [18] the toxicity predicted by use of the LFD was compared with that predicted by use of the most frequently used screening method, which is a bioassay. In fact, measurement of toxin concentration can be regarded as less informative than immediate measurement of toxicity, though the two are related, when mixtures of different toxins are considered. Conversely, the same is not true when a single toxin is determined, because it could not be representative of the overall toxicity of the sample; broad selectivity is therefore indispensable for LFDs developed in this field. The authors screened more than 3000 samples from different countries during a five-year survey and emphasized the close agreement between the two methods, thus demonstrating the value of the LFD as a screening tool for extensive monitoring of PSP.

In addition to validation and comparative studies, production of a monoclonal antibody for DA and its exploitation in the development of a qualitative lateral-flow immunoassay for detecting ASP toxins in mussels was described by Tsao et al. [42]. The optimized dipstick was prepared by pipetting an OVA conjugate of the target toxin as a T-spot and using the selected mAb (GNP-labelled) as the reporter. After a 10-min development, the strip was visually evaluated by the naked eye, providing an indicator range between 1 and 5 ng mL^{-1}, which is well below the statutory maximum admissible level for DA in the tissue of mussels. The assay was used to assess the presence of DA in mussel samples, which were extracted with aqueous methanol followed by centrifugation and dilution of the extracts with phosphate buffer to eliminate interference from the organic solvent in the assay.

STX, besides being a marine contaminant of concern for food safety, is also listed as a chemical weapon; LFDs intended for its detection could, therefore, also be used as disposable tools for field checks against terrorism (for example in airports, frontier checks, etc.). For this purpose, Komano et al. [19] investigated the effect of several potential interfering agents, for example white flour, alkali, acids, oxidants, and reducing reagents, in an assay for determining STX by use of a commercial LFD. The assay proved to be sufficiently robust, although some of tested agents (hydrochloric acid, formaldehyde, sodium hydroxide, wheat flour) invalidated the test and hypochlorite produced false negatives.

Available literature on the LFIA of phycotoxins is summarised in Table 3.

Advances toward multi-residue analysis and high sensitivity

Multi-analyte LFDs

One of the benefits of lateral flow technology is the easy implementation of multi-residue analysis. In theory, it suffices to add one or more T-line to an existing LFD and to mix the partner labelled antibodies to increase the number of analytes to be determined. This is true in so far as cross-reactivity between the target compounds to be simultaneously determined is negligible and samples could be treated in the same manner, as reported in the papers of Shim and co-workers [63, 66]. In their work, the authors established optimized conditions for simultaneous detection of OTA and ZEA, and of OTA and AFB1 in corn by means of two LFDs, which completely resembled individual assays and performed likewise. Comparison of the multi-analyte and single target devices developed by Kolosova's group and published separately [23, 29] also confirms that simultaneous detection of the two analytes could be achieved by transferring optimum conditions for each separate LFIA into a single device. As part of a project funded by the European Union, an LF device which enables the simultaneous detection of up to six Fusarium toxins (DON, ZEA, T-2/HT-2, and Fms) in cereals has been developed and fully validated [68]. Also in this case, the authors developed antibodies directed toward each separate toxin and merely mixed them after GNP-labelling. The strips are prepared by dispensing four test lines, each made by individual toxin conjugate, and a unique control line. Results are instrumentally evaluated and enable discrimination between positive and negative samples according to European legislation in force.

In 2007 Goryacheva et al. had already reported LFIAs as a means of achieving multi-detection by immunochemical methods [69]. Nevertheless, and despite encouragement of

Table 3 Overview of the literature concerning lateral-flow immunoassays developed for measuring phycotoxins

Target	detection	signal reporter	cut-off / LOD	commodities	ref
DA	visual	GNP	1-5 μg L^{-1} (buffer)	mussels	42
DA	visual	GNP	20,000 μg kg^{-1} (sample)	shellfish	41
MCs	instrumental	GNP	0.05 μg L^{-1} (buffer)	tap water, river water	16
MCs	visual /instrumental	GNP	5 μg L^{-1} / 1 μg L^{-1} (water sample)	drinking water, salt water, mussels	35
MCs	instrumental	Fluorescent liposomes	0.06 μg L^{-1} (buffer)	water bloom	37
MCs	instrumental	Fluorescent dye	0.05 μg L^{-1} (buffer)	water (from cell culture)	15
MCs	instrumental	Fluorescent dye	0.2 μg L^{-1} (buffer)	water (from cell culture)	70
MCs	instrumental	Fluorescent dye	0.2 μg L^{-1} (buffer)	-	67
OA	visual	GNP	80,000 μg kg^{-1} (sample)	mussels	31
OA	visual	GNP	160,000 μg kg^{-1} (sample)	bivalve molluscs	32
PbTxs	visual	GNP	10 μg L^{-1} (buffer)	molluscs	30
STXs	visual	GNP	400 μg kg^{-1} (sample)	shellfish, phytoplankton	[18]
STXs	visual / instrumental	GNP	12 μg L^{-1} (buffer)	-	[19]
STXs	visual	GNP	400 μg kg^{-1} (sample)	bivalve molluscs	[40]

multi-detection on the basis of economy and increased rapidity as criteria, and because it would, furthermore, serve to individuate the co-occurrence of mycotoxins in food, which is regarded as a major objective [63], little research has been conducted on this topic (as already pointed out in 2009 by Ngom et al. [14]). Just as before, over the last two years, very little effort has been made in this direction.

Signal enhancement and non-GNP reporters

Besides the standard approach of using GNPs as signal reporters in lateral-flow assays, several researchers have investigated the potential benefits of using different labels, for example fluorescent dyes, liposomes encapsulating visible or fluorescent dyes, quantum dots, magnetic nanoparticles, and silver–gold nanoparticles.

Kim et al. [16] used a fluorescent reporter to label both the antibody and the antigen; consequently two different assay formats (direct and indirect competitive immunoassay) were developed and compared. The approach in which the antigen was immobilized and antibodies were labelled (indirect competitive format) proved to be more sensitive and more highly reproducible in this case, hence it was applied to measurement of MC in tap water and river water. Calibration was achieved by serial dilution of the target in PBS and by plotting the signal of the T-line divided by the signal of the C-line (fluorescence measurement adjusted for the area of the line) against MC concentration. To maintain the C-line as constant as possible, streptavidin was sprayed on the membrane and some fluorescent-labelled biotin was added to the fluorescent-labelled antibody. If the membrane had been saturated with a solution containing BSA, Tween 20, and PVA, and the sample pad had been soaked with PBS, with BSA and Tween 20 added, no matrix interference was observed when measuring MC in tap water. Authors particularly emphasized the function of the surfactant, which strongly reduced non-specific binding of the fluorescent label to the membrane.

Fluorescent labelling has been used by Pyo's group to prepare an LFD for measuring MC also; the assay had an LOD of 200 pg mL^{-1} and was able to detect all major MCs [15, 67, 70]. The fluorescent LFD had lower detection limits than a parallel device in which GNPs were used as signal reporters. However, the observed sensitivity increase could also have been accounted for by the fact that the fluorescent LFD was provided with instrumental detection whereas results from GNP-based LFD were evaluated visually, which generally reduces detectability, as discussed above. Conversely, the fluorescent LFD is limited by the need for an instrumental reading.

High sensitivity was also achieved when liposomes encapsulating sulforhodamine B were used as reporters in the determination of microcystins and nodularins [37]. In this system, the analytical signal was considered as the average T-line and C-line fluorescence, determined instrumentally. To improve sensitivity, after strip development (15 min), the dipsticks were dried in an oven for 5 min to destroy liposomes and enable dye release, which reduced self-quenching. By this stratagem, a tenfold increase in sensitivity was obtained compared with an LFD with GNP reporters. Nevertheless, in this circumstance also, the GNP system was visually evaluated, thus sensitivity was surely underestimated. Use of dye-encapsulating liposomes was first proposed by Ho and Wauchope [54], who described a device in which liposomes were tagged and covalently linked to AFB1; an anti-AFB1 antibody was deposited on to the

membrane to form the test zone, whereas the control zone was missing. Negative samples determined the focusing of liposomes in the test zone, so that colour could be revealed and related to the amount of AFB1 in the sample. The absolute limit of detection of such a device was 18 ng AFB1 and the test could be completed in a total of 12 min, including sample preparation.

The fluorescence of quantum dots (QDs) was exploited by Wang et al. [47] to prepare QD-labelled aptamers used as the reporters in the development of an innovative LFD for measuring OTA. The T-line was made up of a DNA probe which is able to hybridize with the aptamer, and the C-line was composed of a DNA probe binding the 18-polyA tag on the 5′- aptamer end. When OTA was present in the sample, it inhibited binding of the labelled-aptamer to the T-line so that interpretation of results was as normal. The device proved to be suitable for measuring OTA in red wines at the ng mL^{-1} levels both by visual or instrumental reading.

The increment of the specific activity of the reporter (i.e. the intensity of colour/fluorescence per molecule of labelled antibody) should enable reduction of the number of antibodies themselves which should also mean a consequent increase of assay sensitivity. Sharp signal enhancement can be achieved when mixing use of gold nanoparticles with silver, as first revealed in the work of Liu et al. [71] on a nanoparticle-based immunoassay with ICP detection. In 2010, Liao and Li [56] first described a visual device which exploited the same principle. They prepared nanoparticles with a silver core and a gold shell which were used as the reporters in the construction of a LFD for AFB1. The toxin was determined in cereal and nut samples and performances were compared with those of a GNP-based LFIA and with those obtained through a classic microwell-based immunoassay. The authors demonstrated that the newly developed LFD was comparable with the GNP-LFD in terms of stability of components and reproducibility of signals. On the other hand, it enabled substantial enhancement of sensitivity, so values as low as 0.1 ng mL^{-1} AFB1 could be measured. More recently, Wei et al. [72] further confirmed the potential of the combined use of silver and gold in nanoparticles. Their application was for a proteic target (abrin-a), so the scheme of the assay was quite different. Nevertheless, the increased sensitivity obtained is impressive and could be of major interest in the development of LFIA for low-molecular-mass toxins and, particularly when very low detection limits must be achieved, for example for aflatoxin M1.

With the expectation of increasing the useful signal, magnetic nanogold microspheres with an Fe_2O_3 core and a gold shell have also been proposed [55]. The magnetic core of particles enabled the authors to simplify separation steps during labelling of the antibodies, and their microdimensions enhanced the colour. A threefold increase in sensitivity was achieved for visual detection of AFB2 compared with use of simple gold colloid nanoparticles. However, the authors did not discuss the possible adverse effects on capillary flow of the increased particle dimensions (micro rather than nanometric), which could affect reproducibility and rapidity or would imply the requirement of specifically designed materials.

A lateral flow dipstick in which the signal is not generated by focussing of coloured particles has recently been described; in this an enzymatic label has been used [73]. In the proposed assay, unlabelled anti-FM antibodies were mixed with HRP-labelled secondary antibodies and the sample. On completion of the migration on to the membrane, where the usual assay occurred, a chemiluminescent substrate was added, and the luminescence developed at the T and C-lines was recorded by means of a portable CCD camera. Quantitative measurement of FMs in maize was achieved with fivefold improvement of LOD in comparison with the corresponding GNP-based LFIA developed with the same immunoreagents.

Conclusions and outlook

Although LFIAs are still regarded in some ways as emerging technology for mycotoxin detection [38, 39], several examples of fully developed devices have been described in the literature and are available as commercial kits. The applicability of these systems to a variety of food and feed matrices has also been demonstrated by comparison with reference analytical techniques, for example liquid chromatography, or with well-accepted screening methods, for example ELISAs. Annual updates of state-of-the-art techniques emphasize growing interest in the field and the increasing relevance of this technology over more established screening techniques [6, 7, 57, 74]. Notwithstanding, the research is directed toward obtaining an effective, functioning device, often at the expense of true innovation, except in a few rare cases. Among conceivable routes of development, some could be regarded as more attainable, for example:

1. detectability improvement, including strategies for achieving lower detection limits (for example modifying the format of the assay, tailoring the mycotoxin conjugate used as the competitor in the T-line, and varying the probe selected for antibody labelling);
2. ruggedness, which means the reproducibility and stability of strips, irrespective of prevalent ambient conditions, to ensure effective usability, and congruence of interpretation of visual observation; and
3. adaptation of extraction procedures (which are expected to affect the mismatching observed between fortified

and naturally contaminated samples and to contribute to determining selectivity toward matrix components), with the further objective of limiting the use of hazardous chemicals to render the assays effectively applicable outside the laboratory.

The relevance of phycotoxins to food safety assessment is gaining recognition and new analytical devices for monitoring such hazardous metabolites are appearing, although bioassays are traditionally and prevalently used for this purpose [9, 75]. Limitations to the development of LFIA, as for other immunoassays for this class of compounds, lie in the lack of availability or cost of the pure toxins in such quantities as to enable the preparation of conjugates and obtain antibodies. Furthermore, phycotoxin monitoring implies detection of groups of compounds, which is a major disadvantage for overly selective immunochemical techniques. Nevertheless, potential benefits of disposable point-of-use tests and of rapid and economical screening tools would promote research in this field.

Furthermore, recent breakthroughs in research in the immunoassay field could also encourage more innovation in the design of LFIAs for mycotoxins and phycotoxins, for example replacing antigens with mimotope peptides [76], with undoubted advantages in terms of economics and safety, and improving and/or tailoring antibody performance by exploiting phage display technology [77, 78]. Particularly useful would be strategies directed at identification of synthetic selective recognition systems which would overcome the need to produce specific antibodies toward toxins. Aptamers with selective binding properties towards OTA have been shown to replace antibodies in the development of an effective LFD [45] and this approach could be expected to extend to other toxins. Molecularly imprinted polymers as synthetic selective ligands for mycotoxin and phycotoxins have also been described [79, 80]; their application in immunochemical methods of analysis is, however, still challenging.

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