

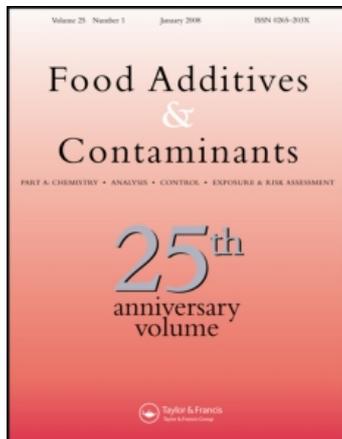
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Rapid and advanced tools for mycotoxin analysis: a review

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The problems associated with mycotoxin contamination of foods and feeds are well established and, in many cases, have been known for a long time. Consequently, the techniques for detecting known mycotoxins are quite advanced and range from methods for directly detecting the toxins themselves, based upon physical characteristics of the toxins, to methods for indirectly detecting the toxins, such as immunoassays. This review focuses on recent technologies that can be used to detect mycotoxins and, as such, is not a comprehensive review of the mycotoxin analytical literature. Rather, the intent is to survey the range of technologies from those that are instrument intensive such as modern chromatographic methods to those that require no instrumentation, such as certain immunoassays and biosensors. In particular, mass spectrometric techniques using ambient ionization offer the intriguing possibility of non-destructive sampling and detection. The potential application of one such technique, desorption electrospray ionization (DESI), is demonstrated for fumonisin B₁ on maize. While methods for detecting mycotoxins are quite advanced, the need remains for assays with increased throughput, for the exploration of novel detection technologies, and for the comprehensive validation of such technologies as they continue to be developed.

Keywords: liquid chromatography/mass spectrometry (LC-MS); immunoassays; screening – biosensor; screening assays; mycotoxins

Introduction

What do we mean by 'rapid' and 'advanced'?

Mycotoxins in human and animal food supplies have been a recognized safety issue for many years. As a result, a sustained international effort has been made to develop and improve methods for detecting and measuring these toxins in commodities, foods, and feeds. Describing advances made even within the past 10 years would require multiple volumes, and a comprehensive review is beyond the scope of this paper. Fortunately, the literature has been surveyed annually for a number of years as part of the AOAC International's General Referee Reports on mycotoxins (Trucksess 2006; Shephard 2007, 2008), and more recently published elsewhere as annual updates (Shephard et al. 2009). Reviews have also been published recently on novel technologies for mycotoxin detection (Goryacheva et al. 2007; Krska et al. 2008; Maragos 2009) and analytical methods (Turner et al. 2009). The purpose of this review is to provide a condensed summary of methods that are both advanced and rapid. The term 'rapid' can have different meanings depending upon the perspective and expectations of the analyst and the context of the analytical environment. Analysts should exercise

caution when evaluating descriptions of rapid methods. In many reports, the determinative step of the assay, the actual measurement of the toxin, is indeed very quick, but the steps leading up to the measurement such as the sample preparation, extraction, and isolation of the toxin may not have been included in the estimate of the assay's speed. Finally, many reports of rapid methods are descriptions of 'proof-of-concept' assays that have measured the toxin(s) in simple solutions such as buffers, without the potentially confounding materials present in sample matrix. Such reports are necessary to the advancement of technology and are very valuable, but provide little insight into how the assay would perform under conditions of expected use. Likewise, the term 'advanced' is subjective. Many analysts would surely consider advanced assays to include those that use the most modern, novel, technologies. However, methods that have been through the arduous process of being developed, evaluated, and have undergone validation by multiple laboratories could also claim to be advanced, as they are the furthest along in the progression from 'proof-of-concept' assays to useful analytical tools. This review will focus on the application of relatively recent, modern, technologies for the

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detection of mycotoxins. Specific application of a novel ambient ionization interface for use in the detection of fumonisin B₁ in maize will also be presented as one example of the direction that current technologies are headed.

Mass spectrometry-based assays

Some of the earliest methods for detecting mycotoxins were chromatographic, and chromatographic assays have continued to evolve with improvements in instrumentation. There is extensive literature on this subject, with several excellent reviews (Shephard 2009; Pascale and Visconti 2008; Turner et al. 2009). With improvements in detector sensitivities and lowered costs of instrumentation, the affordability of certain chromatographic techniques has improved dramatically. This spurred development in areas such as liquid chromatography-mass spectrometry (LC-MS), which has expanded substantially in recent years. Methods in this area have been recently reviewed (Sforza et al. 2006; Songsermsakul and Razzazi-Fazeli 2008; Larsson 2008). The use of 'ultra'-performance liquid chromatography (UPLC) to shorten analysis time (Beltrán et al. 2009) can only be expected to continue. Many high-performance liquid chromatography (HPLC)-MS methods have been described that detect multiple mycotoxins within a single chromatographic run (Nielsen and Smedsgaard 2003; Cavaliere et al. 2007; Ren et al. 2007; Rudrabhatla and Wood 2007; Sulyok et al. 2007; Spanjer et al. 2008). Components of a sample matrix can lead to the suppression or enhancement of the ionization process. Uncontrolled, such effects can interfere with the ability of the detector to quantify the analytes accurately. For this reason, many MS-based methods incorporate clean-up and/or chromatographic steps to reduce the amount of matrix present during elution of the analyte. Nonaka et al. (2009) have recently demonstrated the use of automated solid-phase microextraction (SPME) clean-up to streamline sample preparation before LC-MS analysis of aflatoxins from food samples. The impact of matrix effects can also be controlled through the use of internal standards, or matrix-matched external standards. An example is the use of isotopically labelled standards (Häubli et al. 2006; Rychlik and Asam 2008). While LC-MS in its many forms has dominated the recent chromatographic literature, other chromatographic approaches continue to be developed, such as high-performance thin layer chromatography (Caputo et al. 2007).

Matrix-assisted laser desorption ionization (MALDI)-MS allows the analysis of analytes co-crystallized with a matrix on a metal plate. Analyte ionization is initiated by focusing an ultraviolet (UV) light laser on a dried sample mixed

with matrix. Typically, the matrix is an organic acid that strongly absorbs the UV light. Modern MALDI-MS instrumentation facilitates the extremely high-throughput analysis of samples. Equipment is available for the automated deposition of analyte-matrix mixtures on sample plates for MALDI-MS analysis. Earlier, MALDI-MS was applied to the analysis of aflatoxins from peanuts (Ramos Catharino et al. 2005). More recently, Elostá et al. (2007) have described the analysis of several tricothecenes from barley and malt extracts. While MALDI-MS is attractive due to its convenience and sensitivity, reliable quantitation can often be difficult to obtain. Li and Gross (2004) have discussed approaches to the improvement of quantitation with MALDI-MS by carefully controlling the deposition of the analyte-matrix mixture on the sampling metal plate.

Ion mobility spectrometry (IMS) coupled to corona discharge ionization offers many of the advantages of atmospheric pressure ionization techniques coupled to mass spectrometry. However, required instrumentation is simpler and often much less expensive. IMS instruments have been constructed to be conveniently adapted for portability in the field. Recently, IMS has been utilized for the analysis of aflatoxins from pistachios (Sheibani et al. 2008). Sensitivity compared favourably with LC-MS techniques.

Ambient ionization mass spectrometry

Traditionally, mechanisms for producing ions for mass spectrometry have included electron ionization and chemical ionization, which involve creation of the ions under vacuum. More recently, techniques have been developed that allow ionization to occur at ambient pressure and outside of the mass spectrometer. Two commercial devices, based upon different techniques – desorption electrospray ionization (DESI) (Takás et al. 2004; Cooks et al. 2006) and direct analysis in real time (DART) (Cody et al. 2005) – have been developed. A generic scheme encompassing both processes is shown in Figure 1. The DART ionization source is based on the interactions between excited state atoms, or molecules, with the sample and atmospheric gases. A gas such as nitrogen or helium is subjected to an electrical discharge, producing ions, electrons, and excited state (metastable) atoms and molecules. From these the neutral gas molecules, including the metastable gases, are selected and directed toward the surface of the sample. The dominant mechanism of ionization may depend on the gas used. One mechanism involves the transfer of energy from the excited state metastable gas to the sample (analyte), while another involves the formation of ionized water clusters followed by proton transfer reactions (Cody et al. 2008). The resulting ions are swept into the MS

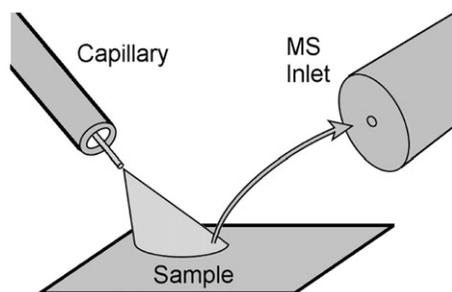


Figure 1. Generic representation of an ambient ionization source for a mass spectrometer. Specific sources available commercially use either a metastable gas or electrospray to bombard the sample and ionize the analytes. The desorbed, ionized, sample is swept up into the inlet of the mass spectrometer for detection.

interface for subsequent detection. The analysis of deoxynivalenol (DON) in beer using the DART source has been described (Hajslova et al. 2008). DON was isolated from beer using a commercial immunoaffinity column (IAC). A sampling stick was immersed into the purified extract and then introduced into the DART source. An internal standard of $^{13}\text{C}_{15}$ -DON was included in the examination of a single beer sample containing $166\ \mu\text{g l}^{-1}$.

DESI of fumonisin B₁

The DESI source utilizes the impact of electrosprayed solvent droplets upon sample surfaces to generate analyte ions from the sample surface under ambient conditions. Analyte ions are sampled into a mass spectrometer in a manner identical to those used for other atmospheric ionization techniques such as electrospray ionization and atmospheric pressure chemical ionization. An early demonstration of the DESI technique showed the analysis of small molecules from plant matrices (Talaty et al. 2005). In our laboratory, a DESI source was constructed and fitted to a ThermoFinnigan LCQ – Classic ion-trap mass spectrometer. The source was constructed readily to allow presentation of samples mounted on glass microscope slides.

To evaluate operation of the DESI source, fumonisin B₁ (FB₁) standards were applied directly to glass slides and to filter paper attached to glass slides with double-sided tape. Figure 2a shows a spectrum acquired from filter paper spotted with $10\ \mu\text{l}$ of $20\ \text{ng ml}^{-1}$ FB₁. To illustrate the utility of DESI for interrogation of grain samples with minimal sample preparation, $10\ \mu\text{l}$ of $20\ \text{ng ml}^{-1}$ FB₁ was deposited on the surface of maize kernels. Upon drying, the kernels were subjected to DESI-MS. The DESI-MS spectrum from the intact maize kernel is shown in Figure 2b. To demonstrate the effect of the DESI process on intact

kernels, ten maize kernels (B73 cultivar) were subjected to the DESI process for 30 s. Another control set of ten kernels was not examined by DESI-MS. Following the DESI-MS analysis, both sets of maize kernels were subjected to a germination test. After 5 days, nine out of ten of the DESI-MS-analysed kernels had germinated, while ten out of ten of the control kernels germinated. The DESI process is gentle enough to allow MS analysis, while leaving a good share of the kernels viable.

Membrane-based immunoassays

Analytes that are too small to allow for the binding of more than one antibody are typically measured in assays where labelled and unlabelled analytes compete. There are a large number of enzyme-linked immunosorbent assays (ELISAs) that have been developed for mycotoxins. The technology has become well established, and so will not be covered here. Many of the biosensors, immunosensors, and test strips are essentially modifications of the two basic forms of ELISA: where either the antigen or the (anti-toxin) antibody is immobilized. A form of ELISA that has been used successfully in rapid test kits has been flow-through ELISA, where the assay is conducted on a membrane or on gel-based columns. The on-column approach has been explored for several mycotoxins and foods (Goryacheva et al. 2008, 2009). With the membrane-based devices, applied sample and reagents flow through the membrane to an underlying absorbent pad or are removed by vacuum (De Saeger et al. 2006; Goryacheva et al. 2007). Notably, Schneider et al. (2004) developed a prototype device with an enzymatic label that allowed the simultaneous detection of seven mycotoxins: aflatoxin B₁ (AFB₁), FB₁, T-2 toxin, roridin A, DON, diacetoxyscirpenol, and ochratoxin A (OTA). Recently, there has been significant effort to produce immunochromatographic test strips for mycotoxins. The most common are termed lateral flow devices (LFD) because the reagents flow lengthwise through the membrane (i.e. chromatographically) rather than perpendicularly to the membrane. Despite their appearance the LFD are technologically advanced, intricate devices. A generic design for an LFD is depicted in Figure 3. When sample is applied to such a device, the liquid moves by capillary action through the conjugate pad and the membrane and is drawn toward the absorbent pad located near the end of the device. As the sample passes through the conjugate pad, the toxin has the opportunity to interact with a labelled primary antibody (anti-toxin). The mixture passes over two lines of capture reagents. In many cases, the first of these is a solid-phase toxin-protein antigen (e.g. AFB₁-protein). As the mixture reaches the first line, there is competition between the

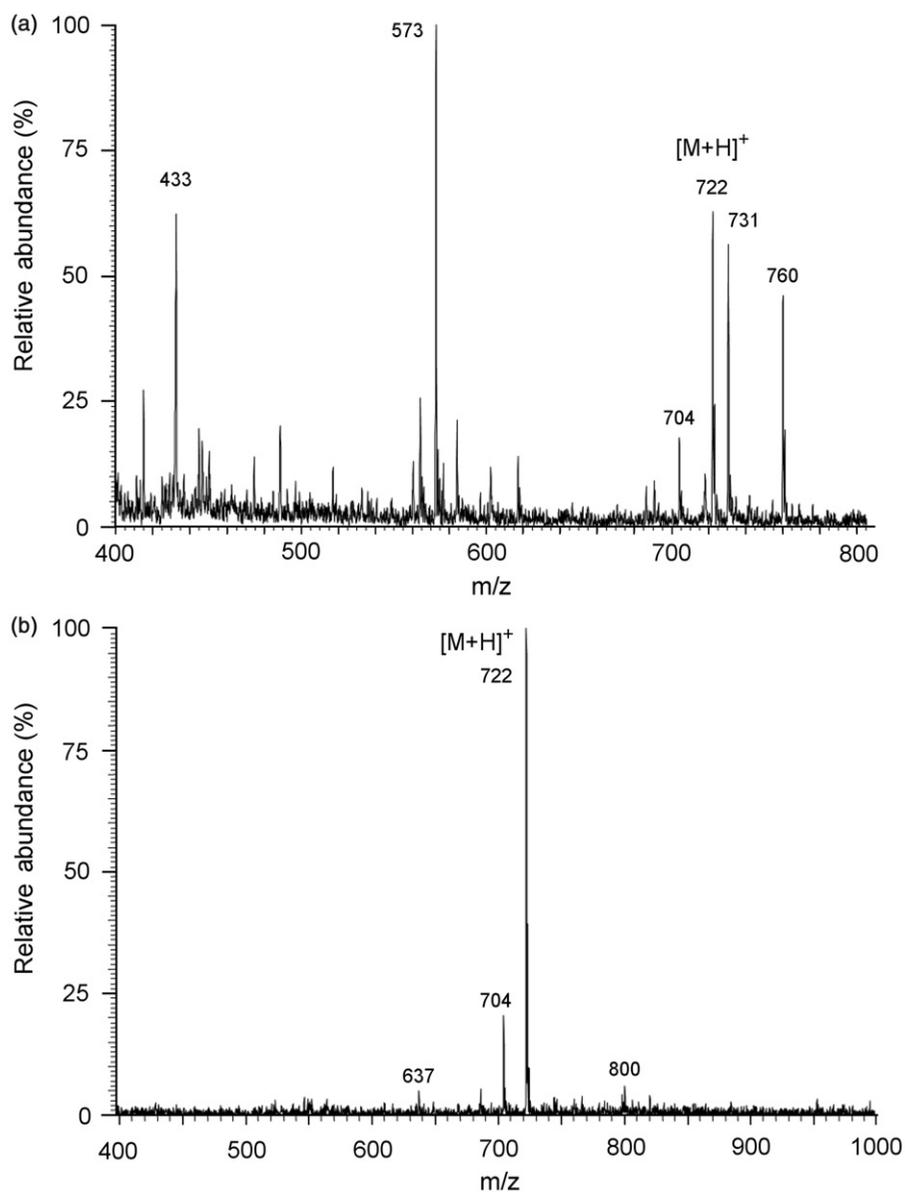


Figure 2. DESI-MS spectrum of fumonisin B₁. (a) Spectrum collected after applying 0.2 ng FB₁ to filter paper and (b) spectrum collected after applying 0.2 ng to a maize kernel.

toxin and the immobilized antigen for the labelled primary antibody. If there is little toxin, then there will be maximal binding of the label at this site. Unbound label continues to move towards a second material immobilized further up the strip, such as a secondary antibody (species specific and directed against the primary antibody). As the labelled antibody passes over the secondary antibody it binds, serving as a control for the assay. The label in the LFDs can be of several types, although gold colloid is frequently used to avoid the need for a substrate addition and incubation steps typical of enzymatic assays. The LFD for mycotoxins have been recently reviewed (Goryacheva et al. 2007; Maragos 2009). Such LFD are commercially available for most of the major

mycotoxins including the aflatoxins, DON, fumonisins, OTA, T-2 toxin, and zearalenone (ZEN). The LFD are advanced devices, both because of their construction and the fact that the reagents exist in a delicate balance in order to optimize sensitivity while retaining a strong visual signal. To facilitate the interpretation of results from LFDs, several manufacturers have introduced hand-held devices capable of scanning the strip and estimating toxin content from the colour intensities of the lines. Published applications of LFDs include detection of aflatoxins in pig feed (Delmulle et al. 2005), in rice, corn and wheat (Xiulan et al. 2006), in chillies (Saha et al. 2007), and in rice, barley, and feed samples (Shim et al. 2007). Fumonisin B₁ has been detected in corn, rice, barley,

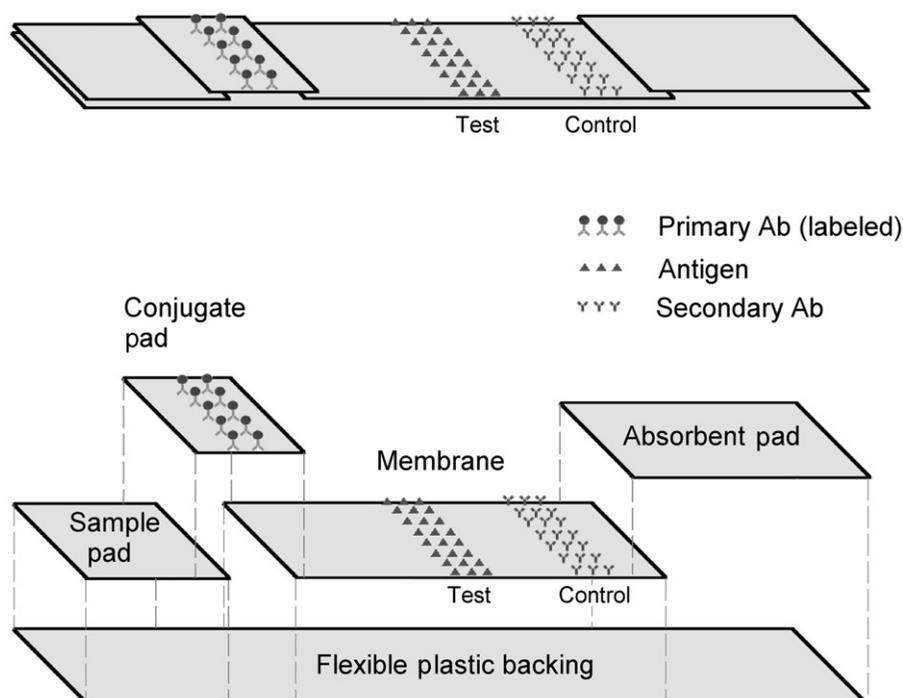


Figure 3. Components of a generic lateral flow device. Sample extract is added to the sample pad and flows, through capillary action, through the conjugate pad and membrane towards the absorbent pad. The presence of toxin in the sample inhibits the binding of conjugate to the 'test' (immobilized toxin-protein) line, but does not inhibit the binding of conjugate to the 'control' (anti-species antibody) line.

oats, peanuts, and sorghum using an LFD (Wang et al. 2006). Deoxynivalenol and ZEN have been detected in spiked wheat (Kolossova et al. 2007) and an LFD has recently been applied to the detection of T-2 toxin in naturally contaminated wheat and oats (Molinelli et al. 2008). An interesting variation on the LFD design has been to use a mimotope peptide, which mimics OTA, rather than an OTA-protein conjugate as the test antigen (Lai et al. 2009). In the latter paper, preliminary tests were conducted to detect OTA in cereal and soybean samples.

Electrochemical immunoassays

ELISAs typically use a colorimetric or fluorimetric endpoint. However, they can also use substrates with products that can be measured electrochemically. As with the more traditional ELISAs, the assays can be conducted in formats with either the test antigen or the antibody immobilized. Generally, the immunoreagents are immobilized onto the surface of an electrode. Detection can take a number of forms, including differential pulse voltammetry, cyclic voltammetry, chronoamperometry, electrochemical impedance spectroscopy, and linear sweep voltammetry. The advantages of electrochemical assays may include the low cost of production of the electrodes, amenability to miniaturization, and amenability to multiplexing.

The development of electrochemical immunoassays has recently been a very active area of research. Several of the reports use the substrate 1-naphthylphosphate, which yields the product 1-naphthol that can be detected by oxidation at the surface of the electrode. Other substrates can also be used. Interesting variations upon the concept include electrodes printed with a conductive polymer upon which the immunoreagents are immobilized. An assay for AFB₁ has been reported by Universal Sensors using this technique. Electrodes can also be configured to perform in multiplexed or microtitre plate formats (Pembernton et al. 2006; Piermarini et al. 2007). Applications of electrode-based sensors have included AFB₁ in milk (Micheli et al. 2005; Parker and Tothill 2009; Parker et al. 2009), AFB₁ in barley (Ammida et al. 2006), AFB₁ in spiked rice (Tan et al. 2009), and OTA in wheat (Alarcon et al. 2006). The latter report found a good correlation between the sensor and an HPLC method. Many variations of the technique exist. In one of these antigen-coated magnetic beads were used to develop an enzyme-linked immuno-magnetic electrochemical (ELIME) assay for AFB₁ in corn flour (Piermarini et al. 2009). As an alternative to measuring an enzymatic product, Tan et al. (2009) developed an assay where the deposition of metallic silver was detected. In that assay the substrate (ascorbic acid 2-phosphate) was converted to ascorbic acid, which in turn reduced silver ions to metallic silver that deposited

onto the surface of the electrode. Other variations of electrochemical assays do not require separation of the bound and unbound enzymatic label (Liu et al. 2006; Sun et al. 2008). In the latter two reports a mycotoxin-specific antibody (or antibody–horseradish peroxidase [HRP] conjugate) was immobilized onto the surface of an electrode. Remaining sites on the electrode were coated with HRP, and the level of enzymatic products in the presence of AFB₁ was measured. It was suggested that the mechanism of the assay involved inhibition of the enzyme activity associated with toxin binding by hindering access of the substrate or by hindering the electrical communication between the active site of the enzyme and the electrode. In one of these reports, good agreement was found between the sensor and an ELISA for detection of AFB₁ in spiked human sera or grape samples (Sun et al. 2008). Assays have also been reported that do not require an enzymatic label at all. Two of these involve the technique of electrochemical impedance spectroscopy (EIS). In one report anti-aflatoxin antibody was immobilized onto a platinum electrode coated with a conductive polymer. The association of AFB₁ with antibody was observed through a change in electron transfer resistance (Owino et al. 2007). A second report has described a label-free EIS immunosensor for detection of OTA (Radi et al. 2009), with a limit of detection of 0.5 ng ml⁻¹. Furthermore, direct detection of electro-active toxin degradation products is possible (Ricci et al. 2009). In the latter report, DON or nivalenol (NIV) isolated from wheat extracts using MycoSep columns were hydrolysed and the products detected using screen-printed electrodes. The electrochemical immunoassays are susceptible to many of the same issues as traditional ELISAs, namely factors that affect the antibody–antigen interaction (solvent and matrix effects) and, in certain cases, factors that influence the oxidation/reduction status of the test solution. However, given the number of toxin and matrix combinations that have been successfully tested and the possible advantages from miniaturization and multiplexing, further commercial development of such devices is expected.

Piezoelectric sensors

Recently, several sensors based upon piezoelectric quartz crystals have been described. Such devices work through the application of an alternating current to a quartz crystal, which induces oscillations of the crystal. The frequency of the oscillation depends in part on the thickness of the crystal. As material binds to the surface of the sensor, the thickness is increased. This increase can be assumed to be correlated with the increase in the mass bound per unit area, which is the basis behind these devices. Such sensors, often

called quartz crystal microbalances (QCM), have an advantage in that they do not require the use of labelled reagents. As with other immunoassays, two common formats are with either antibody-immobilized or test antigen (toxin–protein conjugate) immobilized. Several such devices have been described for aflatoxins (Jin et al. 2009a; Jin et al. 2009b; Wang and Gan 2009) and OTA (Tsai and Hsieh 2007; Vidal et al. 2009). Immobilized OTA antibody was the basis for a competitive assay between OTA and OTA–bovine serum albumin (BSA), with a limit of detection of 16 ng ml⁻¹ (Tsai and Hsieh 2007). Another approach, immobilizing the OTA–BSA, rather than the antibody, and using gold-coated quartz crystals yielded a limit of detection (LOD) of 8 ng ml⁻¹ (Vidal et al. 2009). Jin et al. (2009a) and Jin et al. (2009b) used two antigen-immobilized approaches to determine AFB₁. Both involved immobilization of AFB₁–BSA followed by competition with AFB₁ for an AFB₁ antibody. The two reports differ in the secondary detection step: one used a secondary antibody labelled with gold (Jin et al. 2009a), while the other used a secondary antibody labelled with HRP (Jin et al. 2009b). In the latter case, the substrate 4-chloro-1-naphthol was oxidized to benzo-4-chlorohexadienone, which was insoluble and increased the effective mass on the surface of the sensor. With either of the labels the assays were reported to detect 0.01 ng AFB₁ ml⁻¹ in spiked and defatted milk, although the upper range for the assay using the gold label was higher (Jin et al. 2009a; Jin et al. 2009b). Immobilization of the antibody, rather than the antigen, has been used for the detection of AFB₁ by coating the antibody onto magnetic nanoparticles that were held to the surface of the QCM with a magnet (Wang and Gan 2009). Using magnetic particles facilitated regeneration of the sensor, which responded over the range 0.3–7.0 ng AFB₁ ml⁻¹.

Enzyme inhibition assays

Recently, several novel sensors have been described that are based upon the inhibition of enzymes by AFB₁. These differ from immunoassays in at least two respects: first, they are not immunoassays (i.e. no antibody is involved); and second, direct inhibition of the enzyme is observed (instead of observing the inhibition of binding of an enzymatic label, as in ELISAs). AFB₁ has been reported to inhibit competitively porcine neutrophil elastase, a serine protease (Cuccioloni et al. 2008). The binding of AFB₁ to neutrophil elastase was followed in a non-competitive assay using surface plasmon resonance. AFB₁, isolated from spiked maize by solid-phase extraction, bound to immobilized elastase, increasing the sensor response. The method was reported to be linear over the range

1.67–17.8 $\mu\text{g AFB}_1 \text{ kg}^{-1}$ maize (Cuccioloni et al. 2008). Several other biosensors have been reported based on the inhibition of acetylcholinesterase (AChE) by AFB_1 (Arduini et al. 2007; Hansmann et al. 2009; Hossain et al. 2009; Ben Rejeb et al. 2009). It has been reported that AFB_1 inhibits AChE by binding to a peripheral site located at the entrance of the active site (Hansmann et al. 2009). The effect has been used to develop a colorimetric method with a linear range of 10–60 ng $\text{AFB}_1 \text{ ml}^{-1}$ (Arduini et al. 2007). An amperometric biosensor, based on AChE from electric eel, was less sensitive, with 3 $\mu\text{M AFB}_1$ causing 20% inhibition of the AChE (Hansmann et al. 2009). While this is not sensitive enough to be used with foods, the concept is intriguing. An amperometric biosensor for AFB_1 was also developed using screen-printed electrodes containing immobilized choline oxidase. By consuming the choline product of AChE, the choline oxidase generated H_2O_2 , which was detected with an electrode (Ben Rejeb et al. 2009). The sensor was applied to extracts of olive oil. In another interesting report, paper test strips were prepared containing AChE ‘sandwiched’ between two layers of silica sol-gel. The AChE activity on the strip was determined by colorimetric assay. Dose-dependent inhibition was observed, with an LOD of approximately 30 nM AFB_1 (Hossain et al. 2009). It should be noted that AChE can be inhibited by other compounds besides the aflatoxins, e.g. organophosphorus and carbamatic pesticides, which are irreversible inhibitors of the enzyme (Ben Rejeb et al. 2009). At the moment, most of the AChE-based assays remain ‘proof of concept’ rather than practical assays either due to relatively high limits of detection (relative to other AFB_1 detection techniques) or the need for a pre-concentration step.

Biosensor arrays

In order to complete analyses as quickly as possible, it is often desirable to detect multiple toxins within a single assay. The detection of multiple mycotoxins in complex mixtures can be accomplished based on physical properties such as the affinity for chromatographic matrices, the ability to absorb light or fluoresce, or the mass/charge ratio, as discussed above. Assays of this nature essentially involve serial detection of multiple analytes. The separate mycotoxins can also be detected in parallel by simultaneous assays physically separated from one another. Examples of parallel assays are biosensor arrays. While there are a number of such analytical arrays (Seidel and Niessner 2008), we will focus on the two types that currently appear most pertinent for mycotoxins, namely those based upon surface plasmon resonance (SPR) or fluorescence. A substantial literature now exists on the application

of SPR to mycotoxin detection. The SPR technique is based upon the property that binding of materials to a surface can alter the refractive index near that surface. SPR devices measure the small changes in the angle, or intensity, of internally reflected light that result from the binding event. The magnitude of the response is influenced by the amount of material adhering to the surface. An advantage of SPR is that it does not necessarily require competition, or labelled reagents, for detection. The number of devices that use SPR has increased substantially in recent years and a wide variety of devices are commercially available. The application of SPR to mycotoxins has been reviewed (Maragos 2004, 2009; Lacy et al. 2008). Because antibodies are much larger (circa 150 kDa) than mycotoxins (less than 1 kDa), many of the SPR assays have been configured as competitive assays where the binding of mycotoxin-specific antibodies to immobilized toxin–antigens is detected. Assays of this type have been used to detect AFB_1 , AFM_1 , DON, ZEN, OTA, and the fumonisins (Daly et al. 2000, 2002; Moghaddam et al. 2001; van der Gaag et al. 2003; Schnerr et al. 2002; Tüdös et al. 2003; Wang et al. 2009). An alternative to the detection of induced refractive index changes with SPR is to use surface plasmons to excite fluorophores captured on a surface, a technique known as surface plasmon-enhanced fluorescence spectroscopy (SPFS). An SPFS method for the detection of AFM_1 in milk was recently described (Wang et al. 2009). Antigen (AFM_1 -BSA) was immobilized on the surface of the sensor, and the binding of anti- AFM_1 antibody was measured following the addition of a secondary (Cy5-labelled) antibody. The reported LOD was 0.6 pg ml^{-1} in milk. In addition to competitive assays, there are also examples where non-competitive assays have measured the binding of mycotoxins to immobilized antibodies or antibody fragments. This has been demonstrated for FB_1 and ZEN (Mullett et al. 1998; Cho et al. 2005; Chang et al. 2008). Two recent reports also detected direct binding of toxin to the surface of the sensor, but replaced the antibodies with molecularly imprinted polymers for either OTA (Yu and Lai 2005) or ZEN (Choi et al. 2009). Although the technology is promising, there have been only a few applications of SPR to mycotoxins in foods. These have included AFB_1 in ‘grain’ (Daly et al. 2002), DON in wheat (Schnerr et al. 2002; Tüdös et al. 2003); OTA in wheat and wine extracts (Yu and Lai 2005), up to four mycotoxins in unspecified samples (van der Gaag et al. 2003), ZEN in spiked corn (Choi et al. 2009), and AFM_1 in milk (Wang et al. 2009). As with most immunoassays, SPR-based methods can be influenced by matrix effects. In the above reports this has generally been dealt with by increasing the dilution of the sample extract (in cases where the assay is very sensitive), or by clean-up of the extract before the detection step.

Despite the potential for multiplexing of assays, only one report has demonstrated this (van der Gaag et al. 2003), although with the availability of novel instrumentation that can detect multiple channels simultaneously this is an area that warrants further investigation. A technique related to SPR, optical waveguide lightmode spectroscopy (OWLS), has been used to detect AFB₁ and OTA in competitive and non-competitive assays (Adányi et al. 2007).

Microarrays are also available based upon fluorescence, rather than (or in addition to) SPR. Such formats are commonly used in hybridization assays for the detection of genes or polymerase chain reaction products, but have received considerable attention for protein detection as well. Light at the excitation wavelength of a particular fluorophore, or combination of fluorophores, can be provided in a number of ways, including using waveguides based upon the evanescent wave effect. However, it is also possible to provide the incident light without using a waveguide. As with most biosensors, the format of such assays generally begins either with a surface-immobilized antigen or a surface-immobilized antibody. An example of the surface-immobilized antigen approach was described by Janotta and Krska (2005) for the detection of DON and AFB₁ using fluorescently labelled antibodies. A planar waveguide to provide the excitation light has been incorporated into the immobilized antigen approach by the US Naval Research Laboratory. An advantage of a planar waveguide is the ability to imprint arrays upon the surface. The device has been used for the detection of several mycotoxins in commodities and foods, including OTA in spiked barley, wheat pasta, cornflakes, roasted coffee, and red wine (Ngundi et al. 2005), DON in spiked cornmeal, cornflakes, wheat, barley, and oats (Ngundi et al. 2006), and AFB₁ in spiked cornflakes, cornmeal, popcorn, peanuts, peanut butter, and pecans (Sapsford et al. 2006a). As with most immunoassays, matrix effects can be an issue. The array-based approach for detecting multiple mycotoxins was demonstrated with the detection of AFB₁, OTA, FB₁, and DON (Sapsford et al. 2006b). While the SPR and fluorescence-based arrays show considerable promise, as demonstrated by the appearance of applications, the expense of the instrumentation and, in some cases its availability, are hindering the more widespread adoption of the techniques.

Fluorescence polarization immunoassays

Most of the immunoassays developed for mycotoxins are heterogeneous in nature. That is, they require the separation of bound and unbound tracer (labelled toxin, enzyme, fluorophore, etc.). Fluorescence polarization immunoassay (FPIA) is an example of a

homogeneous assay: it is not necessary to separate the tracer from the immunoassay mixture. With FPIA the tracer is typically a mycotoxin labelled with a fluorophore such as fluorescein. Fluorescence polarization readers indirectly measure the rate of rotation of a tracer in solution because, at a given temperature, the rate of rotation in solution is inversely related to the space that the tracer occupies (and therefore its size). Unbound tracers of low molecular weight (such as DON-fluorescein) rotate more rapidly in solution, and give a lower fluorescence polarization signal than bound tracers which, after binding to the antibody, rotate at the rate of the much larger immunocomplex. Because of this, the binding of the tracer to the antibody can be directly monitored, without the need to separate bound and unbound tracer (Figure 4). This is an advantage for FPIAs because it eliminates at least one of the steps (washing) generally required for heterogeneous assays. FP immunoassays can be used to measure the rate of association of the toxin with the antibody (kinetic assays) or can be used to measure the competition reaction when it has reached equilibrium (equilibrium assays). As with many competitive immunoassays the signal is inversely proportional to the concentration of free toxin present. FPIAs for most of the major mycotoxins have been developed, including the aflatoxins, fumonisins, DON, OTA, and ZEN. Several of these are available commercially. The topic has been recently reviewed (Smith and Eremin 2008). Most of the recent reports have focused on OTA, ZEN, and DON (Shim et al. 2004; Lippolis et al. 2006; Maragos and Kim 2006; Park et al. 2006; Chun et al. 2009). FPIA are not without their limitations. As with most immunoassays, matrix effects can occur. Matrix effects can be controlled in a number of ways, such as through dilution, clean-up, matrix-matched calibration curves, or data normalization. An example of how matrix effects in naturally contaminated wheat and wheat-based products were controlled by subtracting the response from 'toxin-free' matrix was described by Lippolis et al. (2006). As with all immunoassays, the proper selection of antibody and tracer pairs is essential. Of particular importance with FPIA is attention to the kinetics of the assay, which can depend upon the antibody/tracer pair selected (Maragos et al. 2002; Maragos and Plattner 2002). Unlike the arrays discussed above, the portable FPIA readers only allow the detection of a single mycotoxin at a time, a limitation compared with the multiplexed methods that might be addressed with multiple tracers using different fluorescence emission wavelengths. Despite this, the potential speed of FP assays combined with the portability of the devices, suggests this technology has a promising future.

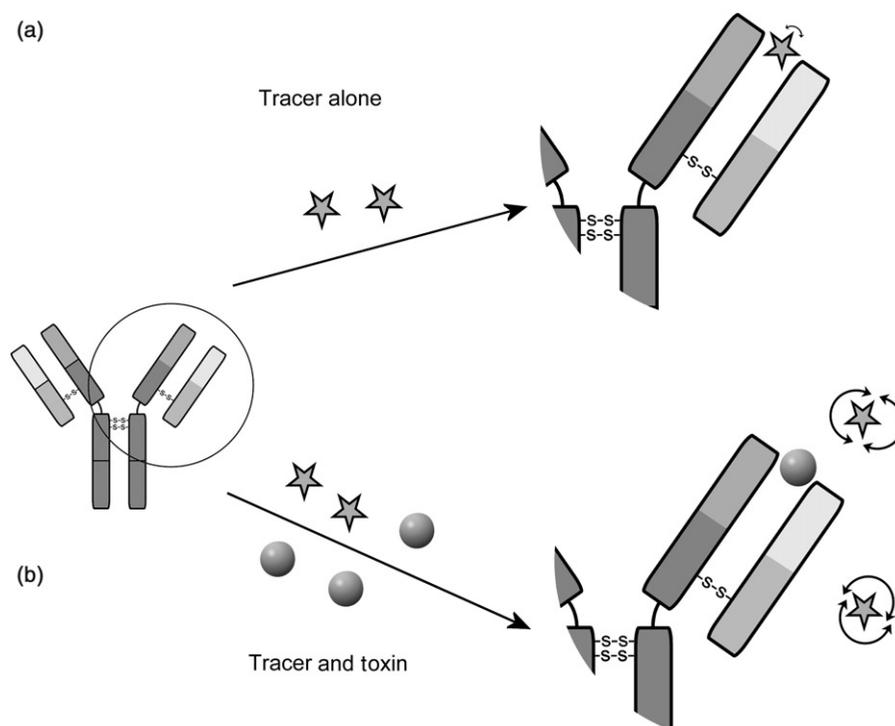


Figure 4. Fluorescence polarization immunoassay in either the absence of toxin (A) or the presence of excess toxin (B). In the presence of toxin (circles), binding of tracer (stars) is reduced. Tracer unbound by the antibody tumbles more rapidly in solution (shown as arrows) than bound tracer, giving a lower fluorescence polarization.

Conclusions

While we have endeavoured to summarize a large variety of very different technologies for mycotoxin detection, we would be remiss if we did not mention that promising technologies are also being developed that estimate fungal contamination. These presumptive tests range from polymerase chain reaction (PCR), transcriptomic-based assays (Lancova et al. 2009), and microarrays (Schmidt-Heydt and Geisen 2007) to tests that link the presence of one or more physical or chemical markers to fungal spoilage (Logrieco et al. 2005). Such tests are very promising for screening for fungal contamination. Early work in this area involved assays based upon visible changes in a commodity occurring with fungal growth. An example of the latter is the bright greenish yellow fluorescence (BGYF) test widely used as a quality measure of maize. More recent presumptive tests include electronic noses and tongues, near infrared devices (transmittance and reflectance), hyperspectral imaging, acoustical methods, and others (Berardo et al. 2005; Presicce et al. 2006; Galvis-Sánchez et al. 2007; Sahgal et al. 2007; Hernández-Hierro et al. 2008; Siuda et al. 2008; De Girolamo et al. 2009; Tripathi and Mishra 2009). Although such assays currently do not detect the toxins themselves, they may nonetheless be very useful for sorting and removal of material containing fungi and therefore could logically be helpful in reducing mycotoxin contamination.

Of the toxin detection technologies described in the preceding review, several are clearly beginning to dominate as real-world applications. In particular the LC-MSⁿ assays and LFD are rapidly becoming more widely used. With the desire to detect as many toxins in a food as quickly as possible, it is expected that technologies that further increase the speed of analysis will increasingly be used in the future. Examples of the latter are combinations of rapid chromatography with mass spectrometry, ambient ionization mass spectrometry, and multiplexed immunoassays having one or more detection technologies such as fluorescence or SPR. The potential of handheld mass spectrometers (Ouyang et al. 2009) is also intriguing. In the current review, the demonstration of the detection of FB₁ using DESI suggests non-invasive chemical detection of mycotoxins on food surfaces is possible, although the feasibility remains to be determined. Given market forces, it is difficult to foresee which of the aforementioned technologies will become commercially viable. Electrochemical sensors, SPR, fluorescence-based sensors, and fluorescence polarization immunoassay have all been applied successfully in laboratories to foods. How widespread these technologies become will depend on market forces. However, from the extent of the literature, it is apparent that interest in these technologies is likely to continue. Challenges remain for mycotoxin analysts, including the development of technologies for the simultaneous extraction of

multiple mycotoxins, which often have very different physical characteristics, from foods. In this regard, new extraction techniques such as the use of detergents (Maragos 2008) and supra-molecular solvents (Ballesteros-Gómez et al. 2009) need further exploration. Additionally, many of the 'proof-of-concept' assays described in this review need to be tested with foods or, where they have been tested, begin the extensive process of method validation necessary to yield methods that can be used for real samples.

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Mention of trade names or commercial products in this review is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

References

- Adányi N, Levkovets IA, Rodriguez-Gil S, Ronald A, Varadi M, Szendro I. 2007. Development of immunosensor based on OWLS technique for determining aflatoxin B₁ and ochratoxin A. *Biosens Bioelectronics*. 22:797–802.
- Alarcon SH, Palleschi G, Compagnone D, Pascale M, Visconti A, Barna-Vetro I. 2006. Monoclonal antibody based electrochemical immunosensor for the determination of ochratoxin A in wheat. *Talanta*. 69:1031–1037.
- Ammida NHS, Micheli L, Piermarini S, Moscone D, Palleschi G. 2006. Detection of aflatoxin B₁ in barley: comparative study of immunosensor and HPLC. *Analyt Lett*. 39:1559–1572.
- Arduini F, Errico I, Amine A, Micheli L, Palleschi G, Moscone D. 2007. Enzymatic spectrophotometric method for aflatoxin B detection based on acetylcholinesterase inhibition. *Analyt Chem*. 79:3409–3415.
- Ballesteros-Gómez A, Rubio S, Pérez-Bendito D. 2009. Potential of supramolecular solvents for the extraction of contaminants in liquid foods. *J Chromatogr A*. 1216:530–539.
- Beltrán E, Ibáñez M, Sancho JV, Hernández F. 2009. Determination of mycotoxins in different food commodities by ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry. *Rapid Comm Mass Spectrom*. 23:1801–1809.
- Ben Rejeb I, Arduini F, Arvinte A, Amine A, Gargouri M, Micheli L, Bala C, Moscone D, Palleschi G. 2009. Development of a bio-electrochemical assay for AFB₁ detection in olive oil. *Biosensors Bioelectronics*. 24:1962–1968.
- Berardo N, Pisacane V, Battilani P, Scandolaro A, Pietri A, Marocco A. 2005. Rapid detection of kernel rots and mycotoxins in maize by near-infrared reflectance spectroscopy. *J Agricult Food Chem*. 53:8128–8134.
- Caputo D, de Cesare G, Fanelli C, Nascetti A, Ricelli A, Scipinotti R. 2007. Innovative detection system of ochratoxin A by thin film photodiodes. *Sensors*. 7:1317–1322.
- Cavaliere C, Foglia P, Guarino C, Motto M, Nazzari M, Samperi R, Laganá A, Berardo N. 2007. Mycotoxins produced by *Fusarium* genus in maize: determination by screening and confirmatory methods based on liquid chromatography tandem mass spectrometry. *Food Chem*. 105:700–710.
- Chang HJ, Choi SW, Chun H. 2008. Expression of functional single-chain variable domain fragment antibody (scFv) against mycotoxin zearalenone in *Pichia pastoris*. *Biotech Lett*. 30:1801–1806.
- Cho YJ, Chun HS, Kim CJ, Kim CT, Hong JY. 2005. Detection of fumonisin B₁ by a batch type surface plasmon resonance biosensor. *Food Sci Biotech*. 14:698–699.
- Choi SW, Chang HJ, Lee N, Kim JH, Chun HS. 2009. Detection of mycoestrogen zearalenone by a molecularly imprinted polypyrrole-based surface plasmon resonance (SPR) sensor. *J Agricult Food Chem*. 57:1113–1118.
- Chun HS, Choi EH, Chang HJ, Choi SW, Eremin SA. 2009. A fluorescence polarization immunoassay for the detection of zearalenone in corn. *Analyt Chim Acta*. 639:83–89.
- Cody RB, Laramée JA, Durst HD. 2005. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Analyt Chem*. 77:2297–2302.
- Cody RB, Laramée JA, Nilles JM, Durst HD. 2008. Direct analysis in real time (DART) mass spectrometry. In: *JEOL application notebook*. 4th ed. p. 1–6. Available from: <http://www.jeolusa.com/>
- Cooks RG, Ouyang Z, Takats Z, Wiseman JM. 2006. Ambient mass spectrometry. *Science*. 311(5767):1566–1570.
- Cuccioloni M, Mozzicafreddo M, Barocci S, Ciuti F, Pecorelli I, Eleuteri AM, Spina M, Fioretti E, Angeletti M. 2008. Biosensor-based screening method for the detection of aflatoxins B₁–G₁. *Analyt Chem*. 80:9250–9256.
- Daly S, Dillon P, Manning B, Dunne L, Killard A, O'Kennedy R. 2002. Production and characterization of murine single chain Fv antibodies to aflatoxin B₁ derived from a pre-immunized antibody phage display library system. *Food Agricult Immunol*. 14:255–274.
- Daly SJ, Keating GJ, Dillon PP, Manning BM, O'Kennedy R, Lee HA, Morgan MRA. 2000. Development of surface plasmon resonance-based immunoassay for aflatoxin B₁. *J Agricult Food Chem*. 48:5097–5104.
- De Girolamo A, Lippolis V, Nordkvist E, Visconti A. 2009. Rapid and non-invasive analysis of deoxynivalenol in durum and common wheat by Fourier-transform near infrared (FT-NIR) spectroscopy. *Food Addit Contamin A: Chem*. 26:907–917.
- De Saeger S, Sibanda L, Paepens C, Lobeau M, Delmulle B, Barna-Vetro I, Van Peteghem C. 2006. Novel developments in rapid mycotoxin detection. *Mycotox Res*. 22:100–104.
- Delmulle BS, De Saeger SMDG, Sibanda L, Barna-Vetro I, Van Peteghem CH. 2005. Development of an immunoassay-based lateral flow dipstick for the rapid detection of aflatoxin B₁ in pig feed. *J Agricult Food Chem*. 53:3364–3368.
- Elosta S, Gajdosova D, Hegrova B, Havel J. 2007. MALDI TOF mass spectrometry of selected mycotoxins in barley. *J Appl Biomed*. 5:39–47.

- Galvis-Sánchez AC, Barros A, Delgadillo I. 2007. FTIR-ATR infrared spectroscopy for the detection of ochratoxin A in dried vine fruit. *Food Addit Contamin.* 24:1299–1305.
- Goryacheva IY, Basova EY, Van Peteghem C, Eremin SA, Pussemier L, Motte JC, De Saeger S. 2008. Novel gel-based rapid test for non-instrumental detection of ochratoxin A in beer. *Analyt Bioanalyt Chem.* 390:723–727.
- Goryacheva IY, De Saeger S, Eremin SA, Van Peteghem C. 2007. Immunochemical methods for rapid mycotoxin detection: evolution from single to multiple analyte screening: a review. *Food Addit Contamin.* 24:1169–1183.
- Goryacheva IY, Karagushcheva MA, Van Peteghem C, Sibanda L, De Saeger S. 2009. Immunoaffinity pre-concentration combined with on-column visual detection as a tool for rapid aflatoxin M₁ screening in milk. *Food Contr.* 20:802–806.
- Hajslova J, Vaclavik L, Cajka T, Poustka J, Schurek J. 2008. Analysis of deoxynivalenol in beer. In: *JEOL applications notebook*. 4th ed. p. 55–56. Available from: <http://www.jeolusa.com/>
- Hansmann T, Sanson B, Stojan J, Weik M, Marty JL, Fournier D. 2009. Kinetic insight into the mechanism of cholinesterase inhibition by aflatoxin B₁ to develop biosensors. *Biosensors Bioelectronics.* 24:2119–2124.
- Häubli G, Berthiller F, Krska R, Schuhmacher R. 2006. Suitability of a fully ¹³C isotope labeled internal standard for the determination of the mycotoxin deoxynivalenol by LC-MS/MS without clean up. *Analyt Bioanalyt Chem.* 384:692–696.
- Hernández-Hierro JM, García-Villanova RJ, González-Martín I. 2008. Potential of near infrared spectroscopy for the analysis of mycotoxins applied to naturally contaminated red paprika found in the Spanish market. *Analyt Chim Acta.* 622:189–194.
- Hossain SMZ, Luckham RE, Smith AM, Lebert JM, Davies LM, Pelton RH, Filipe CDM, Brennan JD. 2009. Development of a bioactive paper sensor for detection of neurotoxins using piezoelectric inkjet printing of sol-gel derived bioinks. *Analyt Chem.* 81:5474–5483.
- Janotta M, Krska R. 2005. Entwicklung von mykotoxin screening methoden mittels protein microarrays. *Arbeitsgemeinschaft Lebensmittel Veterinar Agrarwesen.* 2:42–45.
- Jin X, Jin X, Chen L, Jiang J, Shen G, Yu R. 2009b. Piezoelectric immunosensor with gold nanoparticles enhanced competitive immunoreaction technique for quantification of aflatoxin B₁. *Biosensors Bioelectronics.* 24:2580–2585.
- Jin X, Jin X, Liu X, Chen L, Jiang J, Shen G, Yu R. 2009a. Biocatalyzed deposition amplification for detection of aflatoxin B₁ based on quartz crystal microbalance. *Analyt Chim Acta.* 645:92–97.
- Kolosova A, De Saeger S, Sibanda L, Verheijen R, Van Peteghem C. 2007. Development of a colloidal gold-based lateral-flow immunoassay for the rapid simultaneous detection of zearalenone and deoxynivalenol. *Analyt Bioanalyt Chem.* 389:2103–2107.
- Krska R, Schubert-Ullrich P, Molinelli A, Sulyok M, MacDonald S, Crews C. 2008. Mycotoxin analysis: an update. *Food Addit Contamin.* 25:152–163.
- Lacy A, Dunne L, Fitzpatrick B, Daly S, Keating G, Baxter A, Hearty S, O'Kennedy R. 2008. Rapid analysis of coumarins using surface plasmon resonance. *J AOAC Int.* 89:884–892.
- Lai W, Fung DYC, Yang X, Renrong L, Xiong Y. 2009. Development of a colloidal gold strip for rapid detection of ochratoxin A with mimotope peptide. *Food Contr.* 20:791–795.
- Lancova K, Bowens P, Stroka J, Gmuender H, Ellinger T, Naegeli H. 2009. Transcriptomic-based bioassays for the detection of type A trichothecenes. *World Mycotoxin J.* 2:247–257.
- Larsson L. 2008. Use of mass spectrometry for determining microbial toxins in indoor environments. *J Environment Monit.* 10:301–304.
- Li YL, Gross ML. 2004. Ionic-liquid matrices for quantitative analysis by MALDI-TOF mass spectrometry. *J Am Soc Mass Spectrom.* 15:1833–1837.
- Lippolis V, Pascale M, Visconti A. 2006. Optimization of a fluorescence polarization immunoassay for rapid quantification of deoxynivalenol in Durum wheat-based products. *J Food Protect.* 69:2712–2719.
- Liu Y, Qin Z, Wu X, Jiang H. 2006. Immune-biosensor for aflatoxin B₁ based bio-electrocatalytic reaction on micro-comb electrode. *Biochem Eng J.* 32:211–217.
- Logrieco A, Arrigan DWM, Brenzel-Pesce K, Siciliano P, Tothill I. 2005. DNA arrays, electronic noses and tongues, biosensors and receptors for rapid detection of toxigenic fungi and mycotoxins: a review. *Food Addit Contamin.* 22:335–344.
- Maragos CM. 2004. Emerging technologies for mycotoxin detection. *Toxin Rev.* 23:317–344.
- Maragos CM. 2008. Extraction of aflatoxins B₁ and G₁ from maize by using aqueous sodium dodecyl sulfate. *J AOAC Int.* 91:762–767.
- Maragos CM. 2009. Biosensors for mycotoxin analysis: recent developments and future prospects. *World Mycotox J.* 2:221–238.
- Maragos CM, Jolley ME, Nasir MS. 2002. Fluorescence polarization as a tool for the determination of deoxynivalenol in wheat. *Food Addit Contamin.* 19:400–407.
- Maragos CM, Kim EK. 2006. Cross-reactivity of six zearalenone antibodies in a hand-held fluorescence polarisation immunoassay. In: Njapau H, Trujillo S, van Egmond HP, Park DL, editors. *Mycotoxins and phycotoxins: Advances in determination, toxicology and exposure management*. Wageningen (The Netherlands): Wageningen Academic. p. 91–100.
- Maragos CM, Plattner RD. 2002. Rapid fluorescence polarization immunoassay for the mycotoxin deoxynivalenol in wheat. *J Agricult Food Chem.* 50:1827–1832.
- Micheli L, Grecco R, Badea M, Moscone D, Paleschi G. 2005. An electrochemical immunosensor for aflatoxin M₁ determination in milk using screen-printed electrodes. *Biosensors Bioelectronics.* 21:588–596.
- Moghaddam A, Løbersli I, Gebhardt K, Braunagel M, Marvik OJ. 2001. Selection and characterisation of recombinant single-chain antibodies to the hapten aflatoxin-B₁ from naive recombinant antibody libraries. *J Immunol Meth.* 254:169–181.
- Molinelli A, Grossalber K, Führer M, Baumgartner S, Sulyok M, Krska R. 2008. Development of qualitative and semiquantitative immunoassay-based rapid strip tests for

- the detection of T-2 toxin in wheat and oat. *J Agricult Food Chem.* 56:2589–2594.
- Mullett W, Lai EPC, Yeung JM. 1998. Immunoassay of fumonisins by a surface plasmon resonance biosensor. *Analyt Biochem.* 258:161–167.
- Ngundi MM, Qadri SA, Wallace EV, Moore MH, Lassman ME, Shriver-Lake LC, Ligler FS, Taitt CR. 2006. Detection of deoxynivalenol in foods and indoor air using an array biosensor. *Environ Sci Tech.* 40:2352–2356.
- Ngundi MM, Shriver-Lake LC, Moore MH, Lassman ME, Ligler FS, Taitt CR. 2005. Array biosensor for detection of ochratoxin A in cereals and beverages. *Analyt Chem.* 77:148–154.
- Nielsen KF, Smedsgaard J. 2003. Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography-UV-mass spectrometry methodology. *J Chromatogr A.* 1002:111–136.
- Nonaka Y, Saito K, Hanioka N, Narimatsu S, Kataoka H. 2009. Determination of aflatoxins in food samples by automated on-line in-tube solid-phase microextraction coupled with liquid chromatography-mass spectrometry. *J Chromatogr A.* 1216:4416–4422.
- Ouyang Z, Noll RJ, Cooks RG. 2009. Handheld miniature ion trap mass spectrometers. *Analyt Chem.* 81:2421–2425.
- Owino J, Ignaszak A, Al-Ahmed A, Baker P, Alemu H, Ngila J, Iwuoha E. 2007. Modelling of the impedimetric responses of an aflatoxin B₁ immunosensor prepared on an electrosynthetic polyaniline platform. *Analyt Bioanal Chem.* 388:1069–1074.
- Park JH, Chung DH, Lee IS. 2006. Application of fluorescence polarization immunoassay for the screening of ochratoxin A in unpolished rice. *J Life Sci.* 16:1006–1013.
- Parker CO, Lanyon YH, Manning M, Arrigan DWM, Tothill IE. 2009. Electrochemical immunochip sensor for aflatoxin M₁ detection. *Analyt Chem.* 81:5291–5298.
- Parker CO, Tothill IE. 2009. Development of an electrochemical immunosensor for aflatoxin M₁ in milk with focus on matrix interference. *Biosensors Bioelectronics.* 24:2452–2457.
- Pascale M, Visconti A. 2008. Overview of detection methods for mycotoxins. In: Leslie JF, Bandyopadhyay R, Visconti A, editors. *Mycotoxins detection methods, management, public health and agricultural trade.* Cambridge (MA): CABI International. p. 171–183.
- Pemberton RM, Pittson R, Biddle N, Drago GA, Hart JP. 2006. Studies towards the development of a screen-printed carbon electrochemical immunosensor array for mycotoxins: a sensor for aflatoxin B₁. *Analyt Lett.* 39:1573–1586.
- Piermarini S, Micheli L, Ammida NHS, Palleschi G, Moscone D. 2007. Electrochemical immunosensor array using a 96-well screen-printed microplate for aflatoxin B₁ detection. *Biosensors Bioelectronics.* 22:1434–1440.
- Piermarini S, Volpe G, Micheli L, Moscone D, Palleschi G. 2009. An ELIME-array for detection of aflatoxin B₁ in corn samples. *Food Contr.* 20:371–375.
- Presicce DS, Forleo A, Taurino AM, Zuppa M, Siciliano P, Laddomada B, Logrieco A, Visconti A. 2006. Response evaluation of an E-nose towards contaminated wheat by *Fusarium poae* fungi. *Sensors Actuators B: Chem.* 118:433–438.
- Radi AE, Muñoz-Berbel X, Lates V, Marty JL. 2009. Label-free impedimetric immunosensor for sensitive detection of ochratoxin A. *Biosensors Bioelectronics.* 24:1888–1892.
- Ramos Catharino R, de Azevedo Marques L, Silva Santos L, Baptista AS, Glória EM, Calori-Domingues MA, Facco EMP, Eberlin MN. 2005. Aflatoxin screening by MALDI-TOF mass spectrometry. *Analyt Chem.* 77:8155–8157.
- Ren Y, Zhang Y, Shao S, Cai Z, Feng L, Pan H, Wang Z. 2007. Simultaneous determination of multi-component mycotoxin contaminants in foods and feeds by ultra-performance liquid chromatography tandem mass spectrometry. *J Chromatogr A.* 1143:48–64.
- Ricci F, Flavio P, Abagnale M, Messia MC, Marconi E, Volpe G, Moscone D, Palleschi G. 2009. Direct electrochemical detection of trichothecenes in wheat samples using a 96-well electrochemical plate coupled with microwave hydrolysis. *World Mycotox J.* 2:239–245.
- Rudrabhatla M, Wood JS. 2007. Rapid, quantitative analysis of multiple mycotoxins by liquid chromatography tandem mass spectrometry. *Am Lab.* 39:22–25.
- Rychlik M, Asam S. 2008. Stable isotope dilution assays in mycotoxin analysis. *Analyt Bioanal Chem.* 390:617–628.
- Saha D, Acharya D, Roy D, Shrestha D, Dhar TK. 2007. Simultaneous enzyme immunoassay for the screening of aflatoxin B₁ and ochratoxin A in chili samples. *Analyt Chim Acta.* 584:343–349.
- Sahgal N, Needham R, Cabañes FJ, Magan N. 2007. Potential for detection and discrimination between mycotoxigenic and non-toxigenic spoilage moulds using volatile production patterns: a review. *Food Addit Contamin.* 24:1161–1168.
- Sapsford KE, Ngundi MM, Moore MH, Lassman ME, Shriver-Lake LC, Taitt CR, Ligler FS. 2006b. Rapid detection of foodborne contaminants using an array biosensor. *Sensors Actuators B: Chem.* 113:599–607.
- Sapsford KE, Taitt CR, Fertig S, Moore MH, Lassman ME, Maragos CM, Shriver-Lake LC. 2006a. Indirect competitive immunoassay for detection of aflatoxin B₁ in corn and nut products using the array biosensor. *Biosensors Bioelectronics.* 21:2298–2305.
- Schmidt-Heydt M, Geisen R. 2007. A microarray for monitoring the production of mycotoxins in food. *Int J Food Microbiol.* 117:131–140.
- Schneider E, Curtui V, Seidler C, Dietrich R, Usleber E, Märklbauer E. 2004. Rapid methods for deoxynivalenol and other trichothecenes. *Toxicol Lett.* 153:113–121.
- Schnerr H, Vogel R, Niessen L. 2002. A biosensor-based immunoassay for rapid screening of deoxynivalenol contamination in wheat. *Food Agricult Immunol.* 14:313–321.
- Seidel M, Niessner R. 2008. Automated analytical microarrays: a critical review. *Analyt Bioanal Chem.* 391:1521–1544.
- Sforza S, Dall'Asta C, Marchelli R. 2006. Recent advances in mycotoxin determination in food and feed by hyphenated chromatographic techniques/mass spectrometry. *Mass Spectrom Rev.* 25:54–76.
- Sheibani A, Tabrizchi M, Ghaziaskar HS. 2008. Determination of aflatoxins B₁ and B₂ using ion mobility spectrometry. *Talanta.* 75:233–238.
- Shephard GS. 2007. Mycotoxins. *J AOAC Int.* 90:1B–17B.

- Shephard GS. 2008. Mycotoxins. *J AOAC Int.* 92:1B–16B.
- Shephard GS. 2009. Aflatoxin analysis at the beginning of the twenty-first century. *Analyt Bioanalyt Chem.* 395:1215–1224.
- Shephard GS, Berthiller F, Dorner J, Krska R, Lombaert GA, Malone B, Maragos C, Sabino M, Solfrizzo M, Trucksess MW, van Egmond HP, Whitaker TB. 2009. Developments in mycotoxin analysis: an update for 2007–2008. *World Mycotox J.* 2:3–21.
- Shim WB, Kolosova AY, Kim YJ, Yang ZY, Park SJ, Eremin SA, Lee IS, Chung DH. 2004. Fluorescence polarization immunoassay based on a monoclonal antibody for the detection of ochratoxin A. *Int J Food Sci Tech.* 39:829–837.
- Shim WB, Yang ZY, Kim JS, Kim JY, Kang SJ, Woo GJ, Chung YC, Eremin SA, Chung DH. 2007. Development of immunochromatography strip-test using nanocolloidal gold-antibody probe for the rapid detection of aflatoxin B₁ in grain and feed samples. *J Microbiol Biotech.* 17:1629–1637.
- Siuda R, Balcerowska G, Kupcewicz B, Lenc L. 2008. A modified approach to evaluation of DON content in scab-damaged ground wheat by use of diffuse reflectance spectroscopy. *Food Analyt Meth.* 1:283–292.
- Smith D, Eremin S. 2008. Fluorescence polarization immunoassays and related methods for simple, high-throughput screening of small molecules. *Analyt Bioanalyt Chem.* 391:1499–1507.
- Songsermsakul P, Razzazi-Fazeli E. 2008. A review of recent trends in applications of liquid chromatography-mass spectrometry for determination of mycotoxins. *J Liq Chromatogr Rel Tech.* 31:1641–1686.
- Spanjer MC, Rensen PM, Scholten JM. 2008. LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. *Food Addit Contamin.* 25:472–489.
- Sulyok M, Krska R, Schuhmacher R. 2007. A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. *Analyt Bioanalyt Chem.* 389:1505–1523.
- Sun AL, Qi QA, Dong ZL, Liang K. 2008. An electrochemical enzyme immunoassay for aflatoxin B₁ based on bio-electrocatalytic reaction with room-temperature ionic liquid and nanoparticle-modified electrodes. *Sensing Instrument Food Qual Saf.* 2:43–50.
- Takás Z, Wiseman JM, Gologan B, Cooks RG. 2004. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science.* 306:471–473.
- Talaty N, Takás Z, Cooks RG. 2005. Rapid *in situ* detection of alkaloids in plant tissue under ambient conditions using desorption electrospray ionization. *Analyt.* 130:1624–1633.
- Tan Y, Chu X, Shen GL, Yu RQ. 2009. A signal-amplified electrochemical immunosensor for aflatoxin B₁ determination in rice. *Analyt Biochem.* 387:82–86.
- Tripathi S, Mishra HN. 2009. A rapid FT-NIR method for estimation of aflatoxin B₁ in red chili powder. *Food Contr.* 20:840–846.
- Trucksess MW. 2006. Mycotoxins. *J AOAC Int.* 89:1–15.
- Tsai WC, Hsieh CK. 2007. QCM-based immunosensor for the determination of ochratoxin A. *Analyt Lett.* 40:1979–1991.
- Tüdös AJ, Lucas-van den Bos ER, Stigter ECA. 2003. Rapid surface plasmon resonance-based inhibition assay of deoxynivalenol. *J Agricult Food Chem.* 51:5843–5848.
- Turner NW, Subrahmanyam S, Piletsky SA. 2009. Analytical methods for determination of mycotoxins: a review. *Analyt Chim Acta.* 632:168–180.
- van der Gaag B, Spath S, Dietrich H, Stigter E, Boonzaaijer G, van Osenbruggen T, Koopal K. 2003. Biosensors and multiple mycotoxin analysis. *Food Contr.* 14:251–254.
- Vidal J, Duato P, Bonel L, Castillo J. 2009. Use of polyclonal antibodies to ochratoxin A with a quartz-crystal microbalance for developing real-time mycotoxin piezoelectric immunosensors. *Analyt Bioanalyt Chem.* 394:575–582.
- Wang L, Gan XX. 2009. Biomolecule-functionalized magnetic nanoparticles for flow-through quartz crystal microbalance immunoassay of aflatoxin B₁. *Bioprocess Biosystems Eng.* 32:109–116.
- Wang S, Quan Y, Lee N, Kennedy IR. 2006. Rapid determination of fumonisin B₁ in food samples by enzyme-linked immunosorbent assay and colloidal gold immunoassay. *J Agricult Food Chem.* 54:2491–2495.
- Wang Y, Dostálek J, Knoll W. 2009. Long range surface plasmon-enhanced fluorescence spectroscopy for the detection of aflatoxin M₁ in milk. *Biosensors Bioelectronics.* 24:2264–2267.
- Xiulan S, Xiaolian Z, Jian T, Xiaohong G, Jun Z, Chu FS. 2006. Development of an immunochromatographic assay for detection of aflatoxin B₁ in foods. *Food Contr.* 17:256–262.
- Yu JCC, Lai EPC. 2005. Interaction of ochratoxin A with molecularly imprinted polypyrrole film on surface plasmon resonance sensor. *Reactive Func Polymer.* 63:171–176.