Lateral flow assay applied to pesticides detection: recent trends and progress

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Abstract
Devices based on lateral flow assay (LFA) have been gaining more and more space in the detection market mainly due to their simplicity, speed, and low cost. These devices have excellent sensing format versatility and make these strips an ideal choice for field applications. The COVID-19 pandemic boosted the democratization of this method as a “point of care testing” (POCT), and the trend is that these devices become protagonists for the monitoring of pesticides in the environment. However, designing LFA devices for detecting and monitoring pesticides in the environment is still a challenge. This is because analytes are small molecules and have only one antigenic determinant, which makes it difficult to apply direct immunoassays. Furthermore, most LFA devices provide only qualitative or semi-quantitative results and have a limited number of applications in multi-residue analysis. Here, we present the state of the art on the use of LFA in the environmental monitoring of pesticides. Based on well-documented results, we review all available LFA formats and strategies for pesticide detection, which may have important implications for the future of monitoring pesticides in the environment. The main advances, challenges, and perspectives of these devices for a direction in this field of study are also presented.

Keywords Immunocromatographic strip · Nanoparticles · Environmental monitoring · Point of care testing

Introduction
With growing global environmental concerns coupled with the 2030 agenda goals for sustainable development, the need for detection and monitoring of pesticides in the environment has been widely reported. Traditional methods of pesticide detection, such as gas chromatography (GC), gas chromatography with mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC), are known to be highly sensitive and reliable. However, these methods are complex, time-consuming, with robust equipment and skilled labor, being impractical in most cases. Therefore, quick, simple, and inexpensive techniques have received considerable attention.

One of the quick and sensitive methods that have been used to monitor pesticides is the enzyme-linked immunosorbent assay (ELISA) (Lee et al. 2003; Jiang et al. 2011), but this technique is not suitable for field analysis as it requires multiple steps and trained technicians. In recent years, strategies for the use of optical transducers (Homola 2008; Zeng et al. 2014; Saylan et al. 2017; Tsagkaris et al. 2021), electrochemical (Akyüz and Koca 2019; Nagabooshanam et al. 2019; Liu et al. 2020; Hara and Singh 2021), and microfluidic systems (Tahirbegi et al. 2017; Jang et al. 2020; Nagabooshanam et al. 2020; Xu et al. 2020) have considerably expanded the range of possibilities for detecting pesticides in environmental and food samples. However, the instrument’s cost and reproducibility issues have hampered large-scale applications. Another method used is the LFA, which is a quick and simple alternative that can be performed in a single step and in situ and does not require qualified personnel.
LFA-based devices have excellent versatility of sensing formats and make these strips an ideal choice for field applications. The COVID-19 pandemic boosted the democratization of this method as a POCT, and the trend is that these devices become protagonists for the monitoring of pesticides in the environment. LFA is useful for detecting pathogens, drugs, metabolites, proteins, or nucleic acids (Corstjens et al. 2003; Lai et al. 2007; Niu et al. 2014; Xu et al. 2014; Angelini et al. 2019) and can be used in various areas such as diagnostic medicine, forensic science, aquaculture, agriculture, animal health, food safety, consumer diagnosis, therapeutic, and environmental monitoring and industries (O’Farrell 2009). LFA has been used successfully to detect different types of pesticides such as insecticides (Kim et al. 2011; Liu et al. 2011), fungicides (Luo et al. 2019), and herbicides (Byzova et al. 2010). 

In this review, we present the state of art on the use of LFA in the environmental monitoring of pesticides. We cover pesticide types, their classifications, LFA principles and formats, label types, and a survey of all available LFA strategies for pesticide detection. The main advances, challenges, and perspectives of these devices for the future of monitoring pesticides in the environment are also presented. 

Pesticides

A pesticide is defined as a product that incorporates a substance or mixture of substances designed to prevent, attract, repel, destroy, or mitigate a pest (EPA). They are commonly classified, on the basis of their chemical structure, mainly in organochlorines, organophosphates, carbamates, pyrethrin and pyrethroids, phenyl amides, phenoxyalkonates, triazines, benzoic acid derivatives, benzonitriles, phtalimide derivatives, and dipyrds. Some characteristics of some of these groups are presented in Table 1.

A significant increase in the use of pesticides worldwide has been promoted by the growing economy of the agricultural sector, being the main consuming countries China, USA, Thailand, Argentina, and Brazil (Sharma et al. 2019). Currently, about two million tons of pesticides are used worldwide (Sarkar et al. 2021). This accelerated increase has led to an overuse of pesticides, which has negative effects on the environment and human health, according to several studies (Yadav and Devi 2017; Sharma et al. 2019; Terziev and Petkova-Georgieva 2019; Alengebawy et al. 2021; Rani et al. 2021).

World Health Organization (WHO) has assigned a classification for pesticides in relation to their hazard based on the median lethal dose by oral or dermal route of entry (Table 2). Exposure to pesticides can be oral, dermal, or respiratory, which determines their level of toxicity. Especially workers linked to the production and application of pesticides are the most affected; however, the general population is exposed to

| Table 1 Classification of pesticides according to their chemical structure |
|-----------------------------------------------|
| Chemical group | Features | Uses | Examples |
|----------------|----------|------|---------|
| Organochlorines | Substances linked with five or more chlorine atoms | Insecticides, acaricides, rodenticide and herbicide | DDT, DDD, DDE, Aldrin, Lindane, Dieldrin |
| Organophosphates | Esters, amides or thio derivatives of phosphoric acid | Insecticides, acaricides, nematicides, fungicides | Methyl parathion, phorate, fenthion, diazinon |
| Carbamates | Compounds derived from carbamic acid | Herbicides, acaricides, nematicides, rodenticides and fungicides | Carbaryl, dimethoate, isothiuron, diazinon |
| Pyrethrins and pyrethroids | Synthetic pyrethroids are obtained by duplicating the structure of natural pyrethrin | Insecticides | Allethrin, butoximate, dieldrin, tetrachlorvinphos, permethrin, cypermethrin |

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a greater or lesser extent (Yadav and Devi 2017; Sabarwal et al. 2018). Pesticide exposure has been linked to a number of health problems such as numerous types of cancer (Sabarwal et al. 2018; Alengebawy et al. 2021; Rani et al. 2021), diabetes (Evangelou et al. 2016), asthma and bronchitis (Ye et al. 2013), endocrine and immune system disorders (Casida and Durkin 2017), and neurological disorders such as Parkinson’s disease and Alzheimer’s disease (Sabarwal et al. 2018; Alengebawy et al. 2021).

The toxicity and persistence of synthetic chemical pesticides has led to the search for more sustainable alternatives for pest control such as biological pesticides or biopesticides, defined by the Environmental Protection Agency as products derived from natural materials such as animals, plants, bacteria, and certain minerals (EPA). Properties such as being environmentally friendly, cost-effective, non-bioaccumulative, biodegradable, and safe for humans and non-target organisms (Casida and Durkin 2017), and neurological disorders such as Parkinson’s disease and Alzheimer’s disease (Sabarwal et al. 2018; Alengebawy et al. 2021).

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**MRLs of pesticides**

Several pesticides are highly toxic and have bioaccumulative properties (UNEP 2007), so their excessive use represents a risk to human health and the ecosystem. Among the health conditions that have been related to exposure to pesticides are damage to the respiratory, reproductive, nervous, and immune systems and cancer (Tahir et al. 2009; Yadav and Devi 2017). Because pesticides can contaminate soils, air, surface, and groundwater, and remain in the food chain, pesticide residues may exist in food and drinking water. For these reasons, many countries have established maximum residue limits (MRLs) specifically established under the recommendations of the Codex Alimentarius Commission and pesticide drinking water maximum concentration level (MCLs).

The MRL is the maximum concentration of a pesticide residue (expressed in mg/kg), resulting from the use of a pesticide in accordance with good agricultural practices. The MRL values differ according to the country, the active ingredient, and the crop. Table 3 presents examples of MRLs established by the regulatory agencies of the European Union, the USA, and Brazil for some food products.

The monitoring of pesticide levels in food and drinking water requires detection techniques sensitive enough to detect the values established in the MRLs and guarantee the food safety of consumers. For this purpose, different detection methods and techniques have been developed in the analysis of water, air, soil, or food (Sørensen et al. 2016).

LFAs have gained popularity as detection systems for analytes such as pesticides thanks to features such as speed, ease of use, and on-site detection.

**LFA: basis and formats**

LFAs are analytical sensors for the detection of one or more analytes based on the recognition of these analytes by bioreceptors (antibodies, antigens, aptamers, etc.) coupled to labels (nanoparticles, enzymes, liposomes, etc.) that will generate a signal (colorimetric, fluorescent, chemiluminescent, etc.) with qualitative or quantitative information of the target analyte (Sajid et al. 2015; Bahadır and Sezgintürk 2016; Koczula and Gallotta 2016; Urusov et al. 2019). A standard LFA device is made up of four components of different materials: the sample pad, the conjugate pad, the detection membrane, and the absorption pad. When performing the test, the solution to be analyzed is placed on the sample pad. The sample flows by capillary action

### Table 2 Classification of pesticides according to their hazardousness (WHO 2020)

| Class       | LD50 for the rat (mg/kg body weight) | Oral | Dermal |
|-------------|-------------------------------------|------|--------|
| Ia          | Extremely hazardous                 | < 5  | < 50   |
| Ib          | Highly hazardous                    | 5–50 | 50–200 |
| II          | Moderately hazardous                | 50–2 000 | 200–2 000 |
| III         | Slightly hazardous                  | Over 2 000 | Over 2 000 |
| U           | Unlikely to present acute hazard    | 5000 or higher |

### Table 3 MRLs for wheat and corn according to different legislation

| Pesticides | MRL for wheat (mg/kg) | MRL for corn (mg/kg) |
|------------|-----------------------|----------------------|
|            | US (USDA) | EU (EC) | Brazil (MAPA) | US (USDA) | EU (EC) | Brazil (MAPA) |
| Glyphosate  | 10        | 30      | 0.05          | 3         | 5       | 1           |
| 2,4-D      | 2         | 2       | 0.2           | 0.05      | 0.05    | 0.2         |
| Atrazine   | 0.05      | 0.1     | –             | 0.05      | 0.2     | 0.25        |
| Chlorpyrifos | 0.01    | –       | 0.2           | 0.01      | –       | 0.1         |
| Malathion  | 8         | –       | 8             | 0.02      | –       | 8           |
| Imidaclorpid | 0.1     | 0.05   | 0.5           | 0.1       | 0.05    | 0.5         |
through the strip. Once the sample reaches the conjugated pad, where a detection bioreceptor conjugated with a label has been immobilized, the analyte interacts with the conjugate forming an analyte-conjugate complex that migrates to the detection membrane. This membrane is generally a porous material like nitrocellulose or fiberglass (Costa et al. 2020), in which there is a capture bioreceptor forming a line called test line, acting as a capture zone for the complex or analyte and through which the qualitative, semi-quantitative, or quantitative result of the test can be determined with the naked eye or with instrumentation. Following the test line is the control line, where another capture bioreceptor has been immobilized to check the functionality of the assay. Finally, the absorbent pad has the function of giving an extra wicking force to ensure that the entire sample travels along the strip; it also serves as a waste reservoir to reduce the visual background signal and prevent the sample from returning (Anfossi et al. 2013; Chen and Yang 2015; Bahadır and Sezgintürk 2016) (Fig. 1).

There are two LFA formats, the competitive or direct and the non-competitive or indirect. Competitive LFA is used for low molecular weight molecules with a single epitope, antigens that have a single binding site for a specific antibody, such as pesticides. If the sample does not contain the analyte (negative result), the labeled reagent on the conjugate pad migrates with the sample through the strip and is captured by the capture bioreceptors immobilized in the test zone producing a signal in the test and control line. If the sample contains the analyte (positive result), it binds to the labeled bioreceptor of the conjugate pad forming a complex, the same that is captured in the control line, leaving little or no analyte to be captured by the test line, with which there is a slight or absence of signal in this zone (Fig. 2). In this format, the detectable response in the test line is inversely proportional to the analyte concentration in the sample.

Noncompetitive LFA, also called sandwich, is used for high molecular weight molecules with various epitopes; the analyte in the sample binds to the detection bioreceptor and then one of its free epitopes binds to the capture bioreceptor in the test line. Then, the result is positive when a marking is produced in the test line (Fig. 2). The detectable response in the test zone is directly proportional to the amount of analyte in the sample. For both formats, the control line is always marked regardless of the result (O’Farrell 2009; Mak et al. 2016).

Fig. 1  A Components of a lateral flow strip. B Schematic of a typical lateral flow test (Rivas et al. 2014)
Low weight molecules that do not induce an immune response, such as pesticides, are known as haptens; they are conjugated with carrier proteins such as bovine serum albumin (BSA) or ovalbumin (OVA) and are used in lateral flow assays as the capture bioreceptors in the test line.

**Bioreceptors**

Among the bioreceptors, antibodies are the most used in lateral flow assays mainly due to their highly specific binding to analytes (Ferrigno 2016). They can be polyclonal (pAb) that can recognize and bind to many different epitopes of a single antigen, monoclonal (mAb) that have monovalent affinity and only recognize the same epitope of an antigen, and bispecific (BmAbs) that possess two binding sites with different antigenic specificity. However, its high manufacturing costs, limited stability, and batch-to-batch variation have promoted the development of alternatives such as aptamers and molecularly imprinted polymers (MIPs).

Aptamers are single-stranded DNA or RNA oligonucleotide sequences designed through a combinatorial selection process called systemic evolution of ligands by exponential enrichment (SELEX). Due to their small size, only ~ 1–2 nm, they have a higher surface density with more binding sites per area, and they are more ideal than Ab to use in sensors for pesticide detection (Morales and Halpern 2018). Cheng et al. (2018) developed an aptamer-based LFA system with nanopairs of fluorophore extinguishers and a spectrum reader for smartphones for rapid and in situ detection of chlorpyrifos, diazinon, and malathion. They obtained a biosensor with high specificity and sensitivity, with detection limits of 0.73 ng/mL, 6.7 ng/mL, and 0.74 ng/mL, respectively (Cheng et al. 2018). Aptamers have several advantages such as high specificity, low molecular weight, easy synthesis, and modification, and aptasensors tend to be more sensitive and specific than antibody-based sensors (Liu et al. 2019a). In addition, because of low molecular weight of pesticide molecules, aptamers are more ideal than Abs to construct LFTS aptasensors for pesticide detection.

MIPs are synthetic polymers with recognition sites where analyte specificity is achieved through non-covalent bonding patterns, electrostatic interactions, or inclusion/exclusion size (Morales and Halpern 2018). They were applied by He, Yahui et al. in a lateral flow assay for the detection of triazophos in water, achieving high sensitivity with a detection limit of 20 μg/L (He et al. 2020). The use of MIPs in lateral flow assays could overcome the high manufacturing costs and limited stability of antibodies (Lowdon et al. 2020).

**Labels**

The labels used in LFA allow qualitative, semi-quantitative, or quantitative detection of the analyte. Depending on their physicochemical characteristics, some labels can generate a direct (visual) signal, while others produce an analytical signal, for which additional instrumentation or steps may be required. Colored nanoparticles, enzymes, fluorescent nanoparticles, and magnetic nanoparticles, among others, have been used in various studies as probes for the detection of pesticides. The advantages and disadvantages of labels used in LFAs are listed in Table 4, and some of them are discussed below.
AuNPs

LFAs that use gold nanoparticles (AuNPs) as labels are the most used because they have good optical signaling, high affinity for proteins and biomolecules and excellent stability (Sajid et al. 2015). A large number of LFAs have been extensively studied using AuNPs as labels for detecting organophosphate (Kim et al. 2011), carbamates (Wang et al. 2005), and pyrethrin and pyrethroid (Kranthi et al. 2009) pesticides. In general, it is possible to obtain fast and sensitive tests, for example, Kim et al. (2011) developed an AuNP-based immunochromatographic strip for the detection of chlorpyrifos achieving a limit of detection (LOD) of 10 ng/mL taking less than 10 min. The AuNP-based LFA developed by Shuo Wang et al. (2009) for the detection of carbaryl had a LOD of 100 μg/L, obtaining a higher sensitivity than ELISA (Parolo et al. 2020). Jianqiang et al. (2020) developed a screening test with AuNPs for the simultaneous detection of carbofuran and 3-hydroxy-carbofuran in water samples and pesticide preparations with a LOD of 10 ng/mL and with a running time of 5 min. (Figure. 3). A study developed by Liqiang Liu et al. (2017a) obtained a AuNP immunochromatographic strip for the detection of acetamiprid (AC) and thiacloprid (TC) in cucumber and apple samples achieving LODs of 5 ng/mL for AC and 2.5 ng/mL for TC in cucumber sample and 30 and 15 ng/mL in apple samples. Gui et al. (2008) made a sensitive strip labeled with AuNP for the detection of triazophos residues with a sensitivity (5 ng/mL) close to that of GC (0.1–5 ng/mL).

In order to improve efficiency and reduce costs, multiplexed systems have been developed where several types of pesticides can be detected in the same test. For the operation of these systems, pesticides must have different physical-chemical properties, and their haptons differ in their chemical structure (Wang et al. 2009). Xu et al. (2012) successfully developed a multiple analysis of imidacloprid and thiamethoxam in one step. According to the study, the results did not demonstrate a cross reaction between imidacloprid and thiamethoxam even at relatively high concentrations. The reached detection limits were 0.5 ng/mL and 2 ng/mL respectively (Xu et al. 2012).

To avoid the reciprocal interference between tests that may exist with the aforementioned strategy, individual strips arranged in parallel have been used that share a single sample. Wang et al. (2014) developed an LFA with semi-quantitative and naked eye simultaneous reading for three analytes imidacloprid, chlorpyrifos-methyl and isocarbophos. The LFA consisted of 4 strips. Each strip with 3 test zones with coated antigens for each analyte with increasing concentrations of antigens on each strip. Five detection ranges and optical detection limits were established for the three analytes and the four concentration thresholds. The test was carried out in 7 min, and the results were consistent with the HPLC results.

Another approach used in multiplexed analysis is bispecific monoclonal antibodies (BmAbs) that have specific binding sites for two antigens with different chemical structures. BmAbs are capable of simultaneously binding to two different analytes. This strategy was used by Guo et al. (2009) for the simultaneous detection of carbofuran and triazophos. They used a BmAb with binding sites for each pesticide. It achieved LODs of 64 and 8 μg/L for carbofuran and triazophos, respectively. The authors also developed a strip that used two mAbs specific for each analyte and obtained a much higher sensitivity (Wang et al. 2014).

Although AuNp-based LFAs are the most widely used, they can present drawbacks such as low sensitivity and a limited detection range (Liu et al. 2012); therefore, many researchers have replaced AuNPs with other labels, developing signal amplification techniques and reading techniques to improve LOD and working range.
Gold nanoparticles have also been used as components in Raman surface enhanced dispersion labels (SERS) that have been included in LFAs with a significant increase in sensitivity and dynamic range. SERS tags are composed of plasmonic nanoparticles, active Raman molecules adsorbed on nanoparticles, and an inorganic layer that is functionalized with antibodies. Thus, Sheng et al. (2021) prepared a surface-enhanced Raman scattering–based lateral flow assay (SERS-LFA) test strip using SERS labels that used core/shell nanoparticles. A SERS signal molecule 4-nitrothiophenol (4-NTP) was encapsulated between a silver-core and a gold shell forming the nanoprobe, and then competitive immune binding was used to detect the three pesticides simultaneously, achieving high sensitivity SERS-LFA test strips, a low detection limit, short detection time, high specificity, and low cost (Fig. 4).

Carbon nanoparticles (CNPs) are used in LFAs as colorimetric labels thanks to their dark color that contrasts with the white of the nitrocellulose membrane, which improves the sensitivity and LODs in relation to LFAs based on AuNPS (Quesada-González and Merkoçi 2015; Calucho et al. 2020). CNPs are the cheapest labels, and their suspensions are very stable and easy to prepare, and no activation is necessary (R). Šmidová et al. (2009) used CNPs as colorimetric labels in an LFA for the detection of thiabendazole in enriched fruit juice samples. The LODs obtained were lower than the allowed MRL (Šmidová et al. 2009).

Luminescent nanoparticles

Luminescent nanoparticles have been widely used in LFAs as labels to improve assay sensitivity (Quesada-González

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**Fig. 3** Scheme of an LFA using AuNPs as labels
and Merkoçi 2015). Within this category we find quantum dots (QDs), up-converting nanoparticles (UCNPs), and silica nanoparticles, among others.

**QDs**

QDs are semiconductor particles with high photostability, high absorption coefficient, and resistance to chemical degradation; they have narrow emission spectra and can be excited by a wide spectrum of light, in addition to present excellent anti-bleaching properties and a size-adjustable fluorescence emission (Chan et al. 2002; Jaiswal and Simon 2004; Huang et al. 2016; Le et al. 2018).

The QDs can be used in the LFAs in two ways, directly, measuring the fluorescence emitted by the QDs in the detection zone, once the test is finished, or indirect (Anfossi et al. 2018; Zamora-Gálvez et al. 2018), in which it is used in the quenching property of QD photoluminescence, where the decrease in fluorescence occurs through a Forster resonance energy transfer (FRET) mechanism (Clapp et al. 2006) involving energy transfer from an energy donor photoexcited to an energy acceptor. Among the acceptor materials, graphene oxide has been shown to have a high quenching efficiency (Morales-Narváez et al. 2012).

Several studies have been conducted with QD-based LFIs for the detection of pesticides with high sensitivity. Wang et al. (2017) developed a QD-based fluorescent LFI with a broad-spectrum antibody for the detection of imidacloprid with cross-reactivity with clothianidin and imidaclothiz, with detection limits of 0.5 ng/mL, 1 ng/mL, and 0.5 ng/mL, respectively.

QD-based LFAs have been shown to improve sensitivity and LODs compared to LFAs using AuNP as labels. Liu et al. (2019b), for example, developed an immunosensor to qualitatively and quantitatively detect acetamiprid. They used a QD-mAb conjugate. The visual detection limit of acetamiprid for a qualitative threshold was set at 1 ng/mL with the naked eye, lower than that presented by a AuNP-based LFA which was 10 ng/mL. In the quantitative determination, a linear range of fluorescence intensity was established from 0.098 to 25 ng/mL. For the quantitative measurement, a strip reader was used, and the results were obtained in 1 h (Liu et al. 2019a) (Fig. 5). LFAs based on QDs, and in general on fluorescent nanoparticles, require additional devices for signal excitation or reading (Calucho et al. 2020), and in some cases may require more time than LFAs with AuNPs.

On the other hand, QD assays can be as fast as AuNP-based LFAs. Zou et al. (2010) developed an immunochromatographic test strip with a QD label and a test strip reader for the rapid and sensitive detection of an organophosphate pesticide metabolite being able to detect a minimum of 1.0 ng/mL of 3,5,6-trichloropyridinol (TCP) standard analyte in 15 min (Zou et al. 2010).

Wu et al. (2019) developed a method for the quantitative visual detection of benzothiostrobin residues in strawberry using CdSe/ZnS (core/shell) QDs as labels. Three test lines were included in the strip with different concentrations of...
antigen. The results were determined visually using UV protective glasses and a gel imager for quantitative analysis, obtaining a visual detection limit of 25 μg/L (Wu et al. 2019).

**UCNPs**

Fluorescent materials work by two energy conversion mechanisms, down-conversion and up-conversion. Down-conversion fluorescence converts light from the ultraviolet light spectrum to the visible light region. On the other hand, up-converting nanoparticles convert near infrared (NIR) light to visible light. This property allows that the background signals that come from autofluorescence when excited with UV light are not generated, increasing the sensitivity of LFAs. Up-converting nanoparticles (UCNPs) possess high chemical stability, tunable emission colors, high photostability, low cytotoxicity, high resistance to photobleaching, multiple and narrow emission bands, and long shelf life (Huang et al. 2016; Tan et al. 2016; Zhang et al. 2019).

Zou et al. (2019) reported a UCNP-based LFA for the detection of multiple residues of three organophosphate pesticides in food samples using a broad-spectrum mAb. The UCNP-mAb conjugate was pre-mixed with the sample. The intensity of the fluorescent signal from the strips was read by a portable machine integrated with the NIR laser system that translated the information to an electronic data processing computer. The results were obtained in 40 min and showed a high sensitivity for parathion, parathion-methyl, and fenitrothion (Zou et al. 2019) (Fig. 6).

UCNP-based LFAs require a NIR laser to promote upconversion. Strip readers have now been developed that integrate a NIR laser and a smartphone due to their processing capabilities similar to those of a computer and high-tech cameras, which makes them more suitable for on-site analysis (You et al. 2017a; Jin et al. 2018; Gong et al. 2019). Jin et al. (2018) developed a portable device based on a smartphone integrated into a competitive LFA using multicolored UCNPs functionalized with aptamers as labels for the multiple detection of different analytes like ions, bacteria, and small molecules in food and water samples. The portable reader designed allowed to detect and quantify multiple targets simultaneously and obtain results through a smartphone. The authors reported a sensitive, specific, and convenient assay for in situ detection (Jin et al. 2018).

Several fluorescence detection methods based on the fluorescence quenching process have been developed using mechanisms such as resonance energy transfer (FRET), as mentioned above; however, this mechanism has high requirements for the distance between absorbers and the fluorescent material (Liu et al. 2017a). The detection method based on the internal filter effect (IFE) is more flexible and simple, without the binding of the absorber with the fluorescent material. IFE is a model of energy conversion without irradiation in spectrofluorometry, which results from the absorption of the excitation and/or emission light by the absorber in the detection system. IFE is caused by the overlap between the absorber absorption band and the excitation and/or excitation bands of the fluorophores (Chen et al. 2018). You et al. (2017b) developed an LFA with competitive UCNPs based on IFE using AuNPs as a fluorescence acceptor for the detection of the neocotinoid insecticide imidaclopid. The UCNPs were coupled to antibodies against the analyte, and the AuNPs were used as labels for the analyte. The results showed an improvement in sensitivity compared to other assays such as ELISA and the fluorescence polarization immunoassay. Furthermore, it is mentioned that the use of UCNPs eliminated the interference of background fluorescence.

**Fig. 5** Schematic of an LFA using QDs as labels for the detection of pesticides. Positive (A, B) and negative (C) result (Liu et al. 2019b)
MNPs

Magnetic nanoparticles (MNPs) are colored labels that can also emit magnetic signals that can be preserved for long periods of time (Sajid et al. 2015). Generally, MNPs have been used to purify the analyte before testing (Liu et al. 2019b); however, their color intensity and the magnetic field they generate make them suitable for use as labels. LFAs occupying MNPs require a reading device for reading the emitted signal. The magnetic field generated by MNPs can be measured and studied with a reader as a useful analytical signal related to a concentration of the target (Quesada-González and Merkoçi 2015). Liu et al. (2011) developed aggregates of \( \text{Fe}_3\text{O}_4 \) magnetic particles that were obtained by crosslinking \( \text{Fe}_3\text{O}_4 \) NPs with poly-L-lysine, improving the sensitivity for the detection of the pesticide paraoxon methyl in relation to the assay that used individual \( \text{Fe}_3\text{O}_4 \) NPs, due to the amplification effect, reducing the detection limit by 40 times reaching 1.7 ng/mL maintaining the specificity of the test; it can be used to detect dangerous substances through the detection of the magnetic signal of the aggregates of \( \text{Fe}_3\text{O}_4 \) particles (Liu et al. 2011) (Fig. 7).

Enzymes

Enzymes can amplify the colorimetric signals depending on the catalytic activity of the enzyme. In LFA, a substrate is added after the test is completed to achieve this purpose by an enzymatic reaction. The most commonly used enzymes for detection are horseradish peroxidase (HRP) and alkaline phosphatase (Chen et al. 2019). Zhang et al. (2006) developed an LFA using AuNP and horseradish peroxidase (HRP) as tracers for the simultaneous qualitative or semi-qualitative detection of carbaryl and endosulfan in food samples, improving the sensitivity 10 times in relation to a test immunochromatographic based solely on AuNP (Zhang et al. 2006). Enzymes are used as chemiluminescent labels as a result of a reaction with a suitable substrate (Sajid et al. 2015). Shu et al. (2017) achieved a quantitative detection of pesticide residues using HRP and alkaline phosphatase (ALP) as chemiluminescent probes to label methyl parathion and imidacloprid haptens. The linear ranges of methyl parathion and imidacloprid were 0.1 to 250 ng/ml, with detection limits of 0.058 ng/ml. The two enzyme-catalyzed chemiluminescence reactions were triggered simultaneously by the injection of coreactants (Fig. 8).

Luciferases enzymes have become important research tools for their ability to emit light (bioluminescence, BL) by oxidation of the substrate. There are two main classes of luciferases used as research tools: firefly luciferase (Fluc) and renilla luciferase (Rluc) (Nakatsu et al. 2006; Loening et al. 2007). Ding et al. (2018) use nano luciferase (Nano-Luc) to generate recombinant tracers for the development of immunoassays, achieving good precision for the detection of imidaclothiz in agricultural samples.

Catalytic nanomaterials with enzymatic activities called nanozymes have been developed with advantages such as good stability, availability, and low cost (Chen et al. 2019).
Peroxidase-like nanozymes have been widely used in the development of biosensors (Wang et al. 2018). Platinum-palladium NP (Pt–Pd) have a catalytic activity equal to peroxidase and have been used as colorimetric labels for monitoring exposure to organophosphate pesticides (Zhao et al. 2018).

Nanosheets 2D

Two-dimensional (2D) nanosheets are materials derived from a wide range of low-dimensional solids that contain atomically thin structures, exfoliated 2D structures, and molecular membranes (Chaudhari et al. 2017). 2D nanosheets have more catalytic sites as they have a larger surface area (Wen et al. 2018). Cheng et al. (2019) developed a bidirectional LFA amplified with Pt–Ni (OH) 2D nanosheets with similar activity to peroxidase for the detection of acetochlor and fenpropathrin. LODs of 0.63 ng/mL and 0.24 ng/mL respectively were achieved. Qualitative results were obtained in 13 min, and quantitative results were obtained in 10 min with a smartphone-based

Fig. 7 Scheme of an LFA with MNPs as labels (Liu et al. 2011)
Fig. 8 Scheme of a multiplex LFA for the detection of pesticides using chemiluminescent labels as enzymes (Shu et al. 2017)

Fig. 9 Scheme of a multiplexed bidirectional LFA for the detection of pesticides with Pt–Ni (OH) nanosheets as labels (Chen et al. 2019)
readout. The results confirmed an improvement in sensitivity and detection ranges (Fig. 9).

**Electrochemical LFA**

The integration of the electrochemical approach in LFAs has been increasing thanks to the advantages that they present as a highly sensitive response in a wide detection range, quantitative determination, low cost, high reproducibility, absence of labels, and low detection limit (Nguyen et al. 2020; Perju and Wongkaew 2021). Du et al. (2012) used disposable screen-printed CNT electrodes for electrochemical measurement for the detection of organophosphorus pesticides through the parallel measurement of the activity of the enzyme acetylcholinesterase (AChE) post-exposure and baseline where they take advantage of the reactivation of phosphorylated AChE to allow the measurement of the total amount of AChE used as a baseline for the AChE inhibition calculation. Quantification of the phosphorylated adduct was performed by subtracting active AChE from the baseline measurement. This LFA achieved a LOD of 0.02 nM (Fig. 10).

A literature review was carried out of all available formats of LFA devices for pesticide detection until the year 2021. The conjugation of nanomaterials with biomolecules provided an excellent platform for the detection of a variety of target analytes. Overall, it has been observed that gold nanoparticles are the most used markers because of their excellent stability, good contrast, high affinity for proteins, and relatively low cost.

However, these markers have a limited detection range, and for this reason, new labels have been explored, such as luminescent nanoparticles, CNPs, QDs, UCNPs, MNPs, enzymes, and 2D nanosheets. Regarding target pesticide recognition strategies, antibodies have high specificity and have been explored for most LFIA-based pesticide detection systems. The limited stability of antibodies and batch-to-batch variation have promoted the development of alternatives such as MIPs and aptamers, which have higher surface density and more binding sites per area.

A multitude of models has been published for almost all classes of pesticides, among which qualitative, semi-quantitative, and quantitative analysis devices are described (Kim et al. 2011; Zhang et al. 2014; Ouyang et al. 2018a) with the inclusion of instrumental and software tools for the measurement of the analyte concentration (Hua et al. 2010; Cheng et al. 2019) in addition to assays for the detection of several analytes simultaneously and with modifications of the traditional design of the lateral flow assays as bidirectional assays are mentioned (Cheng et al. 2019). The use of labels other than gold generally makes the LFA more sensitive. However, it should be borne in mind that the sensitivity of an LFA depends on several factors such as materials used, type of sample, assay design, and reaction time. The detection limits found meet the limits of international legislation for agricultural and environmental samples. Finally, the average analysis time is 10 min, indicating that these devices can be useful for field analysis. LFAs based on various probes for the detection of pesticides are listed in Table 5.

**Economic viability**

One of the main problems of analytical techniques considered the gold standard, such as HPLC and GC-MS and their detectors, is the high cost. This makes these technologies inaccessible to farmers and many laboratories in developing countries. In this sense, many efforts have been focused on developing rapid point of service (POC) tests that feature simplicity, reliability, and above all low cost.

Although the examples cited demonstrate economic feasibility and report them as being low-cost systems, most publications do not include production costs in their discussions. However, all products including labels, biorecognition molecules, strip readers, and complete kits are being provided.
Table 5 Summary of published reports on lateral flow immunoassays applied to pesticides

| No. | Target                                | Conjugate                          | Test Line | Detection limit  | Time  | Reference            |
|-----|---------------------------------------|------------------------------------|-----------|------------------|-------|----------------------|
| 1   | N-methylcarbamate                     | AuNPs                              |           | 0.25 mg/L        | 10 min| Zhou et al. 2004    |
| 2   | Carbaryl                              | AuNPs                              |           | 100 μg/L         | 5 min | Wang et al. 2005     |
| 3   | Endosulfan                            | AuNPs                              |           | 100 μg/L         | 15 min| Zhang et al. 2006    |
| 4   | Triazophos                            | AuNPs                              |           | 10 ng/mL         | 10 min| Guo et al. 2008      |
| 5   | Bromoxynil                            | AuNPs                              |           | 4 ng/mL          | 5 min | Zhu et al. 2010      |
| 6   | Carbaryl Endosulfan                   | AuNPs                              |           | 800 μg/L         | 5 min | Wang et al. 2009     |
| 7   | Cypermethrin                          | AuNPs                              |           | 1,000 μg/L       | 10 min| Zhang et al. 2009    |
| 8   | Dichlorophenyltrichloroethylene (DDT) | AuNPs                              |           | 113.91 ng/mL     | 10 min| Guo et al. 2010      |
| 9   | Chlorpyrifos-methyl                    | AuNPs                              |           | 27 ng/mL         | 10 min| Hua et al. 2011      |
| 10  | Chlorpyrifos                          | AuNPs                              |           | 0.5 ng/mL        | 10 min| Xu et al. 2012       |
| 11  | Chlorpyrifos spiked in sample         | AuNPs                              |           | 0.22 ng/mL       | 10 min| Lee et al. 2013      |
| 12  | Imidacloprid                          | AuNPs                              |           | 0.001 μg/mL      | 10 min| Lee et al. 2013      |
| 13  | O-ethyl O-4-nitrophenylphenylphosphonothioate (EPN) | AuNPs                              |           | 0.005 μg/mL      | 15 min| Zhang et al. 2014    |
| No. | Target               | Nanoparticles type | Conjugate                                           | Test line                  | Detection limit | Time | Reference                  |
|-----|----------------------|--------------------|-----------------------------------------------------|-----------------------------|-----------------|------|----------------------------|
| 17  | Imidacloprid         | AuNPs              | Anti-imidacloprid mAb-AuNPs                         | Imidacloprid hapten-OVA     | 50 μg/L         | 7 min | Wang et al. 2014           |
|     | Chlorpyrifos-methyl  |                    | Anti-chlorpyrifos-methyl mAb-AuNPs                 | Chlorpyrifos-methyl hapten-OVA | 100 μg/L        |      |                            |
|     | Isocarbophos         |                    | Anti-isocarbophos mAb-AuNPs                        | Isocarbophos hapten-OVA     | 100 μg/L        |      |                            |
| 18  | Imidacloprid         | AuNPs              | Nanogold-biotinylated anti-imidacloprid mAb        | Imidacloprid hapten-OVA     | 5 ng/ml         | –    | Fang et al. 2015           |
| 19  | Acetamiprid          | AuNPs              | Anti-acetamiprid mAb-AuNPs                         | Acetamiprid-BSA             | 5 ng/mL         | 5 min | Liu et al. 2017c           |
|     | Tiacloprid           |                    |                                                     |                             |                 |      |                            |
| 20  | Isocarbophos         | AuNPs              | AuNPs-aptamer                                       | Cysteamine                 | 2.48 μg/L       | < 1 min | Liu et al. 2020           |
| 21  | Fenpropathrin methyl | LR AuNPs           | Anti-fenpropathrin methyl-AuNPs                    | Parathion methyl-BSA        | 0.17 ng/mL      | 15 min | Ouyang et al. 2018a       |
| 22  | Carbofuran (CBF)     | AuNPs              | Anti-CBF mAb-AuNPs                                 | Fenpropathrin-BSA           | 0.10 ng/mL      | 5 min | Jiang et al. 2011         |
|     | 3-hydroxy-carbofuran (3-OH-CBF) |          | Anti-3-OH-CBF mAb-AuNPs                            | Hapten-OVA                  | 7–10 ng/mL      |      |                            |
|     |                      |                    |                                                     | 1 ng/mL                    |                 |      |                            |
| 23  | Chlorpyrifos diazinon malathion | AuNSs          | AuNSs-aptamer                                       | QDs-BSA- streptavidin biotinylated complementary Sequences | 0.73 ng/mL, 6.7 ng/mL, 0.74 ng/mL | –    | Cheng et al. 2018         |
| 24  | Imidacloprid         | AuNPs              | mAb-AuNPs                                          | IMI-BSA                     | 0.02 ng/mL      | 10–15 min | Tan et al. 2020          |
|     |                      | Time-Resolved fluorescent nanobeads (TRFNs) | mAb-TRFN                         | IMI-BSA                     | 0.02 ng/mL      | 10–15 min |                            |
| 25  | Thiabendazole        | Colloidal carbon   | Anti-thiabendazole mAb-colloidal carbon             | Thiabendazole-OVA           | 0.005 mg/kg for Apple juice, 0.5 mg/kg for pear and orange juices | –    | Šmídová et al. 2009       |
|     | Methiocarb           |                    | Anti-methiocarb mAb-colloidal carbon                | Methiocarb-OVA              |                 |      |                            |
| 26  | Paraaxon methyl      | Fe3O4NP aggregates | Anti-paraaxon methyl pAb-Fe3O4 NP aggregates        | Paraaxon methyl hapten-OVA  | 1.7 ng/mL       | 15 min | Liu et al. 2011           |
| 27  | Parathion            | UCNPs              | PA-QA1-7B2 mAb-UCNP                                | PA0304-OVA                  | 3.44 ng/mL      | 40 min | Zhang et al. 2019         |
|     | Parathion-methyl     |                    |                                                     |                             | 3.09 ng/mL      |      |                            |
|     | Fenitrothion         |                    |                                                     |                             | 12.49 ng/mL     |      |                            |
| No. | Target                                      | Nanoparticles type                  | Conjugate                                         | Test line                  | Detection limit | Time  | Reference                  |
|-----|---------------------------------------------|-------------------------------------|---------------------------------------------------|----------------------------|-----------------|-------|----------------------------|
| 28  | Acetochlor                                  | Pt-Ni (OH)₂ NSs                     | Anti-acetochlor Ab-Pt-Ni (OH)₂ NSs                 | Acetochlor-BSA             | 0.63 ng/mL      | 13 min | Cheng et al. 2019          |
|     | Fenpropathrin                               |                                     | Anti-fenpropathrin Ab-Pt-Ni (OH)₂ NSs             | Fenpropathrin-BSA          | 0.24 ng/mL      |       |                            |
| 29  | Chlorpyrifos and carbaryl                   | g-C₃N₄/BiFeO₃ NCs                   | g-C₃N₄/BiFeO₃ NCs-chlorpyrifos Ab. g-C₃N₄/BiFeO₃ NCs-carbaryl Ab | Chlorpyrifos-BSA           | 0.033 ng/mL     | –     | Ouyang et al. 2018b        |
| 30  | Trichloropyridinol (TCP)                    | Quantum Dot                         | TCP-QD                                            | TCP mAb                    | 1.0 ng/mL       | 15 min | Zou et al. 2010            |
| 31  | Imidacloprid                                | Quantum Dot                         | Anti-imidacloprid mAb-QD                          | Imidacloprid-OVA           | 0.5 ng/mL       | 30 min | Wang et al. 2017           |
| 32  | Triazophos                                  | (CdSe/ZnS) Quantum Dots             | Anti-triazophos mAb-QD                            | Triazophos hapten-OVA      | 0.508 ng/L      |       | Liao et al. 2019           |
| 33  | Benzothiostrobin                            | CdSe/ZnS Quantum Dots               | Anti-benzothiostrobin Ab-QD                        | Benzothiostrobin-BSA       | 25 µg/L         | 15 min | Wu et al. 2019             |
| 34  | Acetamiprid                                 | Quantum Dot                         | Anti-acetamiprid mAb-QD                           | Acetamiprid-OVA            | 1 ng/mL         | 60 min | Liu et al. 2019a           |
| 35  | Parathion methyl                            | Horseradish peroxidase (HRP)        | HRP hapten 1                                      | Anti-parathion methyl-imidaclorid Ab (bispecific) | 0.058 ng/mL     | 22 min | Zhu et al. 2017            |
|     | Imidacloprid                                | Alkaline phosphatase (ALP)          | ALP Hapten 2                                      |                            |                 |       |                            |
| 36  | Triazophos                                  | MIP                                 | Triazophos hapten-IgG-FITC                         | MIP                        | 20 µg/mL        | 18 min | He et al. 2020             |
| 37  | OP-AChE (organophosphorus agents)           |                                     | Anti-AChE antibody-CNTs/SPCE                      | Anti-AChE antibody-CNTs/SPCE | 0.02 nM        | –      | Du et al. 2012             |
| 38  | Pyrethroid                                  | AbGI                                | MSP-SRG- anti-pyrethroid Ab (APTES-MCM)           |                            | < 1 ppb         | < 5 min | Costa et al. 2020          |
| 39  | Imidacloprid                                | Nano luciferase (NanoLuc)           | N terminus (C2-15-NanoLuc) and C terminus (NanoLuc-C2-15) | Anti-imidacloath monocolonal antibody (mAb) | 6.4 ± 0.4 ng/mL | Short time | Yu et al. 2018       |
| 40  | Chlorothalonil (CHL), imidacloprid (IMI), oxyfluorfen (OXY) | Ag4-NTP@Au | Coating antigen-CHL, coating antigen-IMI, coating antigen-OXY | Anti-mouse IgG             | –               | –      | Sheng et al. 2021          |
by several companies at a very affordable price compared to traditional chromatography techniques. This indicates that production cost is really one of the great advantages of LFA strips. It is worth noting that with the democratization of LFA devices for pesticide detection and monitoring and the entry of new companies on the market, there is a tendency to reduce costs in the near future.

Thus, it is biased to believe that LFA strips can be one of the most effective methods for field applications, mainly because of their simplicity and because it does not require technical knowledge, which are also critical parameters for a POCT device.

Challenges

Despite the numerous advantages highlighted for LFA strips, many advances are needed for the democratization of these systems for the detection of pesticides in environmental samples. Problems such as poor reproducibility and sensitivity to high analyte concentrations are frequently reported by researchers. Also, most LFA devices provide only qualitative or semi-quantitative results. Another challenge is the need for multiplex detection systems, given that there are almost always several pesticides used in the same crop with the availability of various residues in the environment. We found a reduced number of applications in the analysis of multiresidues (Kranthi et al. 2009; Xu et al. 2012; Wang et al. 2014; Ouyang et al. 2018a; Zou et al. 2019; Cheng et al. 2019). One of the reasons is that pesticides are normally small molecules and have only one antigenic determinant, which makes applications with direct immunoassays difficult. In these cases, the competitive immunoassay is the main method of choice for detecting these analytes.

It is worth noting that environmental samples include a wide range of possibilities, such as soil, water, and food from different origins and chemical properties. These matrices may require pre-treatment steps such as centrifugation, dilution, filtration, pH adjustment, etc. The selection of the method for pre-treatment of the sample is decided according to the nature of the matrix and has a direct influence on the quality of the results. The simplification and feasibility of these steps can contribute to gains in sensitivity and reproducibility, providing greater application, especially on large scales. It is important to emphasize that the development of technologies that integrate sample collection, processing, and detection is still on a distant horizon.

Commercialization of LFA for pesticide detection has not yet maintained pace with a large amount of research activity. In addition, the high cost and technical difficulties, such as multiple steps for sample extraction and low reproducibility for detection in complex matrices, are still difficult challenges to overcome.

Finally, there is a gap in the literature on how these devices should be disposed of in the environment. Several devices feature fluorescent labels, heavy metals, and quantum dots that are potentially harmful to the environment when used on a large scale. The disposal must comply with all applicable local, regional, national, and international regulations.

Perspectives

In general, there is a great demand for the development of fast and reliable analytical techniques to control various pesticides in food, agriculture, and environmental samples. Different LFIA-based pesticide detection strategies have been published. New approaches must overcome the challenges presented and provide advances in quantitative and multiple detections of pesticides. We list some important directions for the development of future research: (1) extending detection limits by inserting new markers and tools for signal amplification; (2) improvement in the specificity and sensitivity of LFAs with the development of new recognition elements such as aptamers and MIPs; (3) simultaneous detection of several pesticides in the same matrix; (4) use of machine learning algorithms with integration with electronic devices for intelligent delivery of the result; (5) simplification of sample processing steps, such as extraction and purification, without using pipettes and equipment; (6) integration of collection, processing, and detection steps.

Conclusion

In the last decade, the development of analytical methodologies for detecting pesticides in the environment has brought significant advances. As these are mostly field applications, simple detection methods with non-scientific personnel are enthusiastically pursued.

Lateral flow test strip technology can provide a promising approach for this purpose and is becoming an increasingly exploited tool that can provide compact, lightweight, responsive, and reliable detection devices in the field. Modern developments of LFA with various strategies for pesticide detection were analyzed and discussed, as well as the economic viability, challenges, and perspectives. New technologies have been implemented in the tests to improve the performance of the LFA, enhancing its sensitivity, specificity, and reproducibility. The main driver is the need for new technologies for simplifying the steps of extraction and LFA applications in quantitative and multi-residue analysis. Nevertheless, by solving these
problems, there is no doubt that LFIA-based technologies will be able to offer robust, portable, easy-to-operate, sensitive, and multiplexable devices shortly. These devices will allow the reliable monitoring of different matrices for detecting and quantifying pesticides, which is essential for quick decision-making and compliance with legislation.

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