

# Rapid test strips for analysis of mycotoxins in food and feed

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**Abstract** An overview is given on recent trends and applications of rapid immunodiagnostic tests for screening of food and feed for mycotoxins. Different test formats are discussed, and challenges in the development of lateral-flow devices for on-site determination of mycotoxins, with requirements such as being robust, fast, and cost-effective, are briefly elucidated.

**Keywords** Mycotoxins · Lateral-flow device · Immunodiagnostic assay · Rapid test · Food analysis · Feed analysis

## Introduction

Rapid diagnostic assays have been in use for decades in the clinical and medical sector with, e.g., the pregnancy test strip as one of the first commercially available test strips with widespread use. In the last decade, rapid immunoassay-based tests have also increasingly been used in the food and feed sector, where applications range from the screening for foodborne pathogens, drug residues, antibiotics, and mycotoxins, to allergens and, recently, genetically modified organisms. Amongst these, tests for mycotoxins which allow screening of agricultural commodities with results within 15 min are gaining acceptance and are being firmly

integrated into routine quality-monitoring procedures, because of the need for rapid on-site (pre)-screening [1].

Mycotoxins are toxic natural secondary metabolites produced by several species of fungus, for example *Aspergillus* and *Fusarium*, on agricultural commodities in the field or during storage. These toxins cause food- and feed-borne intoxication, and many are cytotoxic, carcinogenic, mutagenic, or immunosuppressive [2]. Due to the health risks for humans and animals, authorities such as the European Commission or the Grain Inspection, Packers and Stockyards Administration (GIPSA) have addressed the mycotoxin problem by adopting regulatory limits. Regulations are in force for, e.g., aflatoxins, ochratoxin A, and *Fusarium* toxins in selected foodstuffs (EC 1881/2006) [3], and there are recommendations for maximum levels of mycotoxins in feed (EC 2006/576/EC) [4]. In the United States, action levels or advisory levels are in force for, e.g., aflatoxins and deoxynivalenol, respectively [5].

Besides validated official analytical methods for mycotoxin detection based on chromatographic principles [6, 7], rapid screening tests and a number of new techniques such as biosensors are rapidly emerging [8–10]. Enzyme-linked immunosorbent assays (ELISAs) have become a standard tool for rapid monitoring of mycotoxins. Despite a high matrix dependence, microtiter plate ELISAs offer the advantages of speed, ease of operation, sensitivity, and high sample throughput. Nevertheless, faster and more straightforward immunoassay-based tests are preferentially used for applications where on-site use is necessary, because they allow rapid turnover. Fluorescence polarization immunoassays have been developed for mycotoxins such as DON, ZON, and aflatoxins [11] and are commercially available. These instrument-based assays are enzyme-free and homogeneous and make use of a mycotoxin-fluorophore conjugate such as a fluorescein tracer. Such rapid methods for

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analysis of mycotoxins have been reviewed elsewhere [12]. Rapid disposable membrane-based assay tests have been developed in multiple formats, for example test strips [13], flow-through tests [14], and dip sticks [15, 16]. Dip sticks work like an ELISA, with carrier membranes instead of microtiter plates. But similar to ELISA, the performance of one to four working steps such as washing, blocking, sample incubation, and staining requires a total time of 30 min to 3 h to obtain the test results, which cannot compete with a 5–10 min test strip. Flow-through membrane-based immunoassays are comparable with lateral-flow test strips in rapidity and ease of use. But these are qualitative or semi-quantitative tests and interpretation of results may be difficult when the test result is close to the cutoff level [12]. Although dip sticks and flow-through immunoassays have been developed for mycotoxins, they are not as commercially successful as test strips. This review will therefore focus on membrane-based test strips, also called lateral-flow devices (LFDs).

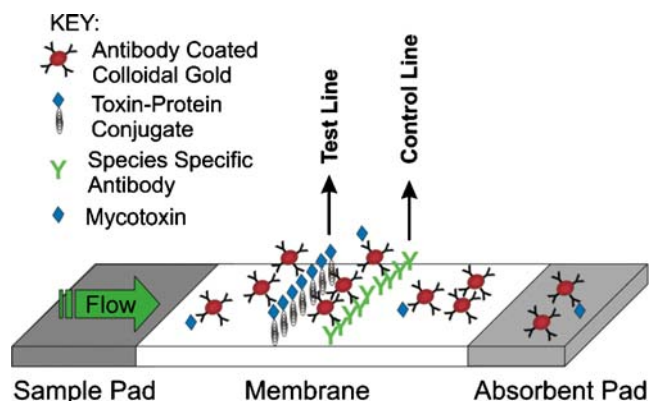
### Lateral-flow devices

LFDs are based on a test format which includes sample flow along an analytical nitrocellulose membrane due to capillary forces and enables fast and easy-to-handle immunoassays which can be both qualitative with a defined cutoff level or quantitative when used with a photometric strip reader. The development of rapid test systems for determination of contaminants such as mycotoxins in food previous to or during production to be implemented into hazard analysis and critical control point (HACCP) systems is of crucial importance. The cutoff level or working range of a mycotoxin rapid test will usually comply with existing regulations, and these tests have the advantage of requiring a relatively small investment in equipment and personnel. However, major restrictions so far are matrix dependence, lack of appropriate specific antibodies, and, therefore, selectivity and sensitivity problems.

Test strips for mycotoxins are based on a competitive immunoassay format in which a labeled antibody is used as signal reagent. Besides the classical enzyme immunoassay approach, a variety of reagents have been used for signaling, for example colored latex particles, colloidal gold particles, fluorescent labels such as, e.g., dye-loaded liposomes [17], carbon nanoparticles [18], and magnetic beads, as previously reviewed elsewhere [19]. Due to their ready availability, ease of production, and ease of conjugate formation with antibodies, colloidal gold is used in most test strips developed for mycotoxins. Colloidal gold particles with a diameter of approximately 40 nm are prepared by controlled reduction of tetrachloroauric(III) acid trihydrate with citric acid trisodium salt using the procedure described by

Frens [20] and Turkevitch [21]. Because of surface plasmon resonance effects, the 40 nm colloidal gold particles have a deep red color, which is exploited for test strip signaling. Conjugation of antibodies is performed after determining, by titration, the required concentration of antibody [22], using non-covalent interactions between colloidal gold particles and proteins, i.e. dative bonding, H-bonding, electrostatic forces, and hydrophobic adsorption.

The test strip components such as sample pad, conjugate pad, analytical nitrocellulose membrane, and absorbent pad are immobilized on a plastic backing card for better handling. The pads which are usually of, e.g., cellulose or glass fiber material will overlap each other and the analytical membrane by a few mm in order to guarantee sample flow along the strip. The absorbent pad at the end of the strip allows absorption of excess liquid, ensuring no backflow on to the membrane (Fig. 1). The signal reagent may either be mixed with sample extract in a microwell or previously immobilized on the strip on the conjugate pad. The test strip can, accordingly, be either a freestanding strip or enclosed within a plastic housing. The test strip is inserted into the well or the sample extract is applied directly to the strip (signal reagent previously immobilized) and the mixed content then migrates on to the nitrocellulose membrane, which contains a test zone and a control zone. In a competitive assay, as shown in Fig. 1, mycotoxin–protein conjugate coated on the test zone captures the free antibody–colloidal gold particle complex, allowing color particles to concentrate and form a visible line. The intensity of the test line is dependent on the analyte concentration and may be measured with a photometric reflectance strip reader. A species-specific antibody coated on the control zone will capture loaded and unloaded antibody–colloidal gold particle complex. One line will therefore always be visible in the control zone regardless of the presence of target analyte, confirming correct test development.



**Fig. 1** Principle of competitive assay in test strip format (freestanding test strip)

Gold colloid-based LFDs have been developed for the most prevalent mycotoxins, for example deoxynivalenol [23], aflatoxin B<sub>1</sub> [24, 25], fumonisin B<sub>1</sub> [26], ochratoxin A [27, 28], and T-2 toxin [13]. Most of the test strips developed are qualitative strips. Nevertheless, a trend can be seen towards (semi)quantitative test strips, driven by a strong demand from the industry, and towards multi-mycotoxin approaches such as a lateral-flow immunoassay for the rapid simultaneous detection of zearalenone and deoxynivalenol [29]. An increasing number of commercially available test kits for mycotoxins confirm the trend towards screening tests which are easy-to-use and allow rapid on-site decision-taking based on quantitative results. Test strip-based test kits for aflatoxins (qualitative and quantitative), deoxynivalenol (semi-quantitative and quantitative), fumonisins (qualitative and quantitative), ochratoxin A (quantitative), and zearalenone (quantitative) are commercially available. GIPSA has a test kit evaluation program to verify the performance of commercially available test kits and provides a listing on its homepage [30]. Nevertheless, problems with reproducibility, reliability with different matrices, and sensitivity may sometimes limit their application [7]. Over or underestimation of mycotoxins when using rapid tests is still an issue often attributed to cross-reactivity of the antibody to closely related fungal metabolites and/or to the matrix itself. Different matrices have been shown to have different effects on the test result, as has been previously shown not only for rapid tests but mainly for standard chromatographic mycotoxin analysis [31]. Rapid membrane-based tests do not include a clean-up step before measurement, which increases speed, although at the expense of accuracy since interfering substances in the sample extract are not removed. Maize is often a more difficult matrix than wheat, because of the higher content of co-extracted fatty components. Measurement changes obtained with maize samples may be substantial compared with wheat samples, with strong signal suppression thus affecting the regression lines [11]. Also, large differences observed between spiked samples and naturally contaminated samples contribute to calibration and validation problems. For qualitative test methods there are no general validation procedures available. Solely the cutoff level is defined as the concentration threshold below which positive identification becomes unreliable [32]. Ten replicates were used to determine the cutoff level of a T-2 toxin test strip with results showing that the test strip was selective and sensitive for the determination of T-2 toxin in wheat and oats [13]. During test strip development the following criteria must be fulfilled:

- a reproducible and color-intense control line;
- a visual detection limit at the desired cutoff in selected commodities, with no test line visible at sample concentrations at and above the cutoff; and

- no background coloring of membrane due to non-specific binding of excess colloidal gold particles.

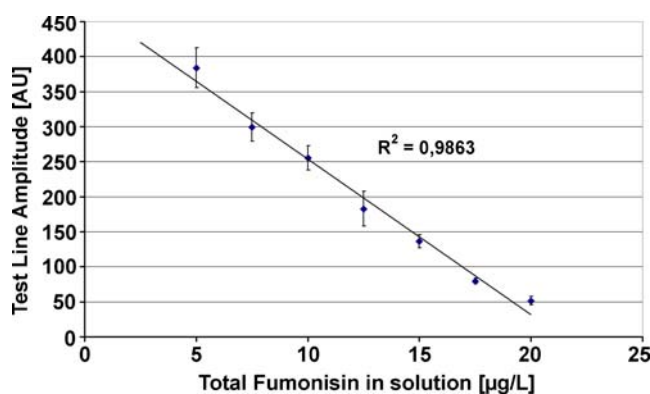
Challenges include adjusting the flow properties of the test strip and, as already mentioned, reducing matrix background interference by optimization of multiple parameters including:

1. type and pore size of analytical membrane;
2. type and concentration of blocking agent for blocking membrane binding sites after spraying of reagents;
3. type of buffer, pH range and ionic strength; and
4. use of surfactants and modifiers for pre or post treatment of test strip materials

to name only test strip development itself. Test strip production showed that the blocking procedure of the NC membrane after spraying the reagent lines was a critical step for obtaining reproducible test results and ensuring longer stability of the test strips. Similar to ELISA, optimization with a selection of reagents such as BSA protein solution, fish gelatin, or conalbumin A is necessary.

The quality of available antibodies is a further issue that should not be neglected [33, 34]. Antibody sensitivity and specificity will have a strong influence on the performance of the developed membrane-based immunoassay [35].

One of the advantages of rapid immunoassay-based tests is that sample clean-up can be omitted. Nevertheless, sample extraction must consider both extraction efficiency of mycotoxins and solvent compatibility with the antibodies applied in the test. The organic solvent tolerance of an antibody to solvents such as methanol or acetonitrile must be tested and will determine the end concentration before performing the test. A further dilution step of the extract



**Fig. 2** Relative reflectance value of the screened test line of a total fumonisin test strip versus the total fumonisin concentration in solution of the extracted maize quality-control material ( $2406 \pm 612 \mu\text{g kg}^{-1}$  FB1 and  $630 \pm 116 \mu\text{g kg}^{-1}$  FB2) ( $n=3$ ). The naturally contaminated maize extract ( $1:80$  with water, therefore  $2000 \mu\text{g kg}^{-1}$  corresponding to  $25 \mu\text{g L}^{-1}$ ) was mixed with blank maize extract ( $1:80$  with water). Further dilution steps were used when sample concentrations were higher

with buffer is usually required, otherwise, e.g., false negative signals may be obtained [13]. For the extraction of mycotoxins, mixtures of methanol and water or acetonitrile and water, which may also contain a modifier such as acetic acid, are commonly used [36]. Extraction procedures for mycotoxin analysis from agricultural commodities such as wheat or maize are known and have been described in many reviews elsewhere. Changes in extraction solvent composition such as, e.g., varying amounts of methanol or acetonitrile in a methanol–water or acetonitrile–water mixture have shown to strongly affect extraction efficiency [36].

Last, but not least, large differences may be observed between spiked samples and naturally contaminated samples with shifts of the cutoff level in the test strip or shifts of relative reflectance readings of the test line in a quantitative test strip. The test strip optimization and validation should therefore be performed using only naturally contaminated material and a reference method, e.g. LC–MS–MS [36], used for sample characterization and monitoring of rapid test performance. Although limited, reference material for quality-control measurements [37] and certified reference materials are available for selected commodities and mycotoxins and should be used to confirm the trueness of developed methods [38, 39]. A maize quality-control material for the fumonisins FB1 and FB2 (Fumonisin FB1+FB2 in Maize Flour; Biopure Referenzsubstanzen, Tulln, Austria) has been used for calibration of a fumonisin test strip ( $n=3$ ) developed in our laboratory, as shown in Fig. 2. The preliminary data presented should briefly illustrate that calibration of optimized test strips which use colloidal gold particles as detector reagent with well characterized material may enable fast quantitative detection of mycotoxins such as total fumonisins in maize (1:80 sample extraction with water) when used in combination with a photometric strip reader. The test performed with an extraction time of only 3 min and a test time of 3 min underlines the rapidity and ease of use of test strips for mycotoxins.

In the last decade, the demand for rapid tests which can be easily integrated into raw material selection or the production chain, e.g. into HACCP protocols, has increased in the food and feed sector. Gold colloid-based immunochromatographic test strips for the detection of mycotoxins fulfill many requirements, being fast, easy to handle, and allowing on-site pre-screening.

## Outlook

Established state-of-the-art chromatography-based methods for determination of mycotoxins are increasingly being complemented by a number of methods for fast and cost-

effective analysis, including rapid test strips. Although problems in test strip development such as insufficient sensitivity, selectivity, or strong matrix dependence may remain, high-quality test strips are rapidly emerging and complementing classical detection methods in which rapid screening is needed. Because the price for simplification is usually lower sensitivity, obtaining extremely good antibodies will remain a major requirement for easy-to-use assays. The optimization and validation of innovative test strips will contribute to meeting contract or legislative specifications for maximum acceptable levels of mycotoxins in foods and feed through effective and rapid screening.

## References

1. Delmulle BS, De Saeger SMDG, Sibanda L, Barna-Vetro I, Van Peteghem CH (2005) *J Agric Food Chem* 53:3364–3368
2. JECFA (2001) Joint FAO/WHO expert committee on food additives, 56th meeting, Geneva, Switzerland, 6–15 February 2001
3. European Commission Regulation No 1881/2006 Off J Eur Union L364:5–24
4. European Commission Recommendation No 2006/576/EC Off J Eur Union L229:7–9
5. FAO (2006) Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper No. 81. Rome, Italy
6. Krska R, Welzig E, Berthiller F, Molinelli A, Mizaikoff B (2005) *Food Addit Contam* 22:345–353
7. Krska R, Schubert-Ullrich P, Molinelli A, Sulyok M, Macdonald S, Crews C (2008) *Food Addit Contam* 25:152–163
8. Krska R, Molinelli A (2007) *Anal Bioanal Chem* 387:145–148
9. Prieto-Simón B, Noguera T, Campàs M (2007) *Trends Anal Chem* 26:689–702
10. Urraca JL, Benito-Peña E, Pérez-Conde C, Moreno-Bondi MC, Pestka JJ (2005) *J Agric Food Chem* 53:3338–3344
11. Maragos CM, Plattner RD (2002) *J Agric Food Chem* 50:1827–1832
12. Zheng MZ, Richard JL, Binder J (2006) *Mycopathologia* 161:261–273
13. Molinelli A, Grossalber K, Führer M, Baumgartner S, Sulyok M, Krska R (2008) *J Agric Food Chem* 56:2589–2594
14. Paepens C, De Saeger S, Sibanda L, Barna-Vetró I, Légli I, Van Hove F, Van Peteghem C (2004) *Anal Chim Acta* 523:229–235
15. Usleber E, Schneider E, Märtlbauer E, Terplan G (1993) *J Agric Food Chem* 41:2019–2023
16. Stephan O, Möller N, Lehmann S, Holzhauser T, Vieths S (2002) *Eur Food Res Technol* 215:431–436
17. Ho J-AA, Wauchope RD (2002) *Anal Chem* 74:1493–1496
18. Van Dam GJ, Wichers JH, Falcao Ferreira TM, Ghatai D, van Amerongen A, Deelder AM (2004) *J Clin Microbiol* 42: 5458–5461
19. Chan CP-Y, Cheung Y-C, Renneberg R, Seydack M (2007) *Adv Biochem Eng/Biotechnol* 109:123–154
20. Frens G (1973) *Nature: Phys Sci* 241:20–22
21. Turkevitch J, Stevenson PC, Hillier J (1951) *Discuss Faraday Soc* 11:55–75

22. Horsiberger M, Rosset J (1977) *J Histochem Cytochem* 25:295–305
23. Kolosova AY, Sibanda L, Dumoulin F, Lewis J, Duveiller E, Van Peteghem C, De Saeger S (2008) *Anal Chim Acta* 616:235–244
24. Delmulle BS, De Saeger SMDG, Sibanda L, Barna-Vetro I, Van Peteghem CH (2005) *J Agric Food Chem* 53:3364–3368
25. Xiulan S, Xiaolian Z, Jian T, Xiaohong G, Jun Z, Chu FS (2006) *Food Contr* 17:256–262
26. Wang S, Quan Y, Lee N, Kennedy IR (2006) *J Agric Food Chem* 54:2491–2495
27. Cho Y-J, Lee D-H, Kim D-O, Min W-K, Bong K-T, Lee G-G, Seo J-H (2005) *J Agric Food Chem* 53:8447–8451
28. Wang X-H, Liu T, Xu N, Zhang Y, Wang S (2007) *Anal Bioanal Chem* 389:903–911
29. Kolosova AY, De Saeger S, Sibanda L, Verheijen R, Van Peteghem C (2007) *Anal Bioanal Chem* 389:2103–2107
30. GIPSA test kit evaluation program (2008) <http://www.gipsa.usda.gov/GIPSA>
31. Häubl G, Berthiller F, Krska R, Schuhmacher R (2006) *Anal Bioanal Chem* 384:692–696
32. CITAC/Eurachem (2002) *Guide to quality in analytical chemistry*. Eurachem Secretariat. Teddington, Middlesex, UK
33. Gathumbi JK, Usleber E, Märtilbauer E (2001) *Lett Appl Microbiol* 32:349–351
34. Dietrich R, Schneider E, Usleber E, Märtilbauer E (2006) *Nat Toxins* 3:288–293
35. Fremy JM, Usleber E (2003) *J AOAC Int* 86:868–871
36. Sulyok M, Berthiller F, Krska R, Schuhmacher R (2006) *Rapid Commun Mass Spectrom* 20:2649–2659
37. FAPAS (2008) <http://www.fapas.com/>
38. Josefs RD, Krska R, MacDonald S, Wilson P, Pettersson H (2004) *Anal Bioanal Chem* 378:1182–1189
39. <http://www.irmm.jrc.be/html/homepage.htm>