Mycotoxin Analysis: A Focus on Rapid Methods

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# Challenges of Mycotoxins and Their control

## Health impacts of mycotoxins

Mycotoxins remain an important global food safety issue. Many hundreds of natural, toxic secondary fungal metabolites that are collectively termed as mycotoxins and have been identified from fungal cultures and some of them are observed in foodstuffs and feedstuffs around the world. FAO has estimated that about 25% of global crops are contaminated by molts and thus affected by mycotoxins. The economic losses are estimated to be billions of dollars. Of the many different mycotoxins only a few specific mycotoxins (or groups) present considerable food safety concerns. These agriculturally-important mycotoxins are aflatoxins, fumonisins, deoxynivalenol (DON), ochtratoxin A (OTA) and zearalenone (ZEA) (Moss, 1991; Steyn, 1995). Among them aflatoxins have a dominant role in terms of incidence in contaminated material.

Contamination with mold and mycotoxins can occur pre- and/or post-harvest if conditions are poor. Mycotoxins occurrence depends on improper condition with high humidity and temperature after harvest and storage. Therefore mycotoxin contamination is a major concern in tropical regions. Because the compounds are chemically stable under conditions usually present during food and feed processing, they are found in raw material as well as processed feedstuffs and foodstuffs. Due to their stability they are also resistant to high temperature and long-term storage. The common occurrence of mycotoxins in foodstuff and feedstuff poses an extensive hazard for human and animal health (Hussein and Brasel, 2001; Rai, 2012; Wild, 2010). The great variability of mycotoxins in structure explains the great variation in clinical symptoms in humans and animals. Mycotoxins toxicity vary from one form to another but generally they are acutely toxic, mutagenic, carcinogenic, teratogenic estrogenic, and immune suppressants (Table 1).

Table 1: Toxic effect of important mycotoxins

|  |  |
| --- | --- |
|  | Toxicity |
| Aflatoxin | pulmonary carcinogen, acute aflatoxicosis (Wild, 2010), liver carcinogen |
| Deoxynivalenol (DON) | abdominal stress, increased salivation, malaise, diarrhea, emesis (Pestka, 2005) |
| Fumonisin | toxicosis in swine (Haschek et al., 2001), equine leukoencephalopathy (Marasas et al., 2001) |
| Ochratoxin (OTA) | nephrotoxic, hepatotoxic, teratogenic in rats (Abdel-Wahhab M A, 2005) |
| T-2 and HT-2 | nausea, emesis, abdominal pain, diarrhea, dermal necrosis (Omurtag and D., 2001) |
| Zearalenone (ZON) | change in the reproductive system of animals such as mice and rats, genotoxic and/or carcinogenic (Scientific Committee on Food, 2000) |

Source: Rai , 2012

While in the developed world mycotoxin exposure has greatly been under control through stringent regulatory enforcement, the potential health implication of mycotoxins are still considerably high in developing countries. Reasons for this are the wide spread occurrence of the mycotoxin at frequently high levels and food consumption patterns that can result in large intake of a single cereal such as corn. Additional negative influences on health impact are concomitant poverty and malnutrition (Shephard, 2008b).

## Prevention and reduction of mycotoxin contamination

Prevention in cultivation and harvest is a possible way to reduce the challagges associated with mycotoxin contamination of crops. In this context biocontrol techniques were investigated to prevent the spreading of toxigenic fungi in corn. Such products are aflaguard® ([www.syngenta-us.com](http://www.syngenta-us.com)) and aflasafe™ ([www.aflasafe.com](http://www.aflasafe.com)). Basically, atoxigenic strains of *Aspergillus flavus* will be applied to the field and replace the toxigenic strains. Aflaguard includes the *A. flavus* AF36 which does not produce aflatoxins. A single application reduces the toxigenic strains in crops by over 80% and leads to a reduction of aflatoxins (Cotty, 2006). Further investigations in the area of such exclusion techniques exist such as Probst et al. who investigated different isolated strains of *A. flavus*. Atoxigenic strains (LOD aflatoxin B1 < 0,5 ppb), co-inoculated with high toxic strains (Probst et al., 2011). Additionally, aflasafe™ (figure 1) was developed from a partnership of the International Institute of Tropical Agriculture (IITA) in Nigeria, the Agricultural Research Service (ARS) of USDA and the Universities of Bonn (Germany) and Ibadan (Nigeria). It includes four native atoxigenic strains adapted to various African countries or agroecosystems. Aflasafe™ will be injected to the crop 2-4 weeks before flowering in the field e.g. a rate of 10 kg/ha will be tossed per hand through small fields. Field testing in Nigeria and Senegal exhibits a reduction of Aflatoxins in products of maize and groundnuts by 80-90% (Bandyopadhyay and Cotty, 2013). In relation to research on aflasafe the “Deutsche Gesellschaft für Internationale Zusammenarbeit” (GIZ) carried out a project in Nigeria in which the efficiency of Aflatoxins biocontrol technology in chili peppers was tested (GIZ, 2012-2013).

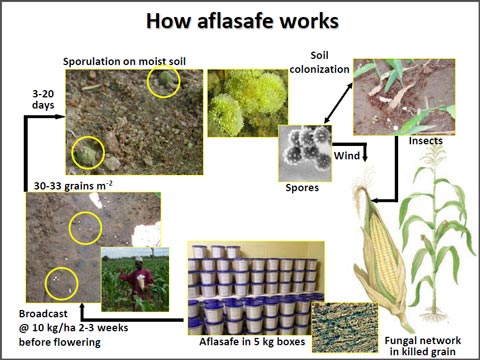


Figure 1: Application of aflasafe™ in the field from [www.aflasafe.com](http://www.aflasafe.com).

Important for the prevention of contaminations with mycotoxin is the fundamental knowledge in genomics, proteomics and metabolomics- so called “omics” - about the fungus and the mycotoxins. The knowledge enables the development of extensive prevention methods (Bhatnagar et al., 2008a; Bhatnagar et al., 2008b). Genomic studies include research in the complete set of genes of an organism. Special methods such as the microarray- or the Expressed Sequence Tags (EST) technology could help to understand the life cycle and the metabolism of the fungus which produces certain mycotoxins (Bhatnagar et al., 2008b).

The study of the whole proteins of a cell as well as their structure and their functions in the physiological pathways of cells is termed as proteomics. Analytical methods to elucidate the proteom are the two-dimensional polyacrylamide electrophoresis (2D-PAGE) followed by a protein cleavage and identification by mass spectrometry particularly the matrix-assisted laser desorption-ionisation time-of-flight (MALDI-TOF) mass spectrometry. Resistance associated proteins (RAPs) e.g. the glyoxylase I which is a stress related aflatoxin resistance protein (Bhatnagar et al., 2008b), are promising in breeding crop varieties with resistance to aflatoxins.

Metabolomic studies complete the understanding of the fungus and the synthesis of mycotoxins pre- and postharvest. Investigations contain metabolites as the results of specific cellular processes in biological organisms. With the “omics” it is possible to get important information about the contamination of plants and crops with a fungus under special environmental conditions, improving understanding of their metabolism and the biosynthesis of mycotoxins. In long-term orientation the investigations could generate knowledge and products to overcome the mycotoxins contamination problem (Bhatnagar et al., 2008b).

## Maximum regulatory limits for major mycotoxins

Ideally, official methods for detection and quantification of contaminants (such as mycotoxins) in food are set for contaminants for which there are accepted maximum limits (MLs). Different regions and countries have set MLs for for mycotoxins in food. In Europe limits of 2ppb (for aflatoxin B1) and 4ppb (for total aflatoxins (B1+B2+G1+G2)) for cereals and cereal products (including maize and maize products) for direct human consumption are in place. Likewise, MLs of 5ppb for Aflatoxin B1 and 10ppb for total aflatoxins are set for maize to be sorted or otherwise processed physically before human consumption. The European Commission further set a method for sampling of cereals and cereals products in view of the prescribed limts. The regulated limits of mycotoxins in the European region are defined in the regulation of the European Community EG-VO 1881/2006. Limits for selected mycotoxins are summarized in Table 39.

Codex Alimentarius Commission is repsonsible for setting maximum limits for mycotoxins in Food and feed at the global level. The Codex Commission has already adopted MLs for my cotoxins as shown below:

1. A maximum level of 10ppb for total aflatoxins in treenuts (almonds, hazelnuts, pistachios and shelled Brazil nuts) “ready to-eat” (IITA 2015: CAC, 2017).
2. ML of 15ppb for total aflatoxins in peanuts and treanuts destined for further processing (IITA 2015: CAC, 2017).,
3. ML of 2000 ppb for fumonisins in maize and maize flour for direct human consumption (CAC, 2014)
4. ML of 4000 ppb for fumonisins in maize for further processing (CAC ,2014)
5. ML of 2000 ppb for Deoxynivalenol in raw cereal grains (wheat, maize and barley) (CAC, 2015)
6. ML of 1000ppb for Deoxynivalenol in flour, semolina, meal and flakes derived from wheat, maize and barley (CAC, 2015)
7. ML of 200 ppb for cereal-based foods for infants and young children (CAC, 2015)

Sampling and analysis methods for these MLs are also prescribed by the Codex Commission. Countries in Africa who have set MLs for aflatoxins in food prescribe 5ppb for aflatoxin B1 and 10 ppb for total aflatoxins (FAO, 2004). In the East Africa region Limits are set for aflatoxins and fumonisins (EAC Standards Office, 2015; IITA, 2015). These are 5ppb for aflatoxins B1 and 10ppb for total aflatoxins and 2000ppb for fumonisins in maize grain, maize flour, wheat grain, wheat flour, Milled rice Macaroni, spaghetti and vermicelli Durum wheat semolina Finger millet flour Maize gluten, Groundnuts (peanuts), Sorghum flour, Pearl millet/bulbrush flour, Dry beans, Dry soybeans, Cassava wheat composite Flour, Composite flour, Pearl millet grains, Green grams, Sorghum grains, Finger millet grains, Faba beans, Rough (paddy) Rice, Brown Rice, Soya protein products and Textured soya protein products

maximum limits for various mycotoxins

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Maximum Limits for various mycotoxins

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| --- | --- | --- | --- | --- |
| Compound | Commodity | maximum level [ppb] | | |
|  |  | B1 | total | M1 |
| Aflatoxins | all food except milk | - | 20 | - |
|  | Milk | - | - | 0,5 |
|  | in feed: corn, corn products, cottonseed meal, and other animal feeds and feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known | - | 20 | - |
| Deoxynivalenol | finished wheat products for consumption by humans |  | 1.000\* |  |
|  | in feed: grains and grain by-products destined for ruminating beef and feedlot cattle older than 4 months and for chickens |  | 10.000\* |  |
| Fumonisins | degermed dry milled corn products (e.g. flaking grits, corn meal, corn flour with fat content of <2.25%, dry weight basis) |  | 2.000\* |  |
|  | in feed: corn and corn by-products intended for equids and rabbits |  | 5.000\* |  |
|  | in feed: corn and corn by-products intended for swine and catfish |  | 20.000\* |  |
|  | in feed: corn and corn by-products intended for breeding ruminants, breeding poultry and breeding mink (includes lactating dairy cattle and hens laying eggs for human consumption) |  | 30.000\* |  |

\*guidance level

# SAM

## 

Fast, cheap, portable and reliable determination methods are required for the analysis of mycotoxins in food and feed – not only for the developing countries but also for farmers and the processing industry in developed countries. Techniques should be reproducible, the methods sensitive and simple for non-scientist persons. Most methods involve time consuming preparation, clean-up and purification and this is more or less independent from the method later used for analytic and quantification such as more instrumental methods or screening methods (Anfossi et al., 2010; Burger et al., 2014; Reiter et al., 2009; Shephard, 2008a, 2009; Whitaker, 2003; Whitaker et al., 2005).

TinvolveMost methods The thus then

2procedures for analysis

## 

### Sampling and sample preparation

The heterogeneous distribution of mycotoxins in cereals, nuts, grains and other products takes an important role in the analysis of mycotoxins in food and feed. A number of wheat kernels can be low contaminated with mycotoxins but a single kernel can be highly contaminated. Studies in corn and peanuts suggest that in one lot only 0.1% of the kernels are contaminated with e.g. aflatoxin and the concentration of these kernels can be extremely high. Because of this wide variation in concentration range in mycotoxins among a few contaminated kernels in a lot, variability in replicated sampling can be high (Biselli, 2006; Schatzki, 2000; Turner et al., 2009; Whitaker, 2003; Whitaker et al., 2005). Adequate sampling and sample-preparation is needed to get a homogenous and representative sample as the fundament for precise determination of mycotoxins.

Solid products, such as kernels and nuts must be ground to powder form with a selected size to enlarge the surface area and make them accessible for extraction solvents. The equipment includes mills, grinder, sieves and filters. Liquids and paste-like solutions must gentle mixed before extraction. Subsamples were taken for extraction and analysis (AOAC, 2000; Beuchat, 1987).

### Extraction, clean-up and purification

Based on the fact that the concentrations of mycotoxins in samples are very low and the determination must be very sensitive a sample extraction, cleaning and purification are needed. Final cleaned-up extracts can be concentrated by evaporating the solvents (e.g. under a nitrogen stream). Techniques in use for extraction include Liquid-liquid extraction and Liquid-solid extraction.

#### Liquid-liquid extraction

Liquid–liquid extraction (LLE) utilizes the solubility or insolubility of mycotoxins in aqueous phase and organic phase (hexane, cyclohexane). Non-polar substances such as lipids and cholesterol can be removed by this approach from the sample solution.

Table 2: Strength and weakness of liquid-liquid extraction (LLE).

|  |  |
| --- | --- |
| Strength | purification, decrease interfering substances |
| weakness | - time consuming  - matrix and mycotoxin dependant  - loss of sample because of adsorption to the glassware |

There are different extraction methods for mycotoxins in addition to the matrix and the type of mycotoxin. High lipophilic or pigmented samples of food and feed require a more complex extraction which includes both clean-up and purification. Based on their chemical property aflatoxins will be extracted with mixtures of organic solvents (acetone, hexane, chloroform, methanol). Other mycotoxins like the fumonisins are polar metabolites. The extraction of them must be basically with water adding organic solvents (Holcomb et al., 1992). An overview about the solvent of choice for some mycotoxins is shown in Table 3.

Table 3: The most effective extraction solvents and alternatives for a number of mycotoxins – reviewed and modified by Biselli (2006).

|  |  |  |
| --- | --- | --- |
| **mycotoxin** |  | **Alternative** |
| **Aflatoxin** | methanol-water (80:20 v/v) | acetonitrile-water, acetone-water |
| **Ochratoxin A** | methanol-water | methyl *tert*-butyl ether (MTBE), acetonitrile-water |
| **Fumonisin** | methanol-acetonitrile-water | acetonitrile-water, methanol-water (75:15 v/v) |
| **Zearalenone** | methanol-water | ethyl acetate, acetonitrile-water (86:14 v/v) |

The official extraction method for the analysis of aflatoxins in peanuts and peanut products, oilseeds and food grains is the CB-method (Contaminants Bureau) (AOAC, 2000). Briefly - in a glass-stoppered flask there water, diatomaceous earth and chloroform are added to a powdered sample. After shaking the solution for 30 min it will be filtered and 50 ml of an extract will be placed on a special prepared column. After loading the column with the extract it will be washed with hexane and anhydrous ether. This fraction will be discarded. Aflatoxin will be eluted with a mixture of methanol-chloroform (3/97 v/v) and evaporated to dryness under a gentle stream of nitrogen or in a water bath. The residue will be used for the subsequent analysis.

Another method is the BF-method (Best Food) (AOAC, 2000). The aflatoxin will be extracted with a mixture of methanol-water-hexane, separated and extracted three times into chloroform. After evaporate to dryness it will be dissolved in a solution of benzene-acetonitrile. Now it can be used in TLC or HPTLC (Jaimez et al., 2000; Richard et al., 1993).

#### Liquid-solid extraction

The liquid-solid extractions of mycotoxins is usually based on the principal of a chromatographic technique. They are unspecific extraction columns such as solid phase extraction (SPE) or specific extraction such as immunoaffinity columns (IAC).

SPE uses small disposable cartridges packed with different stationary phases (e.g. silica gel, octadecylsilane). A sample extract is loaded onto the cartridge and rinsed under reduced pressure. Contaminants or the analyte will be removed with selected solvents. The method is also known as multifunctional column preparation and used as preparation prior to HPLC-analysis (Figure 3).

The specific IAC-extraction is the state-of-the-art method of mycotoxin purification. It enables efficient mycotoxin and specific extraction from different kinds of commodities. The packing material of the column includes antibodies which specific bind to the analyte and passed the interfering substances. The elution of the analyte is achieved with an antibody denaturing solution.

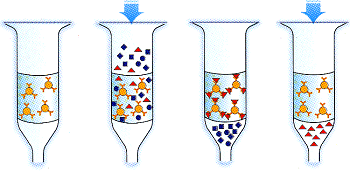


Figure 3: Model for immunoaffinity column (IAC) from European Mycotoxins Awareness Network (European Mycotoxins Awareness).

Other special forms are SPME (solid phase micro extraction), Mycosep™ columns and molecular imprinted polymers (MIPs). Mycosep™ columns keep back the impurities of a sample but allows the mycotoxins to pass (Egmond, 1986; Pittet, 2005; Turner et al., 2009). MIPs results in the application of imprinting techniques. At first monomers will be co-polymerized with a presented target analyt (imprint molecule). The removal of the imprint molecule reveals a molecular memory for the analyte (in shape, size). The special synthetic cavity can be used to separate and to extract analytes from a sample. This extraction method is adapted to the pre-analysis of some mycotoxins (ochratoxin A (Baggiani et al., 2002; Jodlbauer et al., 2002; Turner et al., 2004; Zhou et al., 2004), DON and ZON (Weiss et al., 2003)). It has promising advantages (stability, easy preparation, low cost) but has not been applied widely even on academic level.

Table 4: Strength and weakness of solid phase extraction (SPE).

|  |  |
| --- | --- |
| Strength | - easy handling, low expenditure at time, no scientific stuff is needed  - IAC: specific interactions between mycotoxins and antibodies, only denaturing solvent is needed, robust, large volumes |
| weakness | - consistent method for all mycotoxins not available  - MIPs: inconsistent molecular recognition, limited number of re-use, sensibility of the polymers  - IAC: costs, cross-reactivity, influence antibody activity, use only once |

## Detection and quantification of mycotoxins (Analysis Step)

### State-of-the-art methods

Reference methods for the quantification and qualitative determination of mycotoxins are basically chromatographic systems with different detection applications such as HPLC-UV/DAD or LC-MS. They produce high resolution, sensitive and reproducible results. The disadvantages of these methods are that they are time consuming, high in costs, sophisticated in equipment and with the need of expert scientific knowledge. In general, they also require very extensive pre-analytic clean-up steps prior to analysis. Instrumental methods are usually employed to confirm positive sampling results from screening methods. The basic principal and different applications have been widely published and summarized (Chiavaro et al., 2001; Chu, 1992; Di Stefano et al., 2012; Holcomb et al., 1992; Jansen et al., 1987; Kok, 1994; O'Mahony et al., 2013; Wilcox et al., 2015).

As the most sensitive and reliable method to analyse mycotoxins in different commodities High-Performance-Liquid-Chromatography (HPLC) is denoted as the state-of-the-art analysis. Various HPLC methods have been developed for almost all major mycotoxins in grains, cereals and other food and feed products. Different validated HPLC-methods for the different mycotoxins are reviewed (Gilbert and Anklam, 2002). The focus of this study will be on rapid test methods rather than laboratory based reference methods.

The Association of Analytical Communities (AOAC) released a formal method for aflatoxin determination. After preparation, the samples will be clean-up with immunoaffinity chromatography column (IAC column) followed by reversed phase - HPLC with post column bromiation (LOD: Aflatoxin B1 1ng/g, total Aflatoxin 2,4 ng/g) (Anklam et al., 2002; Stroka et al., 2000). Improvements in the detection tools of chromatographic techniques lead to the more popular determination of mycotoxins by HPLC-MS/MS, e.g. compared to HPLC-FLD or GC-MS no sample derivatization is required. Adapted to the EU and national legislation HPLC-MS/MS,is sensitive, indicates no cross-reactivity and gives the possibility of multiple analyses.

However, under less controlled conditions measurement procedures must be reliable to control the legislation but also easy and just-in-time for use in the field. So in the last years investigations in rapid and simple techniques never become less important. Several more sensitive, specific and simple methods for mycotoxin detection are commercial available and are so called screening methods. They include enzyme-linked immunosorbent assay (ELISA), lateral flow detection (LFD), fluorescence polarization immunoassay (FPI) or thin layer chromatography (TLC). The different methodological approaches have been widely reviewed (Anfossi et al., 2016; Contreras-Medina et al., 2013; Espinosa-Calderón et al., 2011; Hajslova et al., 2011; Manetta, 2011; Rai, 2012; Shephard, 2016; Yazdanpanah, 2011).

### Conventional methods for rapid detection of mycotoxins

## 

Conventional systems which are commercially available for rapid detection of mycotoxins are enzyme-linked immunosorbent assay (ELISA), lateral flow detection (LFD) and fluorescence polarization immunoassay (FPI). In some cases basic fluorometric measurements are used to detect and quantify mycotoxins in food or feed.

The strength and the weakness of these tests are shown in Table 5 giving a first overview.

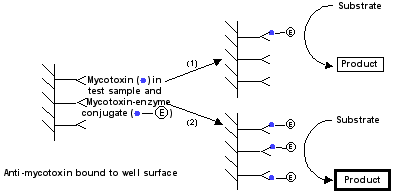
Table 5: Strength and weakness of enzyme-linked immunosorbent assay (ELISA), lateral flow detection (LFD) or fluorescence polarization immunoassay (FPI).

|  |  |  |  |
| --- | --- | --- | --- |
|  | ELISA | LFD | FPI |
| Strength | -easy handling  -low expenditure at time  -sensitive  -multiple analysis | -easy handling  -rapid  -portable  -no special equipment | easy handling  -low expenditure at time  -sensitive  - portable  -quantitative and qualitative |
| weakness | -cross-reactivity  -false-positive because of matrix disruptions  -costs | -not qualitative  -costs | - high costs  - currently only for certain mycotoxins available - in research |

#### Enzyme-linked immunosorbent assay (ELISA)

This assay enables the qualitative, semi-quantitative and quantitative rapid determination of mycotoxins in food and feed. The principle is bases on the use of antibodies and specific color changes. Different forms of ELISA kits are commercially available (e.g. single disposable membrane-based test, microtitre plate and tube assays).

The basic ELISAs are competitive assays. Here a conjugate of an enzyme-coupled mycotoxin or a primary antibody specific for the toxin analyte is used (Figure 5).



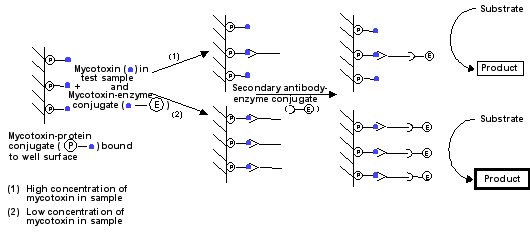


Figure 5: Principle of a competitive ELISA to screen mycotoxins (European Mycotoxins Awareness).

The experiment set-up uses a microtiter plate (Figure 6) which is coated with a mycotoxin-specific-immobilized antibody. In the first step a mycotoxin linked with an enzyme will be added to a sample. This mixture will be applied on the microtiter plate. The amount of the mycotoxin-linked enzyme that binds to the antibody on the plate depends on the amount of mycotoxins in the sample (e.g.the higher the amount of mycotoxins in a sample, the lower the amount of the mycotoxin enzyme conjugate). In the final step the substrate of the enzyme is added which leads to a chromogenic detectable signal. The concentration of this signal is inversely proportional to the concentration of the mycotoxin in the sample.

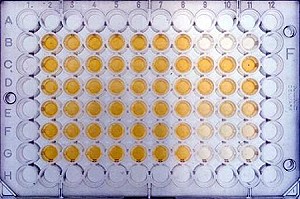


Figure 6: ELISA-microtiter plate after adding the substrate which leads to changes in colors ( here: to yellow) (European Mycotoxins Awareness).

#### Lateral flow detection (LFD)

The lateral flow detection is a form of an immunoassay on a strip to detect the presence or absence of the analyte in a sample. Often they are called “dipstick”-tests. At first a pre-conditioned strip will be wetted. Now the extracted sample will be applied and after running the strip will be detected visualy or with a special reader (Figure 7).

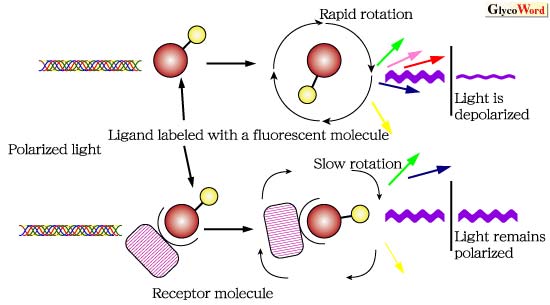


Figure 7: Model of the lateral flow detection-dipstick from Envirologix [**http://www.mycotoxins-rapid-tests.eu/**](http://www.mycotoxins-rapid-tests.eu/)

#### Fluorescence polarization immunoassay (FPI)

This is a newly developed immunoassay based on the indirect measuring the changes of molecule rotation in a solution (Figure 8). There are only two suppliers of FPI to determinate a limited number of mycotoxins.

Basically a fluorochrome labelled mycotoxin with a low molecular weight acts as the antigen. The aggregation with the anti-mycotoxin antibody results in the formation of an immune complex, gaining the weight and slowing the rotation rate of the molecule. That causes an increase in polarization of emitted light which can be detected by fluorescence polarization reading instruments (e.g. the portable Sentry200 from Ellie LLC/ Diachemix).



a)

b)

Figure 8: Principle of fluorescence polarization immunoassay (FPI). a) the rapid rotation of the fluorescence labelled ligand leads to a depolarization of light, b) binding the receptor molecule increases the weight of the fluorescence labelled ligand and slowing its rotation which generates a polarization of light (Glycoforum).

The deficiency of such assays is the problem of cross-reactivity which is not completely deleted and so further researches are needed to evaluate this influence. Cross-reactivity is a general problem of immune methods that clean-up or determine mycotoxins. Antigen-antibody reactions with metabolites or derivates of mycotoxins could not quite be eliminated in all immune methods. In addition to that further tests of different commercially available immunoaffinity columns for mycotoxins are needed. Scientists studied IACs to clean-up deoxynivalenol (DON) and zearalenone (ZON) in regard to the cross-reactivity of antibodies for conjugated mycotoxin forms e.g. glucosids, acetylated forms (Tangni et al., 2010; Versilovskis et al., 2011)

#### Thin Layer Chromatography (TLC)

A very first and well established method for separation and to quantify mycotoxins is the Thin Layer Chromatography (TLC). TLC provides a cheaper alternative to LC-based methods. Especially in developing countries it has an important role for surveillance proposes and control of regulatory limits (Gilbert and Anklam, 2002). Extensive investigations in the field of TLC lead to a high sensitive and well separating method with relatively little technical and methodological efforts (developing tank, coated plate, UV-detector). In summary, after preparation of the sample it is spotted along with standards onto a silica gel plate. It is then separated in a tank with mobile phase e.g. chloroform:acetyl (9:1, v:v) or diethyl ether:methanol:water (96:3:1, v:v:v). After developing the determination can be achieved with long-wave UV-light (Holcomb et al., 1992). Various applications of TLC are described (Turner et al., 2009). Both types - uni-dimensional and two-dimensional TLC ­- are used for quantitative and semi-quantitative determination of mycotoxins (Lin et al., 1998). Despite its ease and simplicity, the method needs a well-controlled laboratory environment and skilled laboratory personal.

Table 6: Detection limits for TLC-methods from [www.eurofinsus.com](http://www.eurofinsus.com).

|  |  |
| --- | --- |
| Compound | limit of detection [ppb] |
| aflatoxin B1, B2, G1, G2 | 2 |
| ochratoxin A | 200 |
| T-2 | 10 |
| Zearalenone | 100 |

Different conditions affect the result of the TLC analysis (Karunyavanij, 1991). For example there are different coatings and binders for the plates depending on the analyte. The plate itself could be glass, aluminum or plastic. Other factors are the pureness of the standards, the manner of spotting the plate and the developing of samples as a chromatogram. The determination of the results can be visual or with densitometry. Different spraying after developing the plate can enhance the visual effects. For example it has been shown that a complexation of sterigmatocystin with projected aluminum chloride on the plate increase the fluorescence intensity 100-fold (Stack and Rodricks, 1971) . Other author reviewed color reaction with iodine starch or Fast Corinth V to get more sensitive results in the analysis of e.g. ZON (Turner et al., 2009). Lin et al. summarized different detection techniques after TLC: UV-light of long and short wavelength, fluorescence quencher, autoradiography, vaporing of iodine or ammonium, exposing to X-ray (Lin et al., 1998).

Table 7: Strength and weakness of Thin Layer Chromatography (TLC).

|  |  |
| --- | --- |
|  | TLC |
| strength | - multiple analysis(Lin et al., 1998; Turner et al., 2009)  -cost efficiency(Espinosa-Calderón et al., 2011; Turner et al., 2009)  - LOD: EU and US 🗸  - rapid (Espinosa-Calderón et al., 2011; Lin et al., 1998)  - repeatable(Espinosa-Calderón et al., 2011)  - little or no clean-up(Pittet, 2005)  - no interfering of the mobile phase(Espinosa-Calderón et al., 2011) |
| weakness | - solutions, reagents for colouring or enhancing the fluorescence  - increase of the costs when IAC is used for purification of the samples |

#### Fluorescence methods

##### Non-specific fluorescence methods

Basic rapid and easy handling determination methods of mycotoxins commonly use the physicochemical natures of mycotoxins e.g. by fluorescence-stimulating. Presumptive tests like the “black light” test (so called Bright Greenish Yellow Fluorescent (BGYF) - test) shows rapid, indirectly, with less equipment and cost but non-specific mycotoxin producing fungi-infections in samples under UV-light (365 nm). Such sorting tests can be execute automatically with optical sorters e.g. SORTEX from Bühler GmbH (Germany) ([www.buhlergroup.com](http://www.buhlergroup.com) ). On the basis of color or other optical properties, contaminated kernels and foreign materials are identified and separated from the stream of seeds (Figure 9). The company Bühler GmbH provides different SORTEX applications addicted to the sample e.g. SORTEX Z+ for rice, grain and beans <https://www.buhlergroup.com/global/en/downloads/SR_Grain_Brochure_2014_EN.pdf>

There is also the Detox aflatoxin laser sorter from [Best Optical and Laser Sorting Equipment](http://www.ferret.com.au/c/Best-Optical-and-Laser-Sorting-Equipment-Heat-and-Control). It makes it possible to detect aflatoxins in various grains and combines various lasers for detection.

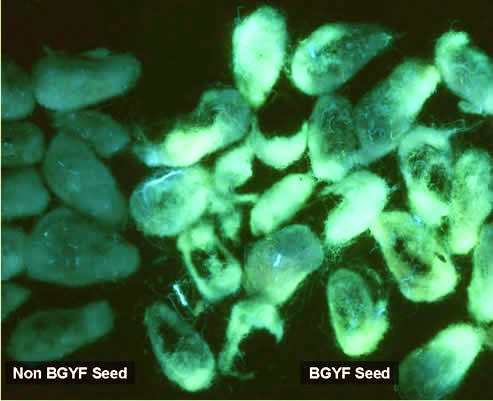


Figure 9: Picture of seeds in the “black light” test. A Bright-Green-Yellow Fluorescence (BGYF) will be reflected from *Aspergillus flavus*- infected seeds. The fluorescence is the result of the reaction of kojic acid (a fungal metabolite) and a host peroxidase. From <http://cals.arizona.edu/research/cottylab/research/epidemiology.html>

Pearson et al. investigated in a high-speed dual-wavelength Sorting to reduce the aflatoxins and fumonisins contamination in yellow corn. The study is based on a sorting with near-infrared (NIR) reflectance spectra (500-1.700 nm). The scientist ascertained a reduction of aflatoxins with an average of 82% (level of aflatoxins in corn > 10 ppb) respectively with an average of 38% (level of Aflatoxins in corn < 10 ppb) by high-speed sorting (filters at 750 nm and 1.200 nm) (Pearson et al., 2004). Further investigations in optical sorting with NIR shows the classification accuracies in the detection and removal of aflatoxins and fumonisins contaminated maize kernel post-harvest (Wicklow and Pearson, 2006). Another rapid and visual method was described by Atas and coworkers. They used hyperspectral imaging with UV and halogen excitation to differentiate between aflatoxin contaminated and non-contaminated chili peppers (Atas et al., 2012).

The application of these optical based techniques is still limited to screening purposes due to high matrix dependence and the lack of appropriate calibration materials.

##### Fluorescence and column separation

Previous reviews described the so called Romer minicolumn method in reference to the AOAC International (AOAC, 2000). Here a special packed column is used.

Table 8: Strength and weakness of Romer minicolumn method.

|  |  |
| --- | --- |
|  | Romer minicolumn method |
| strength | rapid, little equipment and amounts of solutions, easy handling, no special scientific knowledge |
| weakness | sample preparation, less sensitive, less selective, only semi-quantitative, high LOD (limit of detection) |

In the early stages scientists introduced a minicolumn technique to detect aflatoxin in peanuts related to Thin Layer Chromatography (TLC) (Holaday, 1981). Contrary to TLC this method was faster and easier to use. A glass column (Table 9: Holaday “Dip” column) was packed with glass fiber plug to hold the packing material, silica gel and another glass fiber plug and placed in a beaker containing a developing solvent with the sample filtrate. After developing the minicolumn was removed and determination be effected under UV-light (e.g. aflatoxins - blue or bluish - green color). The detection limit was 10 µg/ kg.

Further developments leads to the official technique the Romer minicolumn (Table 9) which is packed with Florisil®. Florisil® is a magnesium silicate (MgO – SiOH 15:85) with a particle size of 150 to 250 µm (Nagl). It is widely used in analysis of feed and food. Magnesium silicate is also used as filler material and parting agent by the industry (E 553a).

In principle, a small glass column will be packed with various layers but basically including the magnesium silicate. For example, for sterigmatocystin analysis the minicolumn will be stuffed with glass wool and stacked with anhydrous sodium sulphate, neutral alumina, Florisil® and again anhydrous sodium sulphate (Ramakrishna and Bhat, 1990). Now the column will be prepared by purging with different organic solvents (e.g. dichlormethan, hexane) under gravity. In the following steps a methanol-sample solution rinsing through the minicolumn. The mycotoxin adsorbs to the layer in the column and can be detected under UV-light. The determination will be carried out by comparing the column with a column of a standard. They are also called “go-no-go” methods because of their semi-quantitative or quantitative but less sensitive determination (Egmond, 1986).

Table 9: Investigations in minicolumn methods to analyse mycotoxins. (AOAC, 2000; Egmond, 1986; Holaday, 1981; Shotwell, 1983).

|  |  |  |
| --- | --- | --- |
| Holaday “Dip“ column (1968) | Velasco column (1972) | Romer minicolumn (official method 975.36 AACC-AOAC method) |
| LOD 10 µg/kg | LOD 5 µg/kg | LOD 5-15 µg/kg |
| 25 min | > 15 min | n.d. |
| 100 mm  glass fiber  glass fiber  silica  4 mm | 250 mm  alumina neutral  silica  5 mm  glass woll  Florisil®  Sand to pass No. 30 sieve  glass woll | alumina neutral  silica  glass woll  Florisil®  calcium sulfate  glass woll  calcium sulfate  150 mm |
| - extraction with chloroform/acetone (97:3 v/v) | - clean-up with ferric chloride solution (pH 4,6)  - extraction with acetone/water (85:15 v/v) | -extraction with acetone/water (85:15 v/v) and filtering  - purification with sodium hydroxide, ferric chloride and chloroform  -sample in chloroform/acetone (9:1 v/v) drain by gravity through MC |
| - blue band 10 mm from the lower and of the MC under UV | - blue band in the interface of Florisil®-silica under UV | - blue band at the top of Florisil® under UV |

##### Fluorescence in solution

Another semiquantitative fluorometric method to analyse mycotoxins is solution fluorometry. After extraction and clean- up with IAC or SPE the elute will be filled in a cuvette, derivatized with e.g. bromine and then measured with a fluorometer (Jansen et al., 1987). In the investigations from (Chiavaro et al., (2002) a sodium bicarbonate solution-methanol mixture was used for the extraction followed by IAC. Determination was performed using a xenon-lamp fluorometer from VICAM. The results were according to a reference RP-HPLC-method.

Table 10: Strength and weakness of solution fluorometry.

|  |  |
| --- | --- |
|  | Fluorometry |
| Strength | rapid, easy handling, inexpensive, sensitive, multiple analysis |
| Weakness | derivatization, fresh derivatization solution every day, equipment, calibration of the system |

Malone et al. describes a similar method for the quantification of aflatoxins in grains and raw peanuts in comparison to LC-analyse (Malone et al., 2000). The results were in a good agreement. In this method the fluorescence of the mycotoxins was enhanced by bromine-derivatization.

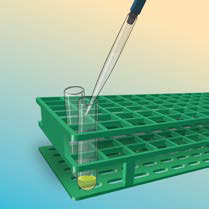


Figure 10: Transferring of the extracted sample in a tube and analyzing of it with the fluorometric test FluoroQuant® from Romer Labs Division Holding GmbH, 2013.

#### Labelling and derivatization

Combining enhancement of fluorescence and better separation of mycotoxins with add-on substances is widely used in toxin analysis to receive more sensitive results. Methodological approaches using different labelling or derivatization strategies are summarized in Table 10.

Table 10: Labeling or derivatization-methods to improve the results of mycotoxin analysis.

|  |  |  |  |
| --- | --- | --- | --- |
| **labeling/ derivatization** | **Contact point** | **Compound** | **Reference** |
| 1,2-diamino-4,5-dichlorbenze (DDB) |  | Monoliformin | (Filek and Lindner, 1996) |
| Iodine | post-column | Aflatoxins | (Jansen et al., 1987; Lemke et al., 1988; Shotwell, 1983) |
| Fluorescein |  | T-2/ HT-2 | (Lippolis et al., 2011; Maragos and Thompson, 1999; Thompson and Maragos, 1996) |
| Bromine | post-column | Aflatoxin | (Espinosa-Calderón et al., 2011; Stroka et al., 2000; Yuan, 2011) |
| Cyclodextrin |  | e.g. Aflatoxins, DON, ZON | (Espinosa-Calderón et al., 2011; Francis et al., 1988; Galaverna et al., 2008) |
| aluminium chloride  spraying and heating | on TLC-plate | DON, Sterigmatocystin | (Egmond, 1986) |
| trifluoroacetic acid |  | Aflatoxins | (Egmond, 1986; Espinosa-Calderón et al., 2011) |
| *o*-phthalaldehyde |  | Fumonisin | (Shephard et al., 1990) |

##### Derivatization with fluorescine, trifluoroacetic-acid, iodine, bromine

The tracer fluorescein can be used in a fluorescence polarization immunoassay for the determination of T-2 and HT-2-toxins in wheat. The labelling not only increases the sensitivity of the test system but also shortens clean-up procedures and incubation-time (Lippolis et al., 2011). Other described techniques are the pre- and post-column derivatization with trifluoroacetic-acid, iodine or bromine (Espinosa-Calderón et al., 2011). Especially in the case of bromine derivatization electrochemical cells can be used. Here the strong oxidizer bromine will be induced by the so called KOBRA® cell. Jansen et al. show a 20-fold increase of the fluorescence intensity of aflatoxin B1 and G1 with post-column iodine derivatization (Jansen et al., 1987).

##### Derivatization with cyclodextrin

A new and promising substance that combines the selective separation with the enhancement of native fluorescence of mycotoxins is cyclodextrin (CD). The cyclic oligosaccharides are formed by 6(-CD), 7 (-CD), 8 (-CD) glucose units linked by -1,4-glycosidic bonds (Figure 11). They are cone-shaped. The pore diameter of -CD, -CD and -CD differs from 4,7 to 7,5 Å. Besides their good solubility in water and dipolar solvents they are able to form inclusion-complexes as host for a wide range of hydrophobic compounds (guest). The complexation affected the guests solubility, stability, physical and chemical properties. The inexpensive substance is widely used in pharmaceutic products (solubility, stability) and in the textile industry (masking tastes and smells) (Galaverna et al., 2008).

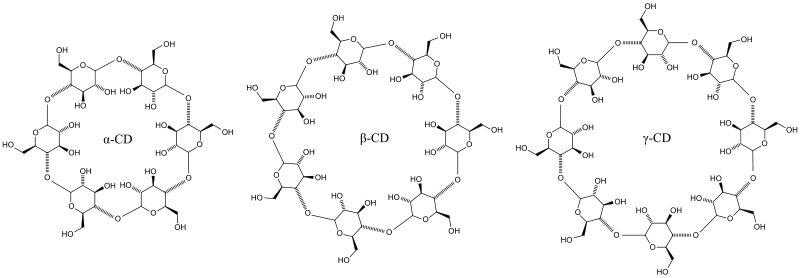
[](http://upload.wikimedia.org/wikipedia/commons/5/51/Cyclodextrin.svg)

Figure 11: Chemical structure of different cyclodextrins with a pore diameter of -CD = 4,7 Å, -CD = 6,8 Å and -CD = 7,5 Å.

The forming of an inclusion complex between different mycotoxins and cyclodextrins leads to an enhancement of the native fluorescence of mycotoxins. This is described below as a result in the interaction of the coumarin structured mycotoxins and cyclodextrin. Otherwise the inclusion results in changes of the polarity and intermolecular rotation, but also in interaction with quenchers (Galaverna et al., 2008). Cucci et al. described a method to analyse aflatoxin M1 in milk with the use of -CD. After cyclodextrin was added the detection limit of analysis were decreased from 25 ng/l to 5 ng/l. Also there was no need to clean-up the samples with IAC before analysis (Cucci et al., 2007).

Maragos et al. investigate a fluorometric method to detect the non-fluorescence T-2 in maize. They derivatized T-2 with pyrene-1-carbonyl cyanide (T-2-Pyr) and studied the enhancement of the fluorescence by adding different CD´s as buffer modifier in capillary electrophoresis laser-induced-fluorescence. The most effective CD was heptakis (2,6-di-*O*-methyl)--cyclodextrin (DIMEB) (Figure 12) (Maragos et al., 2008).

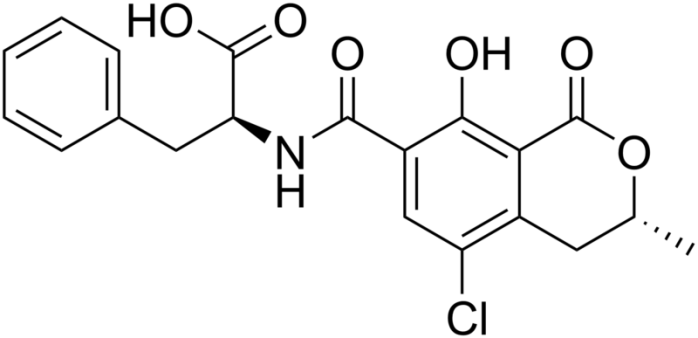


Figure 3: Structure of Ochratoxin A from <http://commons.wikimedia.org/wiki/File:Ochratoxin_A_structure.png>.



Figure 4: Molecular representation of possible interactions between T2-Pyr and DIMEB: (a) 1:1 ratio-, (b) 1:2 ratio. Modelling with HyperChem (Version 7.25) from Maragos et al. (Maragos et al., 2008).

#### Specific fluorescence methods

Referring to the fluorescence methods to analyse mycotoxins the fluorescence polarization immunoassay (FPI) is mentioned above as a rapid screening test. This method based on the indirect measuring of the changes in molecule rotation of a solution as a function in size of molecules. It is one of the methods which are actually in research and shows great promises in the field of rapid, sensitive analysis of mycotoxins. Commercial available test kits are from Diachemix and Aokin AG but only for a limited number of mycotoxins and high in price. The problem of cross-reactivity is not completely deleted.

In principle - the fluorochrome labelled mycotoxin with a low molecular weight acts as the antigen. Aggregation with the anti-mycotoxin antibody results in the formation of an immune complex, gaining the weight and slowing the rotation rate of the molecule. This causes an increase in polarization of emitted light which can be detected by fluorescence polarization reading instruments (e.g. the portable Sentry200 from Ellie LLC/ diachemix).

Table 11: Strength and weakness of fluorescence polarization immunoassay (FPI).

|  |  |
| --- | --- |
|  | FPI |
| strength | - easy handling  - portable |
| weakness | - in research  - expensive equipment |

### Other methods in research use

#### Laser-Induced Fluorescence (LIF)

Espinosa-Calderón et al. reviewed researches concerning the Laser-Induced Fluorescence (LIF) as a fluorescence screening method for mycotoxins (Espinosa-Calderón et al., 2011). A method based on the detection of a mobile phase which includes the sample solution and passes through the detection window of the LIF-detector. Fluorescence which is excited by the laser will be detected. The method enables to analyse samples with very low concentrations. Because of the high costs for the LIF (laser, special dyes for labelling) this method is rarely used.

#### Near infrared spectroscopy (NIR)

Another method in practice and researches is the near infrared spectroscopy (NIR). Petterson et al. described the determination of deoxynivalenol in wheat kernel with a wavelength 570 – 1.100 nm. The detection limit was 400 ppt (Pettersson and Åberg, 2003). While NIR can be used for the determination of aflatoxins at levels between 200-500 ppb in sample with solid or liquid physical conditions this method is not yet established for the detection of aflatoxin in human food at regulatory levels (Jagger et al., 2013).

#### Biosensor techniques

In the last decade different immunochemical assays and assays including biosensor techniques are investigated. Biosensors enables the detection of an analyte in a sample because of the interaction between the analyte and a biological sensitive element e.g. enzyme, tissues, nucleic acids or antibodies. The interaction results in a signal which can be detected by a transducer (e.g. optical or physicochemical detection) and will be transformed in an utilizable measured variable.

A biosensor method to determine mycotoxins is surface plasmon resonance (SPR) (Schnerr et al., 2002; Tudos et al., 2003; van der Gaag et al., 2003). Here the measured variable is the change in mass of mycotoxins which are immobilised at a surface of a sensor chip. The mass change results in the attaching of a specific antibody to the mycotoxins. The advantages get from studies are:

- results compares to LC-MS data

- re-using of the sensor chip without loss in activity (500 times).

Such SPR biosensor protocol described Puiu et al. for the direct measurement of albumin-bound AFB1 in blood samples (Puiu et al., 2012).

#### DNA-based and aptamer-based biosensors

Dinckaya et al. published a DNA biosensor-based method to analyze aflatoxin M1 in samples e.g. in milk. A thiol-modified single stranded DNA (ss-HSDNA) probe was immobilised on a monolayer of cysteamine and gold nanoparticles prepared on gold electrodes. The DNA biosensor particular bound Aflatoxin M1. The detection of the process is carried out with electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) techniques. But there is no information if the method is assignable within the aflatoxin M1 limits of the national and international legislations (Dinckaya et al., 2011).

Another form to use DNA in biosensors are aptamer-based. Aptamers are peptide molecules or DNA, respectively, RNA duplex structures that can bind a special analyte. Chen et al. investigated a DNA duplex structure with an anti-ochratoxin A-aptamer including a fluorophore and a quencher. Binding ochratoxin A to this structure leads to an increase of the fluorescence. With this rapid and high selectively method (only 1 min as per measurment) OTA can be determined with a LOD of 0,8 µg/l (Chen et al., 2012). Similar to that a biosensor DNA-enzyme aptamer was described by Yang et al. The presence of OTA bound to the DNAzyme hairpin leads to open the hairpin structure and activates a horseradish peroxidase-mimicking DNAzyme. This process can be detected with colorimetric measurement at 620 nm in microtiter wells (Yang et al., 2012). Other aptamer-senor-based assays are currently under investigation (Prabhakar et al., 2011; Wu et al., 2011).

#### Electronic nose

A new field on the basis of biosensors are electronic noses developed for the rapid non-destructive analysis of mycotoxins (Cheli et al., 2007; Dell'Orto et al., 2007; Olsson et al., 2002; Tognon et al., 2005). The electronic nose - an array of biosensors detects volatiles emanating- could dinstinguish between the presence and the abscence of the mycotoxins. The fungi growth and their producing of mycotoxins leads to biochemical changes rather to changes in the chemical composition of volatiles. Different volatile molecules act within the electronic nose and generate a special detectable electronic signal. Changes in the relative composition of the molecules lead to changes in the electronic signal. The investigations of Cheli et al. shows that electronic noses can differ between aflatoxins -positive and –negative samples but further quantitative analysis are needed. The model electronic nose needs continuing improvements and research to be a sensitive and reliable method in the mycotoxins analysis due basis of international legislations (Cheli et al., 2009).

## 

use



# Commercially Available Rapid Analysis Test Systems

The following section summarizes some currently used screening test kits for the rapid quantitative analytic of mycotoxins. It is not entitled to be comprehensive. All prices are based on 2012 list prices and are entitled to serve as reference. A complete list of currently approved analytical methods are given in GIPSA (Performance Verified Test Kits – Effective 12/16/2016) <https://www.gipsa.usda.gov/fgis/metheqp/GIPSA_Approved_Mycotoxin_Rapid_Test_Kits.pdf>

## Aokin AG

Table 12: Summarized facts about the rapid analysis test systems from Aokin AG.

|  |  |
| --- | --- |
|  | <http://www.aokin.de/> |
| Principle | FPI |
| equipment | EUR 25.000 (FP-spectrometer, liquid handling workstation) |
| cost per analysis/ consumables | EUR 9 – 15 |
| Portability | No |
| Laboratory | Preperation: yes - Analytics: yes |

Table 13: Overview test systems for various mycotoxins from aokin AG.

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | Matrix | LOD [µg/kg] | detection range [µg/kg] |
| DON | wheat, corn, oats, barley, rye, durum,  cereals, flakes, pasta | 5  (for wheat: 3) | 50-5,000 |
| ZON | wheat, corn, oats, barley, rye, durum,  cereals, flakes, pasta | 1  (for wheat: 10) | 50-5,000 |

- analysis:

- sample preparation: IAC, SPE (QuickClean)

- as order the analysis of DON, ZON, Aflatoxins, Ochratoxin A, Fumonisin, T-2/ HT-2

## Beacon Analytical Systems Inc.

Table 1411: Summarized facts about the rapid analysis test systems from Beacon Analytical Systems Inc.

|  |  |
| --- | --- |
|  | <http://www.beaconkits.com/welcome/category/mycotoxins> |
| Principle | ELISA (plate or tube) |
| equipment | reader (costs not denoted) |
| cost per analysis/ consumables | EUR 18 |
| Portability | No |
| Laboratory | Preperation: yes - Analytics: yes |

Table 15: Overview test systems for various mycotoxins from Beacon Analytical Systems Inc..

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | Matrix | detection range | method |
| Aflatoxin M1 | Milk | 0 – 100 ppt | ELISA |
| Aflatoxin | nuts, grain, grain products | 0 – 100 ppb | ELISA, plate |
| Aflatoxin | corn, peanuts | 0 – 100 ppb | ELISA, tube |
| DON | wheat, barley, malted barley, corn, oats | 0 – 6 ppm | ELISA |
| Fumonisin | corn, corn meal, corn germ meal, corn gluten meal, corn/ soy blend | 0 – 6 ppm | ELISA |
| Ochratoxin A | corn, corn meal, grain | 0 – 25 ppb | ELISA |
| T2 | corn, corn meal, corn germ meal, corn gluten meal, corn/ soy blend | 0 – 500 ppb | ELISA |
| ZON | corn, corn meal, corn germ meal, corn gluten meal, corn/ soy blend | 0 – 1.000 ppb | ELISA |

## Charm Sciences Inc.

Table 16: Summarized facts about the rapid analysis test systems from Charm Sciences Inc..

|  |  |
| --- | --- |
|  | <http://www.charm.com> |
| Principle | LFD |
| equipment | EUR 2.995 (ROSA® EZ-M system) |
| cost per analysis/ consumables | EUR 7-10 |
| Portability | No |
| Laboratory | Preparation: yes - Analytics: yes |

***ROSA® Grain***  
-a variety of ROSA (Rapid One Step Assay) tests to detect mycotoxins (aflatoxin, DON/vomitoxin, fumonisin, ochratoxin, T-2/HT2, and zearalenone) in feed and grain

-lateral flow,quantitative results, all mycotoxins same equipment

*Water Extraction Technology (WET®)*  
-eliminates the use of organic solvents, a non-hazardous powder (composition is not disclosed because of IP – patent has not been obtained) is added to the sample followed by water (e.g., bottled water), materials may be disposed as normal waste providing positive mycotoxin samples do not violate local regulations

-extraction method can be used for multiple commodities, a single extraction for the following mycotoxins: aflatoxin, DON, fumonisin, T2/H2, and zearalenone (ochratoxin requires a separate extraction powder)

**e.g.:*ROSA WET****®****Aflatoxin Quantitative Test:* results in less than 5 min,** disposed as normal waste providing positive mycotoxin samples do not violate local regulations, multiple samples can be run at the same time, uses existing equipment; ROSA ® EZ-M system (Figure 13) does not require end-user instrument calibration, standard curve built in reader



Figure 5: ROSA® EZ-M system from Charm Science which combines to incubate and to analyze lateral flow test strips.

Table 17: Overview test systems for various mycotoxins from Charm Sciences Inc..

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Analyte | Matrix | Method | extraction buffer | detection range | [EUR]/100 pcs. |
| **Aflatoxins** |  | 3, 5, 10 min |  |  |  |
| ROSA WET**®**Aflatoxin Quantitative Test  (USDA(GIPSA)\*-approved) | barley, corn, corn flour, corn germ meal, corn gluten meal, corn meal, corn/soy blend, Distiller’s Dried Grain with Solubles (DDGS), hominy, oats, popcorn, rice bran (defatted), rough rice, sorghum, soybeans, wheat | LFD | Water | n.d. | 765,00 |
| FAST Aflatoxin Quantitative | Corn | LFD | 70% methanol | 0 – 150 ppb |  |
| BEST Aflatoxin Qualitative | Corn | LFD | non-toxic solution called BEST (composition is a trade security) | 10 ppb, 20 ppb |  |
| Aflatoxin P/N Test Qualitative | Corn | LFD | 50% Methanol or 70% Ethanol | 10 ppb, 20 ppb |  |
| **DON** |  | 3, 5, 10 min |  |  |  |
| ROSA DON P/N Test  Quantitative | barley, corn, wheat | LFD | deionized  or distilled water | 0,5ppm, 1 ppm, 2 ppm, 5 ppm |  |
| ROSA DON Quantitative Test | barley, brewer’s rice, buckwheat, corn, corn bran, corn germ meal, corn gluten meal, DDGS, hominy, malted barley, milled rice, oats, palm kernel meal, rapeseed meal, rice bran, rough rice, rye, sorghum, soybean meal, triticale, wheat, wheat bran, wheat flour, wheat midds, wheat red dog | LFD | deionized  or distilled water | 0 – 6 ppm, 6 – 12 ppm, 12 – 24 ppm |  |
| ROSA FAST5 DON  Quantitative | barley, corn, DDGS, malted barley, milled rice, oats, rough rice, sorghum, wheat, wheat bran, wheat flour, wheat midds | LFD | deionized  or distilled water | 0 - 1,5 ppm,  1 – 6 ppm | 830,00 |
| **Fumonisin** |  | 5, 10 min |  |  |  |
| ROSA FAST5 Fumonisin  Quantitative | barley, corn, flaking corn grits, millet, oats, rough rice, sorghum, wheat | LFD | 70% Methanol | n.d. |  |
| ROSA Fumonisin Quantitative | barley, corn, flaking corn grits, DDGS, millet, oats, rough rice, sorghum and wheat | LFD | 70% Methanol | 0 to 1 ppm, 0 to 6 ppm, and 6 to 60 ppm |  |
| ROSA Fumonisin Quantitative | barley, corn, DDGS, hominy, oats, sorghum, soybean meal | LFD | 50% Ethanol | 0 to 1 ppm, 0 to 6 ppm, and 6 to 60 ppm |  |
| **Ochratoxin A** |  | 10 min |  |  |  |
| ROSA Ochratoxin Quantitative Test | barley, corn, corn gluten meal, malted barley, oats, rye, sorghum,  soybean meal, wheat, buckwheat, rice | LFD | 70% Methanol | 0 to 30 ppb, 0 to 150 ppb | 950,00 |

\*USDA(GIPSA)=United States Department of Agriculture (Grain Inspection, Packers and Stockyard Administration)

## Diachemix Inc.

Table 18: Summarized facts about the rapid analysis test systems from Diachemix Inc..

|  |  |
| --- | --- |
|  | <http://www.diachemix.com/en/> |
| Principle | FPI |
| equipment | EUR 26.000 |
| cost per analysis/ consumables | EUR 4-5 |
| Portability | Yes |
| Laboratory | Preparation: yes - Analytics: no |

Table 19: Overview test systems for various mycotoxins from Diachemix Inc..

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | method | detection range | matrix |
| **Aflatoxin** |  |  |  |
| **Aflatoxin FPA qualitative test** | FPA  2 min | ≥ 20 ppb or ≥ 10 ppb | corn |
| **Aflatoxin FPA quantitative test\*** | FPA  2 min | 0 – 100 ppb | grain, nuts |
| **DON Vomitoxin FPA qualitative test** | FPA  2 min | ≥ 1 ppm | wheat |
| **DON Vomitoxin FPA quantitative test\*** | FPA  15 min | 0 – 6 ppm  (LOD 0,17 ppm) | wheat |
| **Fumonisin FPA qualitative test\*\*** | FPA  1-2 min | ≥ 1 ppm | corn |

\*<10 kits EUR 503,2; >10 kits EUR 407,00 ;\*\*presently not available for sale



Figure 6: Portable fluorescence polarization reader Sentry® 100 for determination of various mycotoxins with assays from Diachemix Inc..

## EnviroLogix Inc.

Table 20: Summarized facts about the rapid analysis test systems from EnviroLogix Inc..

|  |  |
| --- | --- |
|  | <http://envirologix.com/artman/publish/index.shtml>  for EU: <http://www.mycotoxins-rapid-tests.eu/> |
| principle | LFD |
| equipment | Not disclosed |
| cost per analysis/ consumables | Not disclosed |
| Portability | No |
| Laboratory | Preparation: yes - Analytics: yes |

* Sample preparation:
  + 1. Extraction: matrix preparation (e.g. grinding), analyte extraction with water or 50% EtOH
    2. Strips wetting
    3. Running
    4. Detection
    5. Analysis ( qualitative or quantitative)
* which mycotoxins: aflatoxins, fumonisins, ochratoxin A, deoxynivalenol (DON), zearalenon (ZON) > simultaneous detection of mycotoxins
* other possible analytes: genetically modified organism (*GMO; e.g. Soybean, corn*), plant disease, domestic molds
* results in less then 10 minutes, non-specialized operators
* equipment: test kit, QuickScan with software, standard PC-platform with a MS-Windows interface

Table 21: Overview test systems for various mycotoxins from EnviroLogix Inc..

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | Matrix | detection range (ppb) | extraction buffer |
| **Aflatoxin** |  |  |  |
| *Aflatoxins B and G quantitative* | corn, wheat | 2,5 – 180 | 50% ethanol |
| *aflatoxin residues quantitative* | corn, wheat | 3 – 180 | Water |
| *aflatoxin residues quantitative* | dried distilled grains with soluble (DDGS) | 10 – 450 | 50% ethanol |
| *B1 and B2*  *qualitative* | Corn | 20 | 50% ethanol |
| **DON** |  |  |  |
| *quantitative* | corn, wheat, oats | 200 - 10.000 | Water |
|  | Barley | 200 - 10.000 | Water |
|  | DDGS | 200 - 10.000 | Water |
|  | corn, corn gluten meal, DDGS; wheat, wheat bran, wheat midds, whole-wheat flour, white wheat flour; barley, malted barley; milled rice, rough rice; oats | 300 - 12.000 | Water |
| *qualitative* | Corn | 500 – 2.000 | Water |
| **ZON** |  |  |  |
| *quantitative* | Corn | 50 – 520 | 50% ethanol |
| **Fumonisin** |  |  |  |
| *quantitative* | corn and corn by-products in food and animal feeds | n.d. | 50% ethanol |
| **Ochratoxin A** |  |  |  |
| *quantitative* | Wheat | 0 – 150 ppb | water based |

## EuroProxima B.V.

Table 22: Summarized facts about the rapid analysis test systems from Europroxima B.V..

|  |  |
| --- | --- |
|  | <http://europroxima.com/> |
| Principle | LFD, ELISA |
| equipment | reader ( not denoted) |
| cost per analysis/ consumables | LFD: EUR 20, ELISA: EUR 500/plate |
| Portability | no |
| Laboratory | Preparation: yes - Analytics: yes |

-LFD: Zearalenone (ZON) and Deoxynivalenol (DON) a colloidal gold based flow-through immunoassay is used, 10 pieces EUR 220-275 + charge ( approx. EUR 20)

*-Further informations about costs for preparation and clean-up: in the kit*

Table 23: Overview Lateral Flow test systems for various mycotoxins from EuroProxima B.V..

|  |  |  |
| --- | --- | --- |
| Analyte | Matrix | detection range (ppb) |
| **Aflatoxins** |  |  |
| *B1* | cereals, soy beans, nuts, derived products | 2 |
| *Total* | cereals, soy beans, nuts, derived products | 4 |
| **DON** | n.d. | n.d. |
| **Ochratoxin A** | cereals, wine, green coffee | 4 |
| **Ochratoxin A in wine** | red, rose, white wine | 2 |
| **ZON** | n.d. | n.d. |

-ELISA (>60 min), 96 well plate EUR 498,50 + charge

Table 24: Overview ELISA test systems for various mycotoxins from Europroxima B.V.

|  |  |  |
| --- | --- | --- |
| Analyte | Matrix | detection range (ppb) |
| **Aflatoxins** |  |  |
| *B1* | n.d. | depends on the kind of sample: 0,3 – 2 |
| *M1* | milk, milk powder, cheese, butter | < 0,006 |
| *Total* | n.d. | < 0,5 |
| **DON** | cereals, beer, silage | depends on the kind of sample: 30 or 50 |
| **Fumonisin** | Maize | 2,3 |
| **Ochratoxin A** | cereals, food and feed | barley, soy: 1  oats: 0,5 |
| **ZON** | Cereals | 0,13 |
| **T 2** | Cereals | 20 - 50 |

## Neogen Corporation

Table 25: Summarized facts about the rapid analysis test systems from Neogen Corporation.

|  |  |
| --- | --- |
|  | <http://www.neogen.com/index.html> |
| Principle | LFD, ELISA |
| equipment | reader:  LFD: EUR 1.700, ELISA: EUR 4.500 |
| cost per analysis/ consumables | LFD: EUR 6, ELISA: EUR 6-7 |
| Portability | no |
| Laboratory | Preparation: yes - Analytics: yes |

(detection: visual or reader, quantitative or qualitative)

*-Multipurpose AccuScan Pro*

* reads and records all Reveal Q+ products
* complete archival recording
* by recording the sample identification, results, time and date, the AccuScan Pro eliminates manual recording and assists with report generation
* export to AccuScan Pro Data Manager software
* provides additional reporting, tracking and trend capabilities

Table 26: Overview test systems for various mycotoxins from Neogen Corporation.

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | Method | detection range | matrix |
| **Aflatoxin** |  |  |  |
| Veratox®  Aflatoxin total  *quantitative* | ELISA  5 min | 5 - 50 ppb | corn, cornmeal, corn gluten meal, corn/soy blend, wheat, rice, milo, soy, whole cottonseed, cottonseed meal, raw peanuts, peanut butter and mixed feeds |
| Veratox® for Aflatoxin HS (High Sensitive)  *Quantitative* | ELISA  20 min | 1 – 8 ppb | corn, cornmeal, corn gluten meal, corn/soy blend, wheat, rice, milo, soy, whole cottonseed, cottonseed meal, raw peanuts, peanut butter and mixed feeds |
| AgriScreen  for Aflatoxin  *qualitative* | ELISA  5 min | 20 ppb | corn, cornmeal, corn gluten meal, corn/soy blend, wheat, rice, milo, soy, whole cottonseed, cottonseed meal, raw peanuts, peanut butter and mixed feeds |
| Reveal®Aflatoxin M1 | LFD  5 min | 500 ppt | milk |
| Reveal® Q+ Aflatoxin total  *quantitative* | LFD  6 min | 2 - 150 ppb | corn, corn products |
| Reveal® Aflatoxin total  *qualitative* | LFD  3 min | 20 ppb | corn, corn gluten meal, corn meal, corn/soy blend, cottonseed, cottonseed meal, hominy, milo, peanuts, popcorn, rice, soy meal and wheat  green test kit: corn |
| **DON** |  |  |  |
| Veratox ®DON 2/3  *quantitative* | ELISA  5 min | 0,5 – 5 ppm | wheat, wheat flour, wheat midds, wheat bran, corn, cornmeal, corn screenings, barley, malted barley and oats |
| Veratox® DON 5/5  *quantitative* | ELISA  10 min | 0,5 – 5 ppm | wheat, wheat flour, wheat midds, wheat bran, corn, cornmeal, corn screenings, barley, malted barley and oats |
| Veratox® DON HS  *quantitative* | ELISA  20 min | 25 – 250 ppb | wheat, wheat flour, wheat midds, wheat bran, corn, cornmeal, corn screenings, barley, malted barley and oats, processed cereal |
| Reveal® Q+ DON  *quantitative* | LFD  3 min | 0,3 – 6 ppm | corn, barley, DDGS( distillers  dried grains with soluble), malted barley, oats and wheat products |
| **Fumonisin** |  |  |  |
| Veratox® Fumonisin  *quantitative* | ELISA  20 min | 1 – 6 ppm | corn, barley, DDGS, milo, popcorn, rice, soybeans and wheat |
| Veratox® Fumonisin 5/10 *quantitative* | ELISA  15 min | 0,5 – 6 ppm | corn, barley, DDGS, milo, popcorn, rice, soybeans and wheat |
| Veratox® Fumonisin HS  *quantitative* | ELISA  15 min | 50 - 600 ppb | corn, barley, DDGS, milo, popcorn, rice, soybeans and wheat |
| Reveal® Q+ Fumonisin  *quantitative* | LFD  6 min | 0,3 – 6 ppm | corn products |
| **Ochratoxin** |  |  |  |
| Veratox® Ochratoxin *quantitative* | ELISA  20 min | 2 – 25 ppb | corn, barley, wheat, green coffee, various dried fruit |
| Veratox® OchratoxinGrain  *quantitative* | ELISA  20 min | 2 – 25 ppb | corn, barley, other grains |
| Reveal® Q+ Ochratoxin *quantitative* | LFD  9 min | 2 – 20 ppb | grain and grain products |
| **T-2/ HT-2** |  |  |  |
| Veratox® T-2/ HT-2  *quantitative* | ELISA  10 min | 25 – 250 ppb | corn, barley, wheat, oats, rye |
| Reveal® Q+ T-2/ HT-2  *quantitative* | LFD  6 min | 50 – 600 ppb | grain and grain products |
| **ZON** |  |  |  |
| Veratox ®  ZON *quantitative* | ELISA  10 min | 25 – 500 ppb | corn, wheat, barley, DDGS |
| Reveal® Q+ ZON  *quantitative* | LFD  6 min | 50 – 1.200 ppb | corn and wheat products |

## R-Biopharm AG

Table 27: Summarized facts about the rapid analysis test systems from R-Biopharm AG.

|  |  |
| --- | --- |
|  | <http://www.r-biopharm.com/products/food-feed-analysis/mycotoxins> |
| Principle | LFD, ELISA |
| equipment | reader: EUR 1.200 |
| cost per analysis/ consumables | LFD: EUR 10-20, ELISA: EUR 4-6 |
| Portability | No |
| Laboratory | Preparation: yes - Analytics: yes |

-RIDASCREEN®: ELISA format enables high-throughput testing of many commodities for most regulated mycotoxins

-RIDA®QUICK: quantitative lateral flow format enables rapid, on-site decisions about the mycotoxin contamination of commodities

**-**AFLACARD**®** /OCHRACARD**®:** specially designed to get a rapid, semi-quantitative result for more complex commodities

-Others:

- Immunoaffinity columns (EUR 10-20/ sample) enable efficient mycotoxin clean-up for all kinds of commodities prior to HPLC, LC- MSMS or ELISA

- PuriToxSR solid phase extraction columns (EUR 1-10/ sample) enable efficient mycotoxin clean-up of many commodities prior to TLC, HPLC, GC or LC-MSMS

- detection with StatFax Reader Model 303 Plus or RIDA®Quick SCAN Model ZG5005

- standard solution: EUR 45 per ml

Table 28: Overview test systems for various mycotoxins from R-Biopharm AG.

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | method | detection range | matrix |
| **aflatoxin** |  |  |  |
| **AFLACARD**®B1  *qualitative* | LFD/card  10 min | n.d. | a wide range of commidities |
| **AFLACARD**®total  *qualitative* | LFD/card  10 min | n.d. | a wide range of commidities |
| RIDA®Quick Aflatoxin *quantitative* | LFD | 4 – 20 ppb | grain, soy flour, nuts, pistachios, coconut flour, sunflower seeds, figs, dates and cashew nuts |
| Aflatoxin RQS *quantitative* | LFD | 4 ppb | corn |
| RIDASCREEN®Aflatoxin M1  *quantitative* | ELISA  1 h 15 min | 5 ppt, 50 ppt | milk, milk powder, cheese |
| RIDASCREEN®Aflatoxin B1 30/15  *quantitative* | ELISA  45 min | 1 ppb | cereals, feed |
| RIDASCREEN®Aflatoxin total  *quantitative* | ELISA  45 min | 1,75 ppb | residues in cereals and feed |
| RIDASCREEN®FAST Aflatoxin *quantitative* | ELISA  15 min | 1,75 ppb | cereals, feed |
| RIDASCREEN®FAST Aflatoxin M1 *quantitative* | ELISA  15 min | 125 ppt | milk, milk powder |
| RIDASCREEN®FAST Aflatoxin SC *quantitative* | ELISA  15 min | 2 ppb | cereals, feed |
| **DON** |  |  |  |
| RIDASCREEN®FAST DON  *quantitative* | ELISA  8 min | 0,2 ppm | wheat, corn, barley,  malted barley, and oats |
| RIDASCREEN®FAST DON SC  *quantitative* | ELISA  8 min | 0,074 ppm | cereals, malt and feed |
| RIDASCREEN® DON  *quantitative* | ELISA  45 min | cereals, malt, feed: 18.5 ppb  beer: 3.7 ppb  wort: 3.7 ppb | cereals, malt, feed, beer and wort |
| RIDA®Quick DON  *semi-quantitative orquantitative* | LFD  5 min | 0,5 ppm, 1,25 ppm | wheat, triticale and corn |
| **Fumonisin** |  |  |  |
| RIDASCREEN® Fumonisin  *quantitative* | ELISA  45 min | 25 ppb | corn, corn products |
| RIDASCREEN®FAST Fumonisin  *quantitative* | ELISA  15 min | 0,222 ppm | corn |
| RIDA®Quick Fumonisin *semi-quantitative* | LFD  5 min | 0,8 ppm, 4 ppm | corn |
| RIDA®Quick Fumonisin RQS  *quantitative* | LFD  5 min | 0,8 ppm, 4 ppm | corn |
| **Ochratoxin A** |  |  |  |
| **OCHRACARD**® | LFD/card  30 min | n.d. | a wide range of commodities |
| RIDASCREEN®  Ochratoxin A 30/15  *quantitative* | ELISA  45 min | cereals and feed: 2.5 ppb  cereals and feed: 1.25 ppb beer pig serum: approx. 50 ppt | cereals, feed, beer and pig serum |
| RIDASCREEN®FAST Ochratoxin A  *Quantitative* | ELISA  15 min | 5 ppb | cereals and feed |
| **T-2** |  |  |  |
| RIDASCREEN®T-2  *quantitative* | ELISA  1 h 30 min | < 5 ppb | cereals and feed |
| RIDASCREEN®FAST T-2  *quantitative* | ELISA  15 min | 20 ppb | cereals and feed |
| **ZON** |  |  |  |
| RIDASCREEN®FAST ZON  *quantitative* | ELISA  15 min | 17 – 41 ppb | cereals and feed |
| RIDASCREEN®FAST ZON SC  *quantitative* | ELISA  15 min | 5 ppb | cereals |
| RIDA®Quick ZON RQS  *quantitative* | LFD  5 min | 75 ppb | corn |

## Romer Labs®

Table 29: Summarized facts about the rapid analysis test systems from Romer Labs®.

|  |  |
| --- | --- |
|  | [www.romerlabs.com/en/products/mycotoxins/](http://www.romerlabs.com/en/products/mycotoxins/)  in Germany: coring system diagnostix ([www.coring.de/catpages.php?s=ae6d867f38f0a9574e3d9689c4754920&nav=2\_4\_0&catPage=4](http://www.coring.de/catpages.php?s=ae6d867f38f0a9574e3d9689c4754920&nav=2_4_0&catPage=4)) |
| Principle | LFD, ELISA, fluorometry |
| equipment | reader:  LFD: EUR not denoted , ELISA: EUR 3.000-4.000, fluorometer: EUR 6.000 |
| cost per kit/ consumables  (per sample not denoted) | LFD: EUR 245, ELISA: EUR 285-630, fluorometry: EUR 373-408 |
| Portability | no |
| Laboratory | Preparation: yes - Analytics: yes |

- Sample preparation for mycotoxin analyses with IAC (StarLine®): aflatoxin B1, B2, G1, G2, M1; ochratoxin A; DON, ZON; T2/HT2 extracted with PBS-buffer

* AgraQuant® ELISA: - quantitative, 10 – 20 min, different kinds of well-plates, detection with StatFax® 303 ELISA Reader or ChroMate® 4300 ELISA Reader

Table 30: Overview ELISA test systems for various mycotoxins from Romer Labs®.

|  |  |
| --- | --- |
| Analyte | quantification range |
| total Aflatoxin | 1 - 20 ppb, 4 - 40 ppb |
| AB1 | 2 - 50 ppb |
| AM1 | 100 - 2.000 ppt, 25 - 500 ppt |
| DON | 250 – 5.000 ppb |
| Fumonisin | 250 – 5.000 ppb |
| Ochratoxin A | 2 – 40 ppb |
| ZON | 25 – 1.000 ppb |
| T2 | – 500 ppb |

- AgraStrip® qualitative and quantitative test kits: - lateral flow detection, 5 min, different kinds of well-plates, detection with AgraVision™

Table 31: Overview Lateral Flow test systems for various mycotoxins from Romer Labs®.

|  |  |
| --- | --- |
| Analyte | quantification range |
| total Aflatoxin | 4 ppb, 10 ppb, 20 ppb, 5 – 100 ppb |
| AM1 | 0,1 – 0,6 ppb |
| DON | 0,25 – 5 ppm |
| Fumonisin | 0,5 – 5 ppm |

- FluoroQuant®: -quantitative fluorometric test, for corn, wheat, milo, popcorn, soybeans, cornsoy blends, raw peanuts, rice, cotton seed™

Table 32: Overview fluorometric test systems for various mycotoxins from Romer Labs®.

|  |  |  |
| --- | --- | --- |
| Analyte | LOD [ppb] | extraction buffer |
| Aflatoxin |  |  |
| FluoroQuant®Afla | 3 | n.d. |
| FluoroQuant®Afla Plus | 1 | acetonitrile |
| FluoroQuant®Afla Plus | 1 | methanol |
| FluoroQuant®Afla IAC | 3 | US Domestic |
| FluoroQuant®Afla IAC | 3 | international |

## Tecnalab s.r.l.

Table 3312: Summarized facts about the rapid analysis test systems from tecnalab.

|  |  |
| --- | --- |
|  | <http://www.tecnalab.it/> |
| Principle | ELISA |
| equipment | n.d. |
| cost per sample/ consumables | n.d. |
| Portability | no |
| Laboratory | Preperation: yes - Analytics: yes |

-rapid quantitative screening with ELISA:

- **BZERO**: fast ELISA test kits with **no calibration curve** for aflatoxin B1 and deoxynivalenol

- **Celer**: fast ELISA test kits for total aflatoxins, aflatoxin B1, fumonisins, zearalenone, deoxynivalenol, T2/HT2 toxins

- **IScreen**: high sensitivity ELISA test kits for aflatoxins and ochratoxin A

### 

## ToxiMet Ltd

Table 34: Summarized facts about the rapid analysis test systems from ToxiMet Ltd.

|  |  |
| --- | --- |
|  | <http://www.toximet.com/> |
| Principle | Fluorometry |
| equipment | EUR 22.500 (ToxiQuant) |
| cost per sample/ consumables | EUR 23 |
| Portability | No |
| Laboratory | Preperation: yes - Analytics: yes |

* rapid testing sequence for aflatoxins and ochratoxin
* high sensitive, simultaneous measuring and identification of toxins
* analyte will be immobilised on a disposable cartridge, excited with UV
* fluorescence will be detected by a spectrometer which outputs quantity of the
* different mycotoxins (chemometrically analyse)
* available for: aflatoxins (B1, B2, G1, G2, total), ochratoxin A
* *costs:*- 1 test with cartridge ToxiSep (clean-up) and ToxiTrace (for analysis): EUR 15-23,

ToxiQuant EUR 7.000 – 22.500

*- analysis:*

- extract toxin from the matrices into a solvent e.g. methanol, filter, pass it through a ToxiSep clean up cartridge

- pass resultant liquid through the ToxiTrace detection cartridge, the cartridge is conditioned with a solvent and the toxin will be immobilized on the cartridge

- after washing place the cartridge in ToxiQuant, follow the symbol driven menu

- after five minutes read the results straight from the screen (no further analysis required)



Figure 7: ToxiQuant, an instrument from ToxiMet Ltd, which automatically scans and analyses mycotoxins of a sample in a cartridge.

- advantages

* Single test simultaneous multi-mycotoxin analysis
* Accurate at the sub parts per billion level
* Cost effective single-test capability.
* non-scientists, No training or sophisticated laboratory required
* Environmentally friendly with very low solvent usage
* 5 min
* operates at temperatures up to 50⁰C
* low maintenance and low running costs
* LOD: EU: 🗸 international: 🗸

- disadvantages

- not portable

## VICAM

Table 35: Summarized facts about the rapid analysis test systems from VICAM.

|  |  |
| --- | --- |
|  | <http://vicam.com/> |
| Principle | LFD, fluorometry |
| equipment | EUR 6.000 (Series-4EX Fluorometer)  (for LFD not denoted) |
| cost per sample/ consumables | fluorometry: EUR 10-15  (for LFD not denoted) |
| Portability | No |
| Laboratory | Preparation: yes - Analytics: yes |

**Fluorometer**

* simple rapid quantitative method delivers parts-per-trillion (ppt) numerical results in as little time as 10 minutes (excluding preparation and extraction)—without costly HPLC or UPLC equipment or special training
* equipment: Series-4EX Fluorometer approx. EUR 6.000
* costs: basic packages with test + fluorometer approx.. EUR 7.950,00; upgrade packages EUR 18,50 – 675,45

Table 36: Overview fluorometric test systems for various mycotoxins from VICAM.

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | detection range | extraction buffer | costs [EUR]  per column |
| AflaTest  (AFB1, AFB2, AFG1, AFG2 and AFM1) (feeds, food, grains, nuts, dairy product) | 0,1 – 300 ppb | salt and methanol/water | 9,97-10,40 |
| AflaB  (AFB1, AFB2, AFG1, AFG2 and AFM1) | 1 – 300 ppb | salt and methanol/water | 12,78 |
| Afla M1FL+  (for milk) | 12,5 - 200 ppt |  | 12,78 |
| [FumoniTest](http://vicam.com/fumonisin-test-kits/fumonitest)™  (Fumonisin B1, B2 and B3) | 0,016 – 10 ppm | salt and methanol/water | 14,32 |
| [OchraTest](http://vicam.com/ochratoxin-test-kits/ochratest)™  (Ochratoxin A) | 0,1 – 100 ppb | methanol/water | 11,25 |
| ZearalaTest | 0,1 – 5 ppm | salt and methanol/water | 12,27 |

**Lateral Flow Reader**

* results in or less than 5 minutes (excluding preparation and extraction), non-specialized operators

****

Figure 8: VertuTM Lateral Flow Reader from VICAM.

* equipment: test kit, VertuTM Lateral Flow Reader (figure 10), standard PC to print or to download

Table 37: Overview Lateral Flow test systems for various mycotoxins from VICAM.

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | Matrix | detection range | extraction buffer |
| Afla-V  (aflatoxin B1, B2, G1, G2 ) | n.d. | 0 -100 bbp | ethanol 70% |
| DON-V | grain, feed | 0 – 5 bbm | Water |
| Fumo-V  (Fumonisin) |  | 0 – 5 bbm | ethanol 70% |

**Qualitative Strip Tests**

* qualitative one-step test kit as first step in detection of contaminations
* visual results in less than 3 minutes (excluding preparation and extraction), non-specialized operators
* detection of aflatoxin (Afla-Check) with range of 10 or 20 ppb
* detection of DON ( DON-Check) with range of 1 ppm
* extraction with water

# Comparison between selected important methods

Table 38: Comparison between selected important methods.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Method | HPLC or LC-MS/MS  [IGV GmbH, Map Milling 2007;2013] | ELISA  [r-biopharm-RIDASCREEN®] | LFD/Dipstick  [EuroProxima] | Fluorescence Polarization Immunoassay (FPI)  [diachemix] |
|  | quantitative | quantitative | qualitative or semi-quantitative | quantitative |
| principle | extraction, clean-up HPLC-UV or –FD with derivatization  ***or*** LC-MS/MS | competitive immunoassay, detection about colour changes in the substrate | form of immunoassay, detect the presence (or absence) of target analyte in sample | based on the measurement of polarization (polarization ⭡= mycotoxin ⭡) |
| time | 3 – 4 d | 45 min | < 10 min | 2 min |
| legislation | EU:🗸  US:🗸 | EU:no  US:🗸 | EU:🗸  US:🗸 | EU:no  US:🗸 |
| investment costs [EUR] | 10.000 - 50.000 | 1.200 | n.d. | 26.000 – 27.000 |
| costs/ sample [EUR] | 50 – 180 | 15 | 22 – 27,50 | 40 - 50 |
| strength | sensitive, reproducible | easy handling, low expenditure at time, sensitive, multiple analysis | easy handling, rapid, portable, no special equipment | rapid, easy handling, portable |
| weakness | high costs and equipment, scientific stuff, time | cross-reactivity, false-positive because of matrix disruptions, costs | not qualitative, costs | in research, ex-pensive equipment |

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# Helpful links and facts

Informations

<http://www.mycotoxins.org/>

<http://www.mycotoxins.info/myco_info/qanda.html>

<http://www.mykotoxin.de/docs/public/tmplt_article.asp?CntID=16&PCat_ID=1&Lang=EN>

<http://www.mold-help.org/content/view/776/>

<http://www.fao.org/docrep/T1838E/T1838E00.htm>

<http://www.sigmaaldrich.com/life-science/cell-biology/cell-biology-products.html?TablePage=9619444>

<http://www.bfr.bund.de/de/presseinformation/2009/01/von_aflatoxin_bis_zearalenon___wissenschaft_macht_lebensmittel_sicher-27754.html>

Legislation

<http://www.romerlabs.com/de/knowledge/mycotoxin-regulations/regulations-europe/>

<http://www.romerlabs.com/de/knowledge/mycotoxin-regulations/regulations-usa/>

<http://europa.eu/legislation_summaries/food_safety/contamination_environmental_factors/index_en.htm>

<http://eur-lex.europa.eu/en/index.htm>

<http://ec.europa.eu/food/food/chemicalsafety/contaminants/legisl_en.htm>

Food

<http://www.codexalimentarius.org/>

<http://www.efsa.europa.eu/en/topics/topic/mycotoxins.htm>

Organisations

<http://www.aaccnet.org/Pages/default.aspx>

<http://www.acs.org/content/acs/en.html>

<http://www.aoac.org/>

<http://www.cen.eu/cen/Pages/default.aspx>

<http://www.fooddrinkeurope.eu/>

<http://www.citac.cc/>

<http://www.european-accreditation.org/home>

<http://www.eurachem.org/>

<http://www.euramet.org/index.php?id=homepage>

<http://www.eurolab.org/>

<http://www.iaf.nu/>

<http://www.foodprotection.org/>

<http://www.ilac.org/>

<http://www.iso.org/iso/home.htm>

<http://www.iupac.org/>

<http://www.measurement.gov.au/Pages/default.aspx>

<http://www.nmkl.org/>

<http://www.oecd.org/index.htm>

<http://www.usda.gov/wps/portal/usda/usdahome>

<http://www.wto.org/english/thewto_e/thewto_e.htm>

http://www.lgcgroup.com/our-science/national-measurement-institute/#.WIe-DYWcHy9

EU-legislation

Links to EU-legislation concerning mycotoxins.

|  |  |
| --- | --- |
| EU-legislation concerning mycotoxins | Compounds |
| <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20100701:EN:PDF> | - maximum levels for DON, Afla, ZON, Fumonisin B1, - B2, Ochratoxin A, T2-HT2 |
| <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0032:20100302:EN:PDF> | - maximum levels for Afla |
| <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:229:0007:0009:EN:PDF> | - maximum levels for DON, ZON,  Fumonisin, Ochratoxin A, T2-HT2 |
| <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:234:0035:0040:EN:PDF> | - Fusarium toxins |
| <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R0401:20100313:EN:PDF> | - DON, Afla, ZON, Fumonisin B1, - B2, Ochratoxin A, T2-HT2 |

Reviewed wavelength of various mycotoxins.

|  |  |  |
| --- | --- | --- |
| mycotoxin | excitation [nm] | emmision [nm] |
| Aflatoxin (Huang and Elmashni, 2007) | 365 | 455 |
| Aflatoxin (Rasch et al., 2007) | 360 | 427 |
| Ochratoxin A (Rasch et al., 2007) | 333/380 | 455/427 |
| Ochratoxin B (Rasch et al., 2007) | 320/367 | 460/422 |
| Zearalenone (Rasch et al., 2007) | 318 | 466 |
| Aflatoxins (Jansen et al., 1987) | 365 | 440 |

Spectrophotometric parameters for various mycotoxins from [**http://www.fao.org/docrep/x5036e/x5036E0c.htm**](http://www.fao.org/docrep/x5036e/x5036E0c.htm)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| mycotoxin | molecular weight | Solvent | absorbtivity | -max (nm) |
| Aflatoxin B. | 312 | Benzene:acetonitrile |  |  |
|  |  | (98:2 v/v) | 19,800 | 353 |
| Aflatoxin B1 | 312 | Chloroform | 22,300 | 353 |
| Aflatoxin B2 | 314 | Benzene:acetonitrile |  |  |
|  |  | (98:2 v/v) | 20,900 | 355 |
| Aflatoxin G1 | 328 | Benzene:acetonitrile |  |  |
|  |  | (98:2 v/v) | 17,100 | 355 |
| Aflatoxin G2 | 330 | Benzene:acetonitrile |  |  |
|  |  | (98:2 v/v) |  | 357 |
| Aflatoxin M1 | 328 | Chloroform | 19,950 | 357 |
| Ochratoxin A | 403 | Benzene:acetic acid |  |  |
|  |  | (99:1 v/v) | 5,550 | 333 |
| Ochratoxin B | 369 | Benzene:acetic acid |  |  |
|  |  | (99:1 v/v) | 6,000 | 320 |
| Ochratoxin A | 431 | Benzene:acetic acid |  |  |
| ethyl ester |  | (99:1 v/v) | 6,200 | 333 |
| Ochratoxin B | 397 | Benzene:acetic acid |  |  |
|  |  | (99:1 v/v) |  | 320 |
| Patulin | 154 | Absolute ethanol | 14,540 | 276 |
| Patulin | 154 | Methanol | 12,880 | 275 |
| Sterigmatocystin | 324 | Benzene | 15,200 | 325 |
| Citrinin | 259 | Chloroform | 16,100 | 322 |
| Zearalenone | 318 | Ethanol | 29,700 | 236 |
| Zearalenone | 318 | Ethanol | 13,909 | 274 |
| Zearalenone | 318 | Ethanol | 6,020 | 316 |