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## Current trends in rapid tests for mycotoxins

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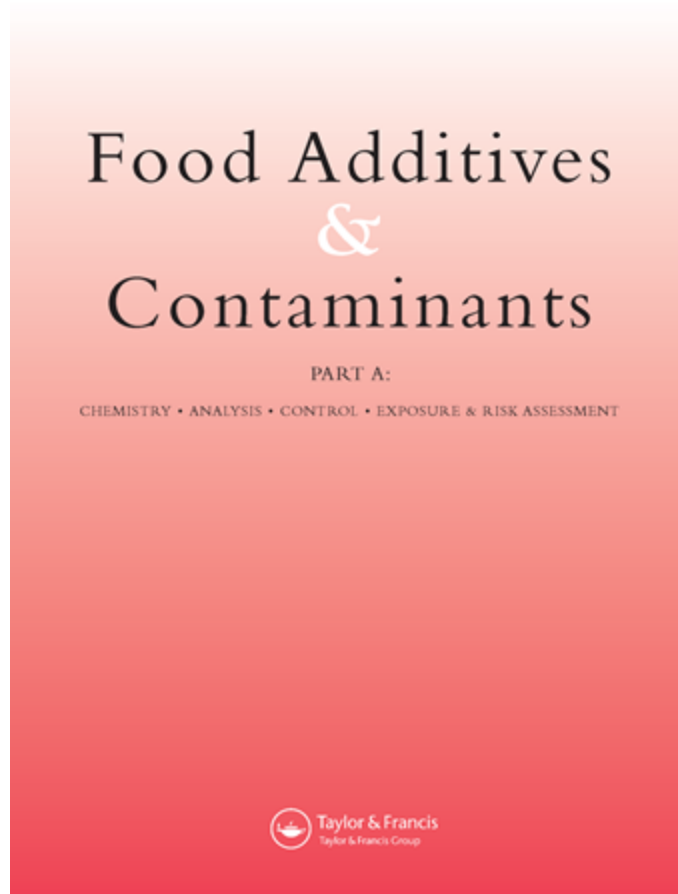
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### Current Trends in Rapid tests for Mycotoxins

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Abstract:	There is an ample number of commercial testing kits available for

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	<p>mycotoxin analysis on the market today, including enzyme-linked immunosorbent assays, membrane-based immunoassays, fluorescence polarisation immunoassays and fluorometric assays. It can be observed from the literature, not only are developments and improvements on going for these assays but there are also novel assays being developed using biosensor technology. This review focuses on both the currently available methods and recent innovative methods for mycotoxin testing. Furthermore, it highlights trends that are influencing assay developments such as multiplexing capabilities and rapid on-site analysis, indicating the possible detection methods that will shape the future market.</p>

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## Current trends in rapid tests for mycotoxins

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### Abstract

There is an ample number of commercial testing kits available for mycotoxin analysis on the market today, including enzyme-linked immunosorbent assays, membrane-based immunoassays, fluorescence polarisation immunoassays and fluorometric assays. It can be observed from the literature that not only are developments and improvements ongoing for these assays but there are also novel assays being developed using biosensor technology. This review focuses on both the currently available methods and recent innovative methods for mycotoxin testing. Furthermore, it highlights trends that are influencing assay developments such as multiplexing capabilities and rapid on-site analysis, indicating the possible detection methods that will shape the future market.

**Keywords:** mycotoxin; immunoassay; biosensor; on-site detection; rapid detection method; multiplex detection

### Introduction

Naturally occurring food and feed contaminants are unavoidable making them a major issue in global food safety, particularly those that pose serious health concerns to humans and animals. Mycotoxins are one such example. These are secondary metabolites of low-molecular weight that are produced by various fungi, the genera of which include

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3 *Aspergillus*, *Penicillium* and *Fusarium* (Marin et al. 2013; Sweeney et al. 1998). Mycotoxins  
4 mainly enter the food chain because of fungal colonization either of pre-harvest susceptible  
5 crops, or during the time between harvesting and drying, or during storage (Alshannaq et al.  
6 2017). The most agriculturally significant mycotoxins in terms of being a health threat to  
7 humans and animals include aflatoxins (AF), fumonisins (FUM), deoxynivalenol (DON) and  
8 other trichothecenes (T2 and HT2 toxins), ochratoxin A (OTA) and zearalenone (ZEN)  
9 (Raiola et al. 2015) (Table 1). The ergot alkaloids (EA), which are produced by fungi of the  
10 genus *Claviceps*, are another class of mycotoxins tested for in food and feed. The three  
11 main groups of EA's are the clavine alkaloids, lysergic acids and ergopeptines. Ergotamine  
12 and ergovaline are some of the EAs most commonly tested for (Crews 2015). Whilst  
13 mycotoxin remediation seems an ideal solution to the problem, many commonly used  
14 physical and chemical methods raise issues regarding safety and nutritional loss of food and  
15 feed (Ji et al. 2016).

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24 The difficulties associated with remediation combined with the health risks of  
25 mycotoxins has provoked other control methods to ensure food safety. This is utilising  
26 accurate and reliable food testing methods to prevent food and feed contaminated with high  
27 levels of mycotoxins reaching the consumer market. The detection of mycotoxins has been  
28 ongoing for approximately 50 years (Wong and Lewis 2017). Maximum levels at which  
29 mycotoxins are permitted in food and animal feed have been set in Regulation (EC)  
30 1881/2006 (EC, 2006a) and subsequent amendments under European legislation and  
31 implemented by Member States. This has subsequently driven the development and  
32 commercialisation of methods for mycotoxin analysis in food and feed.

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38 A myriad of methods for mycotoxin analysis are available and their usage is  
39 dependent on a variety of factors. For example, liquid chromatography tandem mass  
40 spectrometry (LC-MS/MS) is currently extensively used as a confirmatory reference method  
41 as it allows the simultaneous determination of different mycotoxins. Other chromatographic-  
42 based methods exist which can be coupled with ultraviolet (UV), diode array (DAD), and  
43 fluorescence (FLD) detectors including thin-layer chromatography, or high-performance  
44 liquid chromatography (HPLC), the latter of which is more sensitive and reliable, particularly  
45 when coupled FLD's and subject to adequate clean-up (De Santis et al. 2017; Matabaro et  
46 al. 2017; Man et al. 2017). These methods however are limited to large commercial  
47 companies, reference and academic laboratories with skilled technicians and expensive  
48 laboratory equipment. Furthermore, they are generally time-consuming and labour-intensive  
49 (Ahmed et al. 2017). In this regard rapid commercial test kits for mycotoxin analysis are used  
50 as an alternative for more user-friendly, inexpensive, robust detection.  
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The majority of test kits on the market today are based on an immunoassay format which relies on the ability of an antibody to bind to a specific antigen structure (target molecule). As mycotoxins are relatively small molecules (less than 1 kDa) a 'sandwich' type immunoassay in which multiple antibodies bind to a single toxin is not suitable, rather a competitive assay is generally employed (Li et al. 2014; Maragos 2009). In a competitive immunoassay, the analyte of interest from a sample will affect the detection and measurement of a competitor, meaning the result generated is an indirect measurement of the analyte of interest. The properties of the competitor will vary depending on the assay but regardless, every competitor must be capable of triggering a signal that can be detected and measured by the device/reader in place for that assay. Conventional and currently manufactured immunoassay based test kits for screening of mycotoxins can be used by importers, traders, and food and feed manufacturers. These include Enzyme Linked Immuno-Sorbent Assays (ELISAs), Lateral Flow Immunoassays (LFIA) and fluorometric assays that use two sample clean-up methods; immunoaffinity column (IAC) and solid phase extraction (SPE) column clean-up (Alshannaq et al. 2017; Berthiller et al. 2017; Li et al. 2014; Turner et al. 2015) (Table 2).

Assay requirements are evolving which has an impact on the performance of the current above mentioned immunological test kits. The main current trends to be met include portability and multi-toxin detection. The reason for portability becoming increasingly important lies in the growing demand for on-site testing. On-site testing can occur at points in the food production process. For example, upon reception of raw materials to a feed mill, analysis of the material can be conducted in a low technology environment using a portable test kit. Results can be obtained rapidly as samples are not required to be sent off to reference laboratories for analysis. This also prevents the food production process slowing down. On-site testing can therefore be considered a time-efficient and cost-effective choice. Multi-toxin testing eliminates the need to purchase and run multiple single-toxin tests for one sample batch. Therefore, portable multi-toxin testing devices suitable for onsite testing would give added cost and time-saving benefits. Of course, another factor in producing a cost-effective assay lies in the actual fabrication and overall cost of the test itself. Furthermore, the length of time it takes to generate a result is another important factor in time-efficient assays.

This review will give a description of commercially available immunological test kits for mycotoxin analysis as well as highlighting advantages and disadvantages of the methodology. Following this, any progression in immunological test kits in regard to current trends in mycotoxin analysis will be discussed as well as any limitations of the assay hindering progression. Moreover, there are also advancements in the development of

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3 biosensor platforms offering to meet current trends in mycotoxin analysis which will also be  
4 discussed in this review.  
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### 7 **Commercially available Test Kits**

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9 As is evident from Table 2, the market today for mycotoxin test kits is already very  
10 competitive. Some recent developments include the lateral flow devices being sold by R-  
11 Biopharm that utilise a mobile application on a smartphone to analyse colour signal in lieu of  
12 a reader, specifically for AFs, T2/HT2, ZEN and FUM. Another advance in that market from  
13 Randox is the development of Biochip Array Technology (BAT), a chemiluminescent based  
14 assay capable of multiplex analysis. The chips, which are specifically available for mycotoxin  
15 testing, include the Myco10, 7, 5 and flex arrays. The Randox device is laboratory based  
16 and whilst there is a portable device on offer, it is not currently specified to be used with the  
17 mycotoxin specific arrays. Of the companies in Table 2, 67% offer ELISAs, 75% offer lateral  
18 flow devices and 25% offer fluorometric assays. These assays along with some others will  
19 be discussed in more detail below.  
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### 28 **Enzyme-linked Immunosorbent Assay**

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30 ELISAs are traditional for mycotoxin detection. Most commercial ELISAs employ a direct  
31 competitive immunoassay format (*Figure 1(a)*), using an antibody coated 96 well microtiter  
32 plate (Li et al. 2014). Sample/standard is added into the appropriate wells of the plate  
33 followed by an enzyme-coupled mycotoxin conjugate solution. During an incubation period,  
34 competition occurs between any mycotoxin present in the sample/standard and the enzyme-  
35 conjugated mycotoxin for a limited number of antibody binding sites. After incubation the  
36 plate is washed to remove any unbound mycotoxin and an enzyme substrate is added. This  
37 results in a colour reaction occurring between the enzyme substrate and any enzyme-  
38 coupled mycotoxin bound to the antibodies immobilised onto the microtiter plate. The  
39 intensity of the colour is therefore inversely proportional to the mycotoxin concentration in  
40 the standard/sample extract. After another short incubation period with the enzyme substrate  
41 for colour development, the reaction is halted by adding a stop solution. The plate is then  
42 placed immediately into a colorimetric reader for optical density measurement.  
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51 Commercial ELISA kits are high throughput, selective, sensitive assays that require little  
52 sample preparation (Rahmani et al. 2009; Zheng et al. 2006). Assays have also become  
53 more rapid, for example Romer labs offers ELISAs for DON, OTA, AF's, T2, ZEN and FUM  
54 with incubation periods of 15 min. On the other hand, most are between 1 and 2 hours. In  
55 some cases, overestimation of results, due to cross-reactivity of antibodies (Zachariasova et  
56 al. 2014), and false positive results due to the matrix effect, can interfere with ELISA  
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3 readings. To eliminate these effects, most kits specify the matrices to which the ELISA kit  
4 can be applied. However, this can also be considered a disadvantage of the product.  
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7 In terms of meeting current trends, multiplexing abilities of ELISAs have been  
8 demonstrated by Urusov et al. (2015). The group immobilised AFB1, OTA and ZEA in  
9 different wells of a single microplate. The study used a competitive format based on *Figure*  
10 *2(b)*, where analytes in the sample and biotin conjugated antibodies were added to antigen-  
11 coated wells followed by a streptavidin–polyperoxidase conjugate, which through reaction  
12 with bound biotin-antibody produced a high detectable signal. The developed method was  
13 successfully validated using poultry processing products and corn samples spiked with  
14 known quantities of mycotoxins. The LODs for AFB1, OTA and ZEA in these matrices were  
15 0.24, 1.2 and 3 ng/g, respectively. Furthermore, with the advancements in nanotechnology,  
16 McNamee et al. (2017) demonstrated a multiplex nanoarray based on ELISA. ZEA, T2 and  
17 FUMB1 conjugates were nano-spotted into single wells of a microplate. The sensitivity of the  
18 assay was determined by the IC50 values which were 197.4, 0.7 and 216.7 µg/kg in wheat  
19 and 43.6, 0.5 and 25.9 µg/kg in maize for ZEA, T2 and FUMB1 respectively. The group  
20 highlighted the assays comparison to an ELISA protocol, making it easily adaptable by end-  
21 users accustomed to running ELISAs, whilst providing higher sample throughput with high  
22 sensitivity and accuracy.  
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25 Developments in the ELISA technique are also being made with the use of  
26 nanomaterials (Liang et al. 2016; Pei et al. 2018; Xiong et al. 2018). For example,  
27 Hendrickson et al. (2018) used magnetic nanoparticles (MNP) suspended in the reaction  
28 medium as a solid support for antibody binding. This pseudo-homogeneous regime as the  
29 group named it, results in the antibody covering a greater surface area and being more  
30 evenly distributed in the medium which increases antibody-antigen diffusion processes,  
31 reducing the time of the assay. With the use of a magnet, the assay also allows for rapid and  
32 simple separation of the MNP-antibody-antigen complexes. The group referred to this as a  
33 pre-concentration step, which when combined with chemiluminescence detection, achieved  
34 ZEN control in wine with detection limit of 0.03–0.05 ng/g.  
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## 49 **Membrane-based Immunoassays**

### 50 *Lateral Flow Immunoassay*

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52 Lateral flow immunoassays (LFA) also known as lateral flow tests, immunochromatographic  
53 tests or strip tests are typically a competitive format where a labelled antibody is used as a  
54 signal reagent (*Figure 2(b)*). The label can vary from quantum dots (QDs) to luminescent  
55 nanoparticles to amorphous carbon nanoparticles (Zhang et al. 2017). Traditionally it is a  
56 gold nanoparticle (Krska and Molinelli 2009). The strip itself consists of four parts, the  
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3 sample pad, conjugate pad, porous membrane and final absorbent pad. If the strip is  
4 freestanding, the labelled antibody is mixed with the sample extract in a microwell before  
5 application to the sample pad. If the strip is enclosed in plastic housing, the sample is  
6 directly added to the sample pad; it then migrates to the conjugate pad, binding to already  
7 immobilized labelled antibody here. Regardless of application, the sample and labelled  
8 antibodies will migrate to the nitrocellulose membrane, which contains a test zone and a  
9 control zone. In a competitive assay, mycotoxin–protein conjugate is coated on the test zone  
10 and the control zone consists of a 2nd antibody. The control zone will always be visible  
11 regardless of the presence or absence of mycotoxin because the 2nd antibody always  
12 captures an anti-2nd labelled antibody. The anti-2nd labelled antibody resides on the  
13 conjugate pad and flows with the sample and labelled antibodies over the test and control  
14 zones. This is an important part of the assay as it ensures that the sample has in fact flowed  
15 through the device. In a negative sample, the free labelled antibody binds to the mycotoxin–  
16 protein conjugate on the test zone, forming a visible line. In a positive sample, the labelled  
17 antibody will not bind to the mycotoxin-protein conjugate on the test zone as the binding  
18 sites of the antibody will be saturated from the toxin in the contaminated sample. As a result,  
19 no visible line will form (Maragos and Busman, 2010). Line visibility at the test zone is  
20 dependent on the degree of sample contamination. LFAs will have a cut-off level which is  
21 the point of discrimination between positive and negative samples. A positive sample with a  
22 mycotoxin concentration equal to or beyond the assay cut-off level will therefore result in no  
23 visible line in the test zone. This level must meet the regulatory requirements for the  
24 maximum permissible level of contamination.

25 LFAs are strong competitors on the market for mycotoxin detection (Tripathi et al. 2018). The  
26 method can give qualitative and/or semi-quantitative results, is simple, and capable of  
27 generating results within minutes (for example Afla-V by Vicam, 4 min). Furthermore, LFAs  
28 and their readers are easily portable, making them ideal for on-site analysis. In recent years,  
29 LFAs have been demonstrated as capable of multiplex mycotoxin determination. Song et al.  
30 (2014) demonstrated this in a recent paper where an LFA was developed for qualitative  
31 and/or semi-quantitative determination of AFB<sub>1</sub>, ZEN, DON and their analogues (AFs,  
32 ZENs, DONs) in cereal samples. The LFA device had multiple test lines, each with a  
33 different mycotoxin-conjugate (DON-BSA, ZEN-BSA and AFB<sub>1</sub>-BSA). The monoclonal  
34 antibodies adopted were class specific, so the LFA strip could simultaneously detect three  
35 groups of mycotoxins in a single assay. The assay was rapid (15 min) and both visual LODs  
36 and calculated LODs (0.05, 1, and 3 µg/kg, respectively) were lower than the EU maximum  
37 levels. Recoveries also ranged from 80% to 122%. Foubert et al. (2017) used green, orange,  
38 and red epoxy-functionalized silica-coated QDs as a signal reagent, rather than gold  
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3 nanoparticles, conjugated to anti-ZEN, anti-DON, and anti-T2 mAb, respectively, for ZEN,  
4 DON and T2 toxin detection. The LFA also gave a fast result (15 min) with a low false-  
5 negative rate (<5%). Results were easy to interpret visually by having a different colour  
6 correspond to a different toxin. Validation studies on multiplex lateral flow devices have also  
7 been conducted by Lattanzio et al (2013). The study demonstrated the ruggedness of the  
8 test and showed a false positive rate lower than 6 %. Furthermore, the group considered the  
9 test to have significant economic benefits when using it under real-world conditions.

### 16 *Flow-Through Immunoassay*

17 Flow-Through tests are in principle based on the competitive format commonly used in  
18 ELISAs. However, rather than anti-mycotoxin antibody being bound to a microwell, it is  
19 bound to a membrane upon filter paper (*Figure 1(a)*), (Trucksess et al. 1994). The test relies  
20 on the sample extract flowing through filter paper and any mycotoxin present binding to the  
21 antibody on the membrane. Along with the sample extract, enzyme-conjugated mycotoxin is  
22 added, and competition between it and any mycotoxin in the sample occurs for free antibody  
23 binding sites on the membrane. Following this an enzyme substrate solution is added. If the  
24 sample is negative, then the antibody binding sites become saturated with the enzyme-  
25 conjugated mycotoxin and a colour reaction will occur between the enzyme and substrate.  
26 However, if a sample is positive, the mycotoxin will bind to the antibody binding sites and no  
27 colour reaction will take place upon addition of the substrate. Therefore, the colour intensity  
28 is inversely proportional to the amount of toxin present in the sample.

29 Flow-Through immunoassays can give qualitative and/or semi-quantitative results,  
30 are simple and capable of generating results within minutes (for example OCHRACARD by  
31 R-Biopharm, 5 min). In terms of limitations of the test, membrane saturation and high cut-off  
32 values can lead to inaccurate interpretation of results (Beloglazova et al. 2017). Membrane  
33 saturation can occur when the volume of sample used exceeds that which can be absorbed  
34 by the system. This can affect colour development and subsequently the final readings.  
35 However, the addition of more absorption layers can reduce potential membrane saturation.  
36 Furthermore, recent studies have also aimed to optimise cut-off values. From 2000-2010  
37 there have been developments in Flow-Through tests from single to two-analyte analysis  
38 (Paepens et al. 2004, Saha et al. 2007; Sibanda et al. 2000). More recently, multiplexing has  
39 been shown possible with the flow-through approach. (Burmistrova et al. 2014; Ediage et al.  
40 2013). developed a flow-through test for multiplex screening of ZEN, DON, AFB1 and OTA.  
41 The group tested cereal-based feed ingredients and compound feeds (wheat, barley,  
42 soybean, wheat bran, rice, rice bran, maize, rapeseed meal, sunflower meal) and various  
43 types of complete feed (duckling feed, swine feed, broiler feed, piglet feed).

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The developed assay revealed cut-off levels for ZEN, DON, AFB1 and OTA that were 50, 200, 1, and 10 µg/kg, respectively, which comply with European regulations No 401/2006 (EC 2006b) and 519/2014 (EC 2014). Sample pre-treatment involved extraction, dilution and solid-phase extraction by addition of C18 sorbent followed by final filtration of the supernatant. The numbers of false-positive and false-negative outcomes were <5%, which is consistent with the Commission Decision 2002/657/EC (EC 2002). Furthermore, according to Burmistrova *et al* (2014) and Beloglazova *et al* (2017), it is expected that separate test zones in a multiplex Flow-Through assay minimise non-specific interactions between immunoreagents compared to the test zones in a multiplex lateral flow assay. This is due to the sample solution simultaneously contacting the separate immunoreagent test zones in a Flow-Through assay, compared to a lateral flow, where cross-influence of immunoreagents can be affected by the liquid running from the front of the system and passing all test zones (Beloglazova *et al.* 2017). Furthermore, Flow-Through assays and their readers are easily portable, making them ideal for on-site analysis.

Membrane based immunoassays (lateral flow and Flow-Through) are progressing to meet current trends as both have shown the possibility of multiplex testing and both are very suitable for on-site testing. Furthermore the tests are very quick, generating results within minutes.

### Fluorescence Polarization Immunoassay

The fluorescent polarization immunoassay (FPIA) is based on the principle that when a fluorescent molecule in solution is exposed to polarized light at its excitation wavelength the resulting emission is depolarized. The polarization is a measure of the orientation of the fluorescence emission from both horizontal and vertical directions. Small fluorescent molecules have higher rates of rotation and lower polarization than larger molecules. The interaction of a fluorophore with a relatively large molecule such as an antibody reduces the rate of the rotation motion of the fluorophore, resulting in an increase in observed polarization. This polarisation increase can be detected and measured, making this phenomenon suitable for the development of a competitive immunoassay for mycotoxin detection (Smith and Eremin, 2009). In a competitive format the mycotoxin in the sample competes with a tracer (mycotoxin-fluorophore tracer) for binding sites on a mycotoxin-specific antibody (*Figure 1(b)*). In a negative sample, binding of the antibody to the tracer increases polarization whereas in a positive sample, lesser antibody is bound to the tracer, reducing polarization. The polarization value is thus inversely proportional to mycotoxin concentration.

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FPIA has a homologous format conducted in solution phase, which unlike a heterogeneous immunoassay such as ELISA does not require the separation of the free and bound tracer nor the use of an enzymatic reaction. This is beneficial, as no separation and washing steps are required which reduces assay time, increases throughput and eliminates incubation steps for colour development and overall simplifies the method for ease-of-use. On the other hand, matrix effects can cause interference in reading of results if samples with coloured compounds are not correctly pre-treated (Valenzano et al. 2014). Rapid FPIA procedures have been developed since the early 2000's for the determination of many mycotoxins including DON, ZEN, FUMs, AFs and OTA, a review of which was done by Maragos (2009). More recently Bondarenko and Eremin (2012) compared the effect that various fluorescein-based tracers had on the sensitivity of detection of ZEN and OTA. The study found that not only were the best tracers ethylenediamine thiocarbamoylfluorescein (EDF) and aminomethylfluorescein (AMF) but that using OTA-AMF gave a lower detection limit of 1.5 ng/ml compared to OTA-EDF, which was 6 ng/ml in a model system. When carried out using spiked grain samples, LODs for ZEN and OTA with their respective best tracers were 15 and 10 µg/kg, respectively. In 2014, Sheng et al. tested an antiAFB1 mAb with the aim of developing a total AF assay. The antibody had high cross reactivities of 100%, 65.7%, 143%, 111.4% to AFB1, AFB2, AFG1 and AFM1, respectively, the LOD was 13.12 ng/mL and total analysis time was 5 minutes. Li et al. (2016) developed a multi-wavelength fluorescence polarization immunoassay (MWFPIA) for multiplexed detection of DON, T-2 toxin and FUMB1 in maize which were labelled with different dyes for discrimination during detection. Under optimal conditions, the LODs using MWFPIA were 242.0 µg/kg for DON, 17.8 µg/kg for T-2 toxin and 331.5 µg/kg for FUMB1, providing sufficient sensitivity to meet the action levels of these three contaminants in maize as set by the EU. Twenty naturally contaminated maize samples were tested using MWFPIA and HPLC-MS/MS, with correlation coefficients ( $R^2$ ) of 0.97 for DON and 0.99 for FUMB1. FPIA requires sample pre-treatment to decrease the matrix effects and obtain accurate polarisation readings, otherwise there is a risk of overestimation of results. Furthermore, sample pre-treatment increases total assay time (30 min in total for Li et al 2016). Nevertheless, the application is still suitable for onsite analysis provided reliable sample pre-treatment is in place.

### 54 **Fluorometric Assay**

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In a fluorometric assay, after a preliminary solvent extraction from the solid matrix, typically with a mixture of acetonitrile or methanol and water, the mycotoxin extract is commonly cleaned up/enriched by SPE or IAC. The purified sample is then put into a fluorometer for

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3 analysis. Extract purification is required for eliminating matrix effects and potential  
4 fluorescent compounds that could generate false-positive results. Although analysis time  
5 and cost are increased in this case, the analytical method benefits from increased sensitivity  
6 and robustness (Huertas-Pérez et al. 2016; Nilüfer and Boyacıoğlu, 2002). There are many  
7 commercially available SPE and IAC products available for on-site mycotoxin analysis  
8 (Table 2). Şenyuva et al (2010) have also reviewed these products extensively. IAC and  
9 SPE coupled with fluorometric meters give semi-quantitative and results within minutes  
10 (NeoColumn for Aflatoxin <10 min) and do not require expensive laboratory equipment.  
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#### 16 17 *Principle of Immunoaffinity and Solid Phase Extraction clean up columns*

18 IACs work by immobilizing anti-mycotoxin to a solid support contained within a small plastic  
19 column. The solid support is commonly an inexpensive material such as agarose gel or  
20 cellulose, or synthetic organic supports including acrylamide polymers, copolymers or  
21 derivatives, polymethacrylate derivatives and polyethersulfone matrixes. When a sample  
22 extract is passed through the column, any mycotoxin present will selectively bind to the  
23 immobilized antibody and what remains of the extract is flushed out through the column in a  
24 washing step, typically done with water. A solvent is then passed through the column  
25 removing the mycotoxin from the antibody and eluting it from the column. In the now purified  
26 sample extract, the solvent can be evaporated off, leaving a concentrated mycotoxin  
27 sample. An important additional step to this assay is the fluorescent derivatization step to  
28 either enhance the fluorescence of a mycotoxin or render the mycotoxin fluorescent before  
29 measuring in a fluorometer. The natural fluorescence of mycotoxins such as the aflatoxins  
30 may be chemically enhanced via reaction with trifluoroacetic acid, bromine, or iodine (Wacoo  
31 et al. 2014). Other mycotoxins such as DON that are not naturally fluorescent must be  
32 derivatized with zirconyl nitrate and ethylenediamine in methanol for example (Malone et al.  
33 1998).  
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44 SPE makes use of a sorbent agent, known as the 'stationary phase', loaded inside a  
45 plastic column (like that of IAC) or supported on a disc. The sample passes through the  
46 stationary phase and depending on the type of sorbent material being used, either the  
47 analyte of interest will be captured and retained inside the column or disc while impurities  
48 are filtered out, or it will capture matrix contaminants leaving the analyte of interest left in the  
49 eluted sample (Huertas-Pérez et al. 2016). The latter is known as a one-step SPE column,  
50 packed with a porous frit at the top of the column packing, in a durable plastic tube with  
51 plastic caps at both ends. Sample extract is added to the sample reservoir and a rubber  
52 syringe plunger, or a similar device, is used to push the sample extract through the one-step  
53 SPE column. The purified extract collected at either the lower or upper end of the tube  
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3 contains the mycotoxin, which can immediately be derivatized and placed in a fluorometer  
4 for analysis.  
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#### 8 *Usage and developments in IAC and SPE*

9 MycoSep® columns (Romer Labs) are an example of a commercial one step SPE column  
10 clean-up which has been coupled with fluorometry analysis (Malone et al. 1998; Malone et  
11 al. 2000). Companies typically recommend fluorometric readers too, for example, Romer  
12 Labs offer a FQ-Reader with their FluoroQuant Aflatoxin test kits which are based on either  
13 immunoaffinity column clean-up or solid phase extraction. VICAM also offer IAC kits with  
14 their Series-4EX Fluorometer specifically to be used with the following IACs: AflaTest, AflaB,  
15 AflaM1 FL+, FumoniTest, OchraTest and ZearalaTest.  
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20 In a study by Longobardi et al. (2013) the group used a commercial IAC coupled to  
21 SPE for extraction of OTA from red wine samples before direct fluorometric measurement  
22 with a spectrofluorometer. The LOD in spiked red wine samples was 0.2 ng/ml, recoveries  
23 ranged from 94.5-105.4% and the total analysis time was 30 min. A good correlation ( $r^2 =$   
24 0.9765) was observed between OTA levels obtained with the fluorometer and HPLC,  
25 showing the results are reliable, with the added advantage of using a simple benchtop  
26 fluorometer which evidently reduces the cost and time of analysis. Commercial IACs have  
27 also been used in evaluation studies such as that by Li et al. (2014) to evaluate the safety of  
28 food in the Yangtze Delta region of China. The group tested for ZEN, OTA, AFs and AFB1  
29 using four different IACs for each toxin (or group of toxins) and four different preliminary  
30 extraction techniques. As no IAC coupled with fluorometry was available for DON, HPLC-UV  
31 was used. Whilst the study allowed for a reliable assessment of mycotoxins in the Yangtze  
32 Delta region, it exemplifies how a high-throughput, affordable, multiplex method of detection  
33 would have enabled a more cost-effective and time-efficient sample analysis for the group.  
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43 Developments in SPE have branched to usage of engineered receptors. For  
44 example, in a study by Ali et al. (2010) the group successfully used molecularly imprinted  
45 polymer (MIP) as a selective sorbent for the retention of OTA from cereal extracts. The study  
46 compared capacity of the MIP-SPE method with IAC. The capacity in this case was the  
47 maximum amount of compound that was retained by the sorbent. For MIP-SPE the  
48 recoveries of the extraction were linear up to 5000 ng/g<sup>-1</sup>, whereas for IAC, when percolating  
49 an extract containing 900-4300 ng/g<sup>-1</sup> of OTA, only 650ng were detected in the elute. These  
50 results indicate a decrease of recoveries in IAC caused by overloading of the capacity. In a  
51 study by Sergeyeva et al (2017) SPE with an MIP membrane as a stationary phase was  
52 developed and used with a fluorescent sensor system (spectrofluorometer from Perkin-  
53 Elmer, UK) capable of detecting AFB1 within the range 14–500ng/mL<sup>-1</sup>. In this paper, the  
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3 group aimed to highlight the potential in synthesis of nanostructured polymers (1–10 nm  
4 artificial receptor sites) capable of selective recognition of mycotoxins for usage as novel  
5 selective layers in portable biosensor technology. Furthermore, MIP membranes would also  
6 improve the storage stability of the biosensor as biological reagents such as antibodies and  
7 antigens most commonly used are evidently less stable than MIPs.  
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11 Today, most recent developments seen across literature in IAC or SPE are coupled  
12 with LC-MS/MS or HPLC analysis with fluorometry rather than developments in the  
13 traditional fluorometric kits (Wang et al. 2016; Anene et al. 2016). This is likely because LC-  
14 MS/MS and HPLC analysis are the current reference methods of choice capable of multiplex  
15 analysis. In fact, there are even commercial IACs for multiple mycotoxin clean up (Vicam)  
16 that can be used prior to LCMS/MS. In terms of commercial, portable methods, perhaps  
17 SPE integrated with MIP coupled with fluorometric biosensors is the future progression in  
18 SPE technology.  
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### 24 25 **Biosensor Development**

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27 A biosensor device typically has three components (1) the detection layer (2) the transducer  
28 and (3) the output system. The detection layer can either consist of biological material (e.g.  
29 tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids,  
30 natural products etc.), a biologically derived material (e.g. recombinant antibodies,  
31 engineered proteins, aptamers etc.) or a biomimic (e.g. synthetic receptors, biomimetic  
32 catalysts, combinatorial ligands, imprinted polymers) (Chauhan et al. 2016). The detection  
33 layer is integrated within the transducer so that upon interaction with the sample, a binding  
34 event or reaction occurring at the detection layer will generate an electronic signal via the  
35 transducer that is measured by the output system.  
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42 Biosensors today are advancing enormously as analytical devices in food safety with  
43 the integration of many modern developing technologies including but not limited to signal  
44 transduction technology (magneto, piezo, optical and direct electrochemical techniques and  
45 micro-electro-mechanical systems), microfluidics (droplet or digital) and immobilization  
46 technologies (Lin and Guo, 2016). The integration of aptamers in particular with biosensing  
47 technology (aptasensor) is also becoming increasingly prominent. Aptamers are composed  
48 of single stranded oligonucleotides (DNA or RNA), capable of interacting with analytes.  
49 There are many reasons for aptasensor progression in recent years, a comprehensive  
50 review of which was done by Rhouati et al (2017). The review points out how the chemical  
51 production of aptamers is cheaper than that of antibodies. Furthermore, aptamers are easily  
52 labelled (with fluorescent dyes, enzymes) and unlike antibodies, can be regenerated and  
53 reused for other analyses.  
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Some examples of biosensors that have been applied to mycotoxin analysis are listed below and in Table 3. The potential barriers to commercialisation are also discussed.

## Optical Sensors

### *Surface Plasmon Resonance*

Since the early 2000's, commercially-available laboratory-based systems founded on the principle of surface plasmon resonance have been effectively used for the detection of mycotoxins in a variety of matrices including DON in wheat (Meneely et al. 2010), AFM1 in milk (Wang et al. 2009), OTA in cereals and beverages (Yuan et al. 2009), T2/HT2 in cereals (Meneely et al. 2012) and ZEN in sorghum (Edupuganti et al. 2013). SPR is an optical phenomenon that occurs when polarised light hits a metal film between two media of different refractive index. Biosensors based on SPR typically use a thin gold metal film at the interface of a glass prism and a sample solution (Meneely and Elliott 2014). Typically, light coming from the side of higher refractive index (glass prism), is partly reflected and partly refracted. However, above a critical angle of incidence, total internal reflection will occur (Ying et al. 2012). Under these conditions, an electromagnetic field component of the light, the evanescent wave, will penetrate the lower refractive index medium (the sample solution). When there is a thin metal film at the interface between the high and low refractive mediums, the evanescent wave interacts with the free oscillating electrons of the thin metal layer resulting in excitation of surface plasmons. Energy from the incident light dissipates in the metal film, resulting in a decrease in the intensity of the reflected light. This is the phenomenon of surface plasmon resonance (Hodnik et al. 2009). In SPR biosensing, the gold thin film acts as a sensing surface and is modified with biorecognition probes (antigen-conjugate). Antibody is mixed with the sample solution prior to running it over the sensor surface so that any free mycotoxin in the sample will bind to the mixed antibody resulting in no antibody binding to the probes on the sensor surface (*Figure 2(a)*). However, in a negative sample, the free antibody will bind to the probes on the sensor surface. A binding event changes the resonance frequency of the surface plasmons since the refractive index of the medium at the interface is changing. This change in resonance, changes the intensity of the reflected light which is detected by the biosensor device as a binding event and is expressed in arbitrary units known as resonance units. These binding events provide a relative response (relative to the baseline) and are measured against a calibration curve, allowing determination of the sample concentration. Each cycle produces a plot known as a sensorgram, a plot of response against time, detailing binding events in real time which is a key advantage of the technique (Xu et al. 2016). SPR based biosensors are also reliable,



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3 label-free, sensitive and have the added advantage of reusability with regeneration of the  
4 biosensor chip surface.  
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7 There have been variations to the immunoassay format of SPR to include the  
8 combination of SPR with enzyme-derivatised sensors and MIPs. Fluorescence spectroscopy  
9 and the use of gold nanoparticles have also been coupled with SPR for signal enhancement.  
10 Mennely et al. (2014) extensively reviewed SPR methods and highlighted the need for  
11 development and manufacture of portable SPR instruments demonstrating multiplexing  
12 capabilities at lower costs. Since then many studies have been focusing on these aspects of  
13 SPR development. Nevertheless, in a later review by Dahlin, (2015) it was highlighted that  
14 no published studies developing portable or multiplex SPR devices were including a fair  
15 benchmark test against state-of-the-art SPR. A year later, a publication by Joshi et al. (2016)  
16 aimed to address all of the above mentioned issues by developing a 6-plex competitive  
17 inhibition immunoassay for mycotoxins in barley on a prototype portable nanostructured  
18 iSPR instrument which has a nanostructured gold sensor surface, eliminating the need for a  
19 prism. The group first developed a double 3-plex assay, which involved using a well-  
20 established benchtop SPR instrument and two biosensor chips. One chip was used for the  
21 detection of DON, ZEN, T-2 toxin and a second chip for the detection of OTA, FUMB1 and  
22 AFB1. The ovalbumin (OVA) conjugates of mycotoxins were immobilized on the chips via  
23 amine coupling. Upon injection of mixed antibodies at a fixed concentration, with sample or  
24 standard, over a chip with the immobilized mycotoxin–OVA conjugates, the SPR response  
25 was recorded. The chips could be used for up to 60 cycles after regeneration with 10 mM  
26 HCl and 20 mM NaOH after each run. The LODs in barley (in  $\mu\text{g kg}^{-1}$ ) were 26 for DON, 6 for  
27 ZEN, 0.6 for T-2, 3 for OTA, 2 for FUMB1 and 0.6 for AFB1. In accordance with the EU  
28 regulatory limits, results could be validated for DON, T-2, ZEN and FUMB1 while for OTA  
29 and AFB1 sensitivities should be improved. With these point-of-reference results the group  
30 transferred the assay to a 6-plex format (one chip with all six toxins), using the same bio-  
31 reagents, in the nanoplasmonics instrument and compared the two assays. The 6-plex  
32 portable iSPR assay allowed detection of DON (64  $\mu\text{g/kg}$ ), T-2 (26  $\mu\text{g/kg}$ ), ZEN (96  $\mu\text{g kg}$ )  
33 and FUMB1 (13  $\mu\text{g/kg}$ ) at relevant EU levels, although it was less sensitive. The prototype  
34 iSPR was therefore shown to have potential for future development for application in rapid  
35 in-field and at-line screening of multiple mycotoxins.  
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52 From the literature, SPR biosensors from Biacore AB are demonstrated as applicable  
53 for mycotoxin testing. Whilst SPR biosensors are widely studied in academic research, their  
54 commercialisation for mycotoxin analysis is limited by several factors. For example, the  
55 technology and data analysis are not currently usable by non-experts and miniaturisation  
56 needs to be optimised to maintain high sensitivity, both of which limit on-site testing.  
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3 Furthermore, labelling of reagents to achieve higher sensitivities adds to the overall cost of  
4 the potential commercial product. These issues would need to be addressed to achieve an  
5 SPR biosensor equivalent test kit.  
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### 9 10 **Mass-Sensitive Sensors**

11 Mass-sensitive sensors are based on electroacoustic technology and are often referred to  
12 within the scientific literature as electroacoustic sensors. Electroacoustic technology relies on  
13 the transformation of acoustic energy into electric energy or vice versa. This transformation  
14 can occur in piezoelectric material “sandwiched” between two electrodes, one of which is  
15 functionalised (typically coated with antigen-conjugate) to sense the analyte of interest  
16 (*Figure 2(a)*). When a piezoelectric material is exposed to a force and is mechanically  
17 deformed it will generate an electric dipole and an electric voltage. This effect can work in  
18 reverse, meaning that if an alternating voltage is applied to a piezoelectric material, it will  
19 oscillate and this is known as the piezoelectric effect (Ferreira et al. 2009). Oscillation of the  
20 material at its resonant frequency generates an acoustic wave which propagates through the  
21 bulk of the material, in a direction perpendicular to the surface. When a sample meets the  
22 coated electrode, any binding event will result in slowing of oscillation on the piezoelectric  
23 materials surface, thus change the property of the acoustic wave. The wave alteration is  
24 detected as a frequency shift from the resonant frequency. This is directly proportional to the  
25 mass bound on the electrode, the relationship between which was described by Sauerbrey  
26 (1959). Better mass sensitivities are achievable by operating at higher frequencies.  
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#### 38 *Quartz Crystal Microbalance*

39 The development of quartz crystal microbalances (QCM) for bio-sensing relies on the  
40 piezoelectric effect exhibited by quartz crystals (Vashist and Vashist, 2011). A QCM  
41 transducer consists of a thin quartz crystal disk in between two gold electrodes, one of which  
42 is functionalized to sense the analyte of interest. Like SPR, the sensor is typically coated  
43 with antigen-conjugate. QCM has been investigated as a promising transducer for mycotoxin  
44 detection providing real-time analysis and high sensitivity. The technology is also simple to  
45 operate and offers portability due to the small size of the transducer. Similar to SPR, the  
46 functionalised surface of a QCM chip can be regenerated for multiple biosensing runs  
47 (Vashist and Vashist 2011). However, QCM assays have suffered as often insufficient signal  
48 is generated (frequency change) that is needed for detection in the ng-pg range. In a study  
49 by Karczmarczyk et al. (2017) using a QCM based sensing device to detect OTA in red wine,  
50 the group aimed to overcome this issue. To reach a lower limit of detection, the signal was  
51 amplified using secondary antibody conjugated to gold nanoparticles (AuNPs). Initially, due  
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3 to the very low concentration of the primary antibody needed to allow inhibition, the recorded  
4 signal for frequency change was weak ( $\Delta f = 17.97$  Hz). Hence, an additional high mass  
5 provided by Ab2-AuNPs injection was applied resulting in a signal enhancement ( $\Delta f = 52.53$   
6 Hz) and consequently an improvement of immunoassay sensitivity. This resulted in a linear  
7 detection range of 0.2–40 ng mL<sup>-1</sup> with an LOD of 0.16 ng mL<sup>-1</sup>, which is one order of  
8 magnitude lower than LOD specified by EU legislation concerning the limit of OTA in food.  
9 However, the downside to this is that the assay is not label-free and perhaps more time  
10 consuming compared to other biosensor techniques on the market for mycotoxin analysis  
11 such as SPR. Other methods of enhancing signal response have been explored including  
12 the coating process of the QCM transducer. In a study by Chauhan et al. (2016), the group  
13 found that in buffer conditions an LOD of 0.008 ng/ml could be achieved with a label-free  
14 non-competitive assay by using an electrochemical quartz crystal microbalance (EQCM)  
15 based immunosensor coated with self-assembled monolayers of hexandithiol (HDT) and  
16 gold nanoparticles (AuNPs) followed by aflatoxin B1 antibody.

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QCM biosensors are the most well-established acoustic wave type sensors and  
commercially available QSense biosensors by the company Biolin Scientific, Sweden can be  
used for mycotoxin detection. However, commercialisation of a QCM biosensor test kit  
equivalent is limited by several factors. For example, whilst quartz plates are 330-55  $\mu\text{m}$   
thick with a frequency of 5-20 MHz, it is possible to achieve higher mass sensitivities with  
thinner materials. However, attempting to make quartz crystals thinner is an expensive  
process. Higher frequency (1-5 GHz) resonators have been developed by thin film  
technology, through the emergence of Film Bulk Acoustic Resonators (Webner et al. 2006;  
García-Farrera et al. 2017).

#### *Film Bulk Acoustic Resonators*

Whilst biosensors based on thin film bulk acoustic resonators (FBAR) have not yet been  
used in mycotoxin analysis, literature highlights the potential of this technology as a highly  
sensitive technique that could be exploited for the detection of low molecular weight  
compounds like mycotoxins. An FBAR transducer is based on a similar principle to QCM,  
however, unlike QCM, the piezoelectric material used in FBAR transducers is generally a  
thin film (0.5-3 $\mu\text{m}$  thick) of aluminium nitride (AlN) or zinc oxide (ZnO) (Chen et al. 2015;  
Rughoobur et al. 2018). AlN is a popular choice due to its high values of chemical inertness,  
acoustic longitudinal wave velocity, electrical resistivity and its piezoelectric behaviour  
(García-Farrera et al. 2017). With these thin piezoelectric films, FBAR biosensors can  
ultimately provide better mass sensitivities than QCM as the FBAR transducer operates at  
higher frequencies. This nano-manufacturing process is known as the 'bottom-up' approach,

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3 building materials up from atomic- and molecular-scale components. QCM however uses the  
4 'top-down' fabrication process, reducing the size of large quartz crystals. Due to the small  
5 size of the FBAR transducers, many of them can be integrated within a small area which  
6 gives an added advantage of high throughput and cost-effective analysis, with the possibility  
7 of developing a hand held portable device (Zhang et al 2018).  
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### 11 12 13 **Conclusion and Future Outlook**

14 The market today for mycotoxin test kits is already a competitive one with multiple  
15 companies selling variations of similar products. However, judging from the literature it is  
16 likely that within the next decade, the market will consist of test kits that are hand-held digital  
17 biosensors capable of performing multiplex analysis. One contrasting assay development,  
18 for mainly biosensors, is that which regards either the use of label-free detection or the  
19 addition of labels such as nanoparticles, to increase sensitivity. Here, a trade-off between  
20 sensitivity and cost can be made. There is also a chance that using aptamers or MIPs in  
21 commercially available assays will become more popular, however, immunoassays are still  
22 the current detection method of choice.  
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### 31 **References**

- 32 Ahmed, MU, Zourob, M, and Tamiya. E. 2017. Food Biosensors. *The Royal Society of*  
33 *Chemistry*: UK, Cambridge.  
34  
35 Ali, WH, Derrien, D, Alix, F, Pérollier, C, Lépine, O, Bayouhd, S, Chapuis-Hugon, F, Pichon,  
36 V. 2010. Solid-phase extraction using molecularly imprinted polymers for selective extraction  
37 of a mycotoxin in cereals. *Journal of Chromatography A*, 1217: 6668-6673.  
38  
39 Alshannaq, A and Yu, JH. 2017. Occurrence, Toxicity, and Analysis of Major Mycotoxins in  
40 Food. *International Journal of Environmental Research and Public Health*, 14: 632.  
41  
42 Anene, A, Hosni, K, Chevalier, Y, Kalfat, R, Hbaieb, S. 2016. Molecularly imprinted polymer  
43 for extraction of patulin in apple juice samples. *Food Control*, 70: 90-95.  
44  
45 Anfossi, L, Giovannoli, C, Baggiani, C. 2016. Mycotoxin detection. *Current Opinion in*  
46 *Biotechnology*, 37: 120-126.  
47  
48 Beloglazova, NV, Graniczowska, K, Ediage, EN, Averkieva, O and De Saeger, S. 2017.  
49 Sensitive Flow-through Immunoassay for Rapid Multiplex Determination of Cereal-borne  
50 Mycotoxins in Feed and Feed Ingredients. *Journal of Agricultural and Food Chemistry*, 65:  
51 7131-7137.  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 Berthiller, F, Brera, C, Iha, MH, Krska, R, Lattanzio, VMT, MacDonald, S, Malone, RJ,  
4 Maragos, C, Solfrizzo, M, Stranska-Zachariasova, M, Stroka, J, Tittlemier, SA. 2017.  
5 Developments in mycotoxin analysis: an update for 2015-2016. *World Mycotoxin Journal*,  
6 10: 5-29.  
7  
8

9  
10 Bondarenko, AP and Eremin, SAJ. 2012. Determination of zearalenone and ochratoxin a  
11 mycotoxin in grain by fluorescence polarization immunoassay. *Journal of Analytical*  
12 *Chemistry*, 67: 790.  
13  
14

15  
16 Burmistrova, NA, Rusanova, YT, Yurasov, NA, Goryacheva, IY and De Saeger, S. 2014.  
17 Multidetector of mycotoxins by membrane-based flow-through immunoassay. *Food Control*,  
18 46: 462469.  
19  
20

21 Chauhan, R, Sing, J, Sachdev, T, Basu, T and Malhotra, BD. 2016. Recent advances in  
22 mycotoxins detection. *Biosensors and Bioelectronics*, 81: 532–545.  
23  
24

25 Chauhan, R, Singh, J, Solanki, PR, Manaka, T, Iwamoto, M, Basu, T and Malhotra, BD.  
26 2016. Label-free piezoelectric immunosensor decorated with gold nanoparticles: Kinetic  
27 analysis and biosensing application. *Sensors and Actuators B: Chemical*, 222: 804-814.  
28  
29

30 Chen, G, Zhao, X, Wang, X, Jin, H, Li, S, Dong, S, Flewitt, AJ, Milne, WI and Luo, JK. 2015.  
31 Film bulk acoustic resonators integrated on arbitrary substrates using a polymer support  
32 layer. *Scientific Reports*, 5: 9510.  
33  
34

35  
36 Crews, C. 2015. Analysis of Ergot Alkaloids. *Toxins*, 7: 2024–2050.  
37  
38

39 Dahlin, AB. 2015. Sensing applications based on plasmonic nanopores: The hole story.  
40 *Analyst*, 140: 4748-4759.  
41  
42

43 Daly, SJ, Keating, GJ, Dillon, PP, Manning, BM, O'Kennedy, R and Lee, HA. 2000.  
44 Development of Surface Plasmon Resonance-Based Immunoassay for Aflatoxin B1. *Journal*  
45 *of Agricultural and Food Chemistry*, 48: 5097-5104.  
46  
47

48 De Santis, B, Debegnach, F, Gregori, E, Russo, S, Marchegiani, F, Moracci, G and Brera, C.  
49 2017. Development of a LC-MS/MS Method for the Multi-Mycotoxin Determination in  
50 Composite Cereal Based Samples. *Toxins*, 9:169.  
51  
52

53 Edupuganti, SR, Edupuganti, OP and O'Kennedy, R. 2013. Generation of anti-zearalenone  
54 scFv and its incorporation into surface plasmon resonance-based assay for the detection of  
55 zearalenone in sorghum. *Food Control*, 34: 668-674.  
56  
57  
58  
59  
60

1  
2  
3 [EC] European Commission. 2002. Commission Decision of 12 August 2002 implementing  
4 Council Directive 96/23/EC concerning the performance of analytical methods and the  
5 interpretation of results. Official J Eur Union. L221:8-36  
6  
7

8  
9 [EC] European Commission. 2006a. Commission Regulation (EC) No 1881/2006 of 19  
10 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official J Eur  
11 Union. L364:5-24.  
12  
13

14 [EC] European Commission. 2006b. Commission Regulation (EC) Commission Regulation  
15 (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis  
16 for the official control of the levels of mycotoxins in foodstuffs Official J Eur Union. L70:12.  
17  
18

19 [EC] European Commission. 2014. Commission Regulation (EU) No 519/2014 of 16 May  
20 2014 amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots,  
21 spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and  
22 screening methods of analysis. Official J Eur Union. L147:29-43.  
23  
24  
25

26 Ferreira, GN, Da-Silva, A-C and Tomé, B. 2009. Acoustic wave biosensors: physical models  
27 and biological applications of quartz crystal microbalance. *Trends in biotechnology*, 27: 689-  
28 697.  
29  
30

31 Foubert, A, Beloglazova, NV, Gordienko, A, Tessier, MD, Drijvers, E, Hens, Z and De  
32 Saeger, S. 2017. Development of a Rainbow Lateral Flow Immunoassay for the  
33 Simultaneous Detection of Four Mycotoxins. *Journal of Agricultural and Food Chemistry*, 65:  
34 7121-7130.  
35  
36  
37

38 García-Farrera, B, Murillo AE, Melo-Máximo DV, Salas, O, Melo-Máximo, L and Oseguera,  
39 J. 2017. Effect of the Electrode Layer on the Deposition of AlN for Biosensors. *Journal of*  
40 *Bio- and TriboCorrosion*, 3: 15.  
41  
42  
43

44 Hendrickson, O.D, Chertovich, JO, Zherdev, AV, Sveshnikov, PG and Dzantiev, BB. 2018.  
45 Ultrasensitive magnetic ELISA of zearalenone with pre-concentration and chemiluminescent  
46 detection. *Food Control*, 84: 330-338.  
47  
48

49 Hodnik, V and Anderluh, G. 2009. Toxin Detection by Surface Plasmon Resonance.  
50 *Sensors*, 9: 1339– 1354.  
51  
52

53 Ji, C, Fan, Y and Zhao, L. 2016. Review on biological degradation of mycotoxins. *Animal*  
54 *Nutrition*, 2: 127-133.  
55  
56  
57  
58  
59  
60

1  
2  
3 Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2017. Evaluation of certain  
4 food additives (Eighty-fourth report of the Joint FAO/WHO Expert Committee on Food  
5 Additives) WHO Technical Report Series, No.1007  
6  
7

8  
9 Joshi, S, Segarra-Fas, A, Peters, J, Zuilhof, H, A. van Beek, T and Nielen, MWF. 2016.  
10 Multiplex surface plasmon resonance biosensing and its transferability towards imaging  
11 nanoplasmonics for detection of mycotoxins in barley. *Royal Society of Chemistry*, 141:  
12 1307-1318.  
13  
14

15  
16 Joshi, S, Annida, R.M, Zuilhof, H, Van Beek, T.A and Nielen, M.W.F. 2016. Analysis of  
17 Mycotoxins in Beer Using a Portable Nanostructured Imaging Surface Plasmon Resonance  
18 Biosensor. *Journal of Agricultural and Food Chemistry*, 64, 43: 8263-8271.  
19  
20

21  
22  
23 Karczmarczyk, A, Haupt, K, Feller, KH. 2017. Development of a QCM-D biosensor for  
24 Ochratoxin A detection in red wine. *Talanta*, 166: 193-197.  
25

26  
27 Karczmarczyk, A, Dumbiak-Szepietowska, M, Vorobii, M, Rodriguez-Emmenegger, C,  
28 Dostalek, J and Feller, K. 2016. Sensitive and rapid detection of aflatoxin M1 in milk utilizing  
29 enhanced SPR and p(HEMA) brushes. *Biosensors and Bioelectronics*, 81: 159-165.  
30

31  
32 Krska, R and Molinelli A. 2009. Rapid test strips for analysis of mycotoxins in food and feed.  
33 *Analytical and Bioanalytical Chemistry*, 393: 67.  
34

35  
36 Lattanzio, V.M. T, von Holst, C, Visconti, A. 2013. Experimental design for in-house  
37 validation of a screening immunoassay kit. The case of a multiplex dipstick for Fusarium  
38 mycotoxins in cereals. *Analytical and Bioanalytical Chemistry*, 24:7773–7782.  
39

40  
41 Li, W, Powers, S and Dai, SY. 2014. Using commercial immunoassay kits for mycotoxins:  
42 'joys and sorrows'? Rapid methods for mycotoxin detection. *World Mycotoxin Journal*, 7: 417  
43 – 430.  
44  
45

46  
47 Li, R, Wang, X, Zhou, T, Yang, D, Wang, Q and Zhou, Y. 2014. Occurrence of four  
48 mycotoxins in cereal and oil products in Yangtze Delta region of China and their food safety  
49 risks. *Food Control*, 35: 117-122.  
50

51  
52 Li, C, Wen, K, Mi, T, Zhang, X, Zhang, H, Zhang, S, Shen, J and Wang, Z. 2016. A universal  
53 multiwavelength fluorescence polarization immunoassay for multiplexed detection of  
54 mycotoxins in maize. *Biosensors and Bioelectronics*, 79: 258-265.  
55  
56  
57  
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54  
55  
56  
57  
58  
59  
60
- Liang, Y, Huang, X, Yu, R, Zhou, Y and Xiong, Y. 2016. Fluorescence ELISA for sensitive detection of ochratoxin A based on glucose oxidase-mediated fluorescence quenching of CdTe QDs. *Analytica Chimica Acta*. 936: 195-201.
- Lin, X and Guo, X. 2016. Advances in Biosensors, Chemosensors and Assays for the Determination of Fusarium Mycotoxins. *Toxins*, 8: 161.
- Longobardi, F, Iacovelli, V, Catucci, L, Panzarini, G, Pascale, M, Visconti, A and Agostiano, A. 2013. Determination of Ochratoxin A in Wine by Means of Immunoaffinity and Aminopropyl Solid-Phase Column Cleanup and Fluorometric Detection. *Journal of Agricultural and Food Chemistry*, 61: 1604-1608.
- Malone, BR, Humphrey, CW, Romer, TR and Richard, JL. 2000. Determination of aflatoxins in grains and raw peanuts by a rapid procedure with fluorometric analysis. *Journal of AOAC International*, 83: 95-98.
- Malone, BR, Humphrey, CW, Romer, TR and Richard, JL. 1998. One-step solid-phase extraction cleanup and fluorometric analysis of deoxynivalenol in grains. *Journal of AOAC International*, 81: 448-452.
- Marin, S, Ramos, AJ, Cano-Sancho, G and Sanchis, V. 2013. Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemistry Toxicology*, 60: 218-237.
- Man, Y, Liang, G, Li, A, and Pan, L. 2017. Recent Advances in Mycotoxin Determination for Food Monitoring via Microchip. *Toxins*, 9: 324.
- Maragos, CM and Busman, M. 2010. Rapid and advanced tools for mycotoxin analysis: a review. *Food Additives & Contaminants: Part A*, 27: 688-700.
- Maragos, CM. 2009. Biosensors for mycotoxin analysis: recent developments and future prospects. *World Mycotoxin Journal*, 2: 221-238.
- Maragos, C. 2009. Fluorescence Polarization Immunoassay of Mycotoxins: A Review. *Toxins*, 1: 196-207.
- Matabaro, E, Ishimwe, N, Uwimbabazi, E, and Lee, BH. 2017. Current Immunoassay Methods for the Rapid Detection of Aflatoxin in Milk and Dairy Products. *Comprehensive Reviews in Food Science and Food Safety*, 16: 808-820.
- McNamee, SE, Bravin, F, Rosar, G, Elliott, CT, Campbell, K. 2017. Development of a nanoarray capable of the rapid and simultaneous detection of zearalenone, T2-toxin and Fumonisin. *Talanta*, 164: 368-376.



1  
2  
3 Meneely, J, Fodey, T, Armstrong, L, Sulyok, M, Krska, R and Elliott, C. 2010. Rapid Surface  
4 Plasmon Resonance Immunoassay for the Determination of Deoxynivalenol in Wheat,  
5 Wheat Products, and Maize-Based Baby Food. *Journal of Agricultural and Food Chemistry*,  
6 58: 8936-8941.  
7  
8

9  
10 Meneely, JP, Quinn, JG, Flood, EM, Hajšlová, J and Elliott, CT. 2012. Simultaneous  
11 screening for T2/HT-2 and deoxynivalenol in cereals using a surface plasmon resonance  
12 immunoassay. *World Mycotoxin Journal*, 5: 117-126.  
13  
14

15 Meneely, JP and Elliott, CT. 2014. Rapid surface plasmon resonance immunoassays for the  
16 determination of mycotoxins in cereals and cereal-based food products. Special issue: Rapid  
17 methods for mycotoxin detection. *World Mycotoxin Journal*, 7: 491-505.  
18  
19

20  
21 Nilüfer, D and Boyacıoğlu D. 2002. Comparative Study of Three Different Methods for the  
22 Determination of Aflatoxins in Tahini. *Journal of Agricultural and Food Chemistry*, 50: 3375-  
23 3379.  
24  
25

26 Njumbe, EE, Di Mavungu, JD, Goryacheva, IY, Peteghem, CV and De Saeger, S. 2012.  
27 Multiplex flowthrough immunoassay formats for screening of mycotoxins in a variety of food  
28 matrices. *Analytical and Bioanalytical Chemistry*, 403: 265-278.  
29  
30

31 Paepens, C, De Saeger, S, Sibanda, L, Barna-Vetró, I, Léglise, I, Van Hove, F and Van  
32 Peteghem, C. 2004. A flow-through enzyme immunoassay for the screening of fumonisins in  
33 maize. *Analytica Chimica Acta*, 523: 229-235.  
34  
35

36 Pei, K, Xiong, Y, Xu, B, Wu, K, Li, X, Jiang, H and Xiong, H. 2018. Colorimetric ELISA for  
37 ochratoxin A detection based on the urease-induced metallization of gold nanoflowers.  
38 *Sensors and Actuators B: Chemical*, 262: 102-109.  
39  
40

41 Pirincci, S, S, Ertekin, Ö, Laguna, D.E, Özen, F.Ş, Öztürk, Z.Z and Öztürk, S. 2018. Label-  
42 free QCM immunosensor for the detection of ochratoxin A. *Sensors*, 18, 4.  
43  
44

45 Rahmani, A, Jinap, S and Soleimany, F. 2009. Qualitative and Quantitative Analysis of  
46 Mycotoxins. *Comprehensive Reviews in Food Science and Food Safety*, 8: 202–251.  
47  
48

49 Raiola, A, Tenore, GC, Manyes, L, Meca, G and Ritieni, A. 2015. Risk analysis of main  
50 mycotoxins occurring in food for children: An overview. *Food and Chemical Toxicology*, 84:  
51 169-180.  
52  
53

54 Rughoobur, G, Sugime, H, DeMiguel-Ramos, M, Mirea, T, Zheng, S, Robertson, J, Iborra, E,  
55 Flewitt, AJ. 2018. Carbon nanotube isolation layer enhancing in-liquid quality-factors of thin  
56  
57  
58  
59  
60

1  
2  
3 film bulk acoustic wave resonators for gravimetric sensing. *Sensors and Actuators B: Chemical*, 261: 398407.

4  
5  
6  
7 Saha, D, Acharya, D, Roy, D, Shrestha, D and Dhar, TK. 2007. Simultaneous enzyme  
8 immunoassay for the screening of aflatoxin B1 and ochratoxin A in chili samples. *Analytica*  
9 *Chimica Acta*, 584: 343-349.

10  
11  
12 Sauerbrey, G. 1959. Verwendung Von Schwingquarzen Zur Wagung Dunner Schichten Und  
13 Zur Mikrowagung. *Zeitschrift für Physik*, 155: 206–222.

14  
15  
16 Şenyuva, HZ, Gilbert, J. 2010. Immunoaffinity column clean-up techniques in food analysis:  
17 A review. *Journal of Chromatography B*, 878: 115-132.

18  
19  
20 Sergeyeva, T, Yarynka, D, Piletska, E, Lynnik, R, Zaporozhets, O, Brovko, O, Piletsky, S,  
21 El'skaya, A. 2017. Fluorescent sensor systems based on nanostructured polymeric  
22 membranes for selective recognition of Aflatoxin B1. *Talanta*, 175: 101-107.

23  
24  
25 Sibanda, L., De Saeger, S, Van Peteghem, C, Grabarkiewicz-Szczesna J and Tomczak, M.  
26 2000. Detection of T-2 Toxin in Different Cereals by Flow-Through Enzyme Immunoassay  
27 with a Simultaneous Internal Reference. *Journal of Agricultural and Food Chemistry*, 48:  
28 5864-5867.

29  
30  
31 Sheng, YJ, Eremin, S, Mi, TJ, Zhang, SX, Shen, JZ and Wang, ZH. 2014. The Development  
32 of a Fluorescence Polarization Immunoassay for Aflatoxin Detection. *Biomedical and*  
33 *Environmental Sciences*, 27:126-9.

34  
35  
36 Smith, DS and Eremin, SA. 2008. Fluorescence polarization immunoassays and related  
37 methods for simple, high-throughput screening of small molecules. *Analytical and*  
38 *Bioanalytical Chemistry*, 391: 1499.

39  
40  
41 Song, S, Liu, N, Zhao, Z, Ediage, EN, Wu, S, Sun, C, De Saeger, S and Wu, A. 2014.  
42 Multiplex Lateral Flow Immunoassay for Mycotoxin Determination. *Analytical Chemistry*, 86:  
43 4995-5001.

44  
45  
46 Sweeney, M.J. and Dobson, D.W., 1998. Mycotoxin production by *Aspergillus*, *Fusarium* and  
47 *Penicillium* species. *The International Journal of Food Microbiology*, 43: 141-158.

48  
49  
50 Tripathi, P, Upadhyay, N and Nara, S. 2018. Recent advancements in lateral flow  
51 immunoassays: A journey for toxin detection in food. *Critical Reviews in Food Science and*  
52 *Nutrition*, 58: 1715-1734.

1  
2  
3 Trucksess, MW and Stack, ME. 1994. Enzyme-linked immunosorbent assay of total  
4 aflatoxins B1, B2 and G1 in corn: follow-up collaborative study. *Journal of Aoac*  
5 *International*, 77: 655-658.  
6  
7

8  
9 Turner, NW, Bramhmbhatt, H, Szabo-Vezse, M, Poma, A, Coker, R, and Piletsky, S.A. 2015.  
10 Analytical methods for determination of mycotoxins: An update (2009–2014). *Analytica*  
11 *Chimica Acta*, 901: 12-33.  
12  
13

14 Urusov, AE, Zherdev, AV, Petrakova, AV, Sadykhov, EG, Koroleva, OV and Dzantiev, BB.  
15 2015. Rapid Multiple Immunoenzyme Assay of Mycotoxins. *Toxins*, 7: 238–254.  
16  
17

18 Vashist, SK, and Vashist, P. 2011. Recent Advances in Quartz Crystal Microbalance-Based  
19 Sensors. *Journal of Sensors*, 2011: 13.  
20  
21

22 Wacoo, AP, Wendi, D, Vuzi, PC, and Hawumba, JF. 2014. Methods for Detection of  
23 Aflatoxins in Agricultural Food Crops. *Journal of Applied Chemistry*, 2014: 15.  
24  
25

26 Wang, M, Jiang, N, Xian, H, Wei, D, Shi, L and Feng, X. 2016. A single-step solid phase  
27 extraction for the simultaneous determination of 8 mycotoxins in fruits by ultra-high-  
28 performance liquid chromatography tandem mass spectrometry. *Journal of Chromatography*  
29 *A*, 1429: 22-29.  
30  
31

32 Weber, J, Albers, WM, Tuppurainen, J, Link, M, Gabl, R, Wersing, W, Schreiter, M. 2006.  
33 Shear mode FBARs as highly sensitive liquid biosensors. *Sensors and Actuators A:*  
34 *Physical*, 128: 84-88.  
35  
36  
37

38 Wong, YC and Lewis, RJ. 2017. Analysis of Food Toxins and Toxicants. *John Wiley & Sons*  
39 *Ltd: UK*  
40  
41

42 Xiong, Y, Pei, K, Wu, Y, Duan, H, Lai, W and Xiong, Y. 2018. Plasmonic ELISA based on  
43 enzymeassisted etching of Au nanorods for the highly sensitive detection of aflatoxin B1 in  
44 corn samples. *Sensors and Actuators B: Chemical*, 267: 320-327.  
45  
46  
47

48 Zhang, X, Yu, X, Wen, KT, Li, C, Mari, GM, Jiang, H, Shi, W, Shen, J and Wang, Z. 2017.  
49 Multiplex Lateral Flow Immunoassays Based on Amorphous Carbon Nanoparticles for  
50 Detecting Three Fusarium Mycotoxins in Maize. *Journal of agricultural and food chemistry*,  
51 65: 8063-8071.  
52  
53  
54

55  
56 Xu, L, Zhang, Z, Zhang, Q and Li, P. 2016. Mycotoxin Determination in Foods Using  
57 Advanced Sensors Based on Antibodies or Aptamers. *Toxins*, 8: 239.  
58  
59  
60

1  
2  
3  
4  
5  
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48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Li, Y, Liu, X and Lin, Z. 2012. Recent developments and applications of surface plasmon resonance biosensors for the detection of mycotoxins in foodstuffs. *Food Chemistry*, 132: 1549-1554.

Yuan, J, Deng, D, Lauren, DR, Aguilar, M-I and Wu, Y. 2009. Surface plasmon resonance biosensor for the detection of ochratoxin A in cereals and beverages. *Analytica Chimica Acta*, 656: 63-71.

Zachariasova, M, Cuhra, P and Hajslova, J. 2014. Cross-reactivity of rapid immunochemical methods for mycotoxins detection towards metabolites and masked mycotoxins: the current state of knowledge Special issue: Rapid methods for mycotoxin detection. *World Mycotoxin Journal*, 7: 449 – 464.

Zhang, Y, Luo, J, Flewitt, A.J, Cai, Z, Zhao, X. 2018. Film bulk acoustic resonators (FBARs) as biosensors: A review. *Biosensors and Bioelectronics*, 116: 1-15.

Zheng, MZ, Richard, JL, and Binder, J. 2006. A Review of Rapid Methods for the Analysis of Mycotoxins. *Mycopathologia*, 161: 261.

Zhu, Z, Feng, M, Zuo, L, Zhu, Z, Wang, F, Chen, L, Li, J, Sang, G and Luo, S. 2015. An aptamer based surface plasmon resonance biosensor for the detection of ochratoxin A in wine and peanut oil. *Biosensors and Bioelectronics*, 65: 320-326.

Table 1. Health risks to humans associated with mycotoxins (information gathered from Alshannaq &amp; Yu 2017)

Mycotoxin	Major Species of Producing Fungi	Toxic Effect <sup>i</sup>
Aflatoxins B1, B2, G1, G2	<i>Aspergillus flavus</i> and <i>Aspergillus parasiticus</i>	Carcinogenic, teratogenic, hepatotoxic, mutagenic, and immunosuppressive effects
Aflatoxin M1	Metabolite of aflatoxin B1	
Deoxynivalenol	<i>Fusarium graminearum</i> and <i>Fusarium culmorum</i>	Causes nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever
Fumonisin B1, B2, B3	<i>Fusarium verticillioides</i> , <i>Fusarium proliferatum</i>	Neurotoxic, hepatotoxic, and nephrotoxic effects in animals, and possible carcinogen to humans
Ochratoxin A	<i>Aspergillus ochraceus</i> , <i>Penicillium verrucosum</i> , and other <i>Penicillium</i> species	Acutely nephrotoxic and hepatotoxic. Causes immunotoxicity, genotoxicity, neurotoxicity, teratogenicity, and embryotoxicity
T2/HT2	<i>Fusarium langsethiae</i> , <i>Fusarium poae</i> , and <i>Fusarium sporotrichioides</i>	Toxic effects include growth retardation, myelotoxicity, hematotoxicity, and necrotic lesions on contact sites
Zearalenone	<i>Fusarium graminearum</i> and <i>Fusarium semitectum</i>	Strong estrogenic activity resulting in changes.
Ergot alkaloids	<i>Claviceps</i> spp.	Vasoconstriction, delirium, hallucinations, muscle spasms, diarrhoea and convulsions

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<sup>i</sup> A provisional maximum tolerable daily intake (PMTDI) or a Tolerable Daily Intake (TDI) has been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for each mycotoxin. For all AF's Intake should be reduced to levels as low as reasonably possible as it is a genotoxic carcinogen. DON PMTDI is 1 µg/kg bw/day. FUM PMTDI is 2 µg/kg bw/day for FB1, FB2, and FB3 alone or in combination. OTA PMTDI is 0.112µg/kg bw/week. T2 and HT2 Toxins PMTDI is 0.06 µg /kg bw/day. ZEA PMTDI is 0.5 µg/kg bw/day. PATULIN PMTDI 0.4 µg/kg bw/day. Ergot alkaloids TDI 0.06 µg /kg bw (JECFA 2017).

For Peer Review Only

Table 2. Companies that sell commercial test kit products for mycotoxins

Company	Product Type	Mycotoxin					
		AF	DON	T2/HT2	ZEN	OTA	FUM
Elbasience	ELISA	✓	✓	✓	✓	✓	✓
	LFA	✓	✓	-	✓	-	-
Envirologics	ELISA	-	-	-	-	-	-
	LFA	✓	✓	✓	✓	✓	✓
Europroxima	ELISA	✓	✓	✓	✓	✓	✓
	LFA	-	-	-	-	-	-
Helica	ELISA	✓	✓	-	✓	✓	✓
	LFA	-	-	-	-	-	-
Neogen <sup>i</sup>	ELISA	✓	✓	✓	✓	✓	✓
	LFA	✓	✓	✓	✓	✓	✓
R-Biopharm	ELISA	✓	✓	✓	✓	✓	✓
	LFA	✓	✓	✓	✓	✓	✓
Randox <sup>ii</sup>	ELISA	✓	-	-	-	-	-
	LFA	-	-	-	-	-	-
Romer Labs <sup>iii</sup>	ELISA	✓	✓	✓	✓	✓	✓
	LFA	✓	✓	-	✓	-	✓
Tecna Diagnostics	ELISA	✓	✓	✓	✓	✓	✓
	LFA	✓	✓	-	✓	-	✓
Vicam <sup>iv</sup>	ELISA	-	-	-	-	-	-
	LFA	✓	✓	-	✓	✓	✓
Unisensor	ELISA	-	-	-	-	-	-
	LFA	✓	✓	✓	✓	✓	✓

<sup>i</sup> Neogen offer a fluorometric assay for AF's

<sup>ii</sup> Randox offer an ELISA for testing Ergot Alkaloids.

<sup>iii</sup> Romer Labs offer a fluorometric assay for AF's

<sup>iv</sup> Vicam offer fluorometric assay's for AF's, ZEN, OTA and FUM.

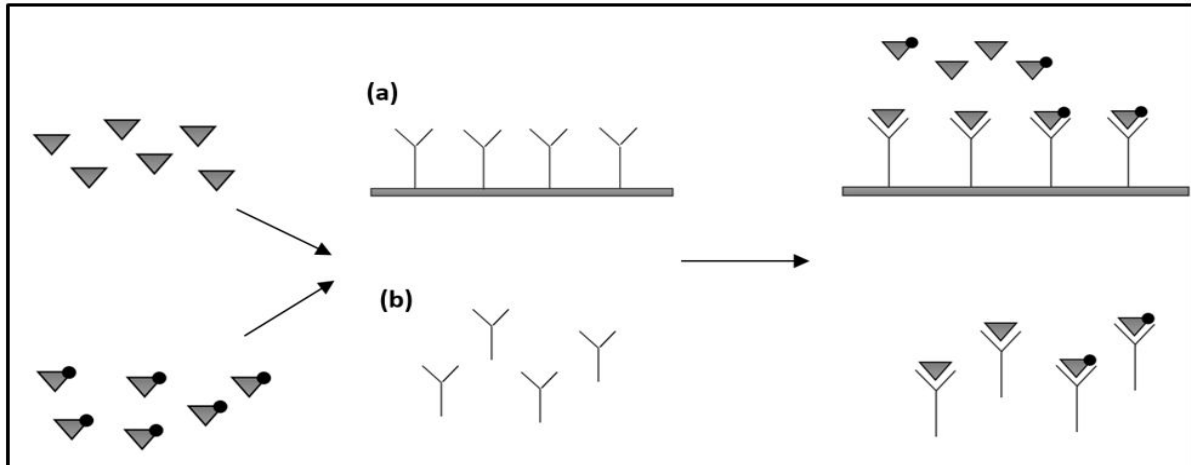
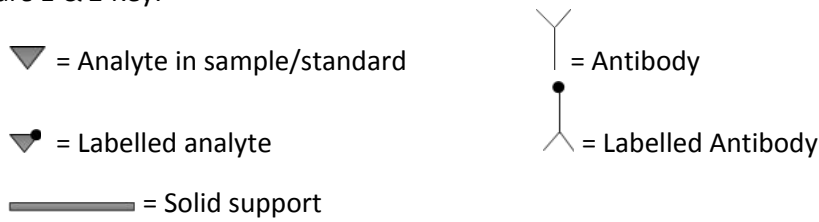
Table 3 Different biosensor techniques developed for mycotoxin analysis

Biosensing Device	Matrix	Analyte	LOD ( $\mu\text{g kg}^{-1}$ )	Reference
SPR	Milk	AFM1	0.018	Karczmarczyk et al 2016
iSPR	Beer and barley	DON, OTA	17, 7	Joshi et al 2016 (2)
iSPR	Barley	DON, T-2, ZEA, FB1	64, 26, 96, 13	Joshi et al 2016 (1)
SPR	Wine and peanut oil	OTA	0.005	Zhu et al 2015
QCM	Buffer	OTA	17.2	Pirincci et al 2018
QCM-D <sup>i</sup>	Red wine	OTA	0.16	Karczmarczyk et al 2017
EQCM	Cereal	AFB1	0.008	Chauhan et al 2016

<sup>i</sup> Quartz crystal microbalance with dissipation monitoring.

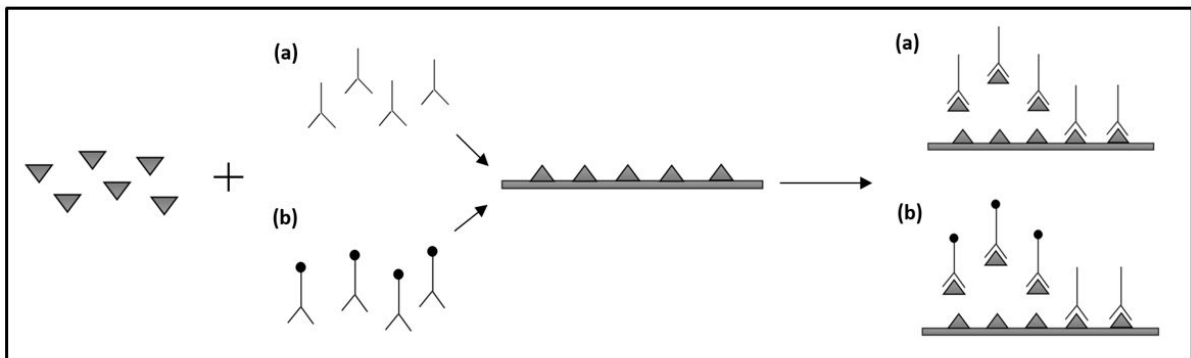


Figure 1 & 2 Key:



1. Enzyme Linked Immunosorbent Assay (a)
2. Fluorescent Polarization Immunoassay (b)
3. Flow Through Immunoassay (a)

Figure 1. A competitive immunoassay format where the antibody is either (a) immobilised on the solid support surface or (b) suspended in solution.



1. Lateral Flow Immunoassay (b)
2. Quartz Crystal Microbalance (a)
3. Surface Plasmon Resonance (a)

Figure 2. A competitive immunoassay format where the antigen is immobilised on the solid support surface and the antibody is either (a) not labelled or (b) labelled.

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