MYCOTOXIN ANALYSIS:
A FOCUS ON RAPID METHODS

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PACA Partnership for Aflatoxin Control in Africa

With support from

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A FOCUS ON RAPID METHODS

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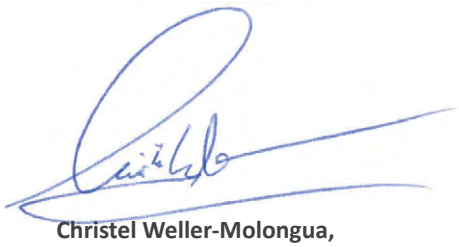
FOREWORD

Mycotoxin contamination of staple and cash crops is a serious developmental challenge that calls for urgent actions. African Union Commission has priority attention to aflatoxin control because aflatoxins are a real threat to the achievement of major global and continental commitments including ending hunger, boosting trade in agricultural commodities and services and eradicating poverty. Aflatoxins thwart Africa's efforts at achieving food security, improving nutrition and health outcomes and attaining agriculture-led economic growth.

The outbreaks of acute aflatoxin poisoning that tragically killed hundreds of people in Eastern African countries in recent years are a cause for concern. Reports show that chronic aflatoxin exposure is attributable to at least one-third of the liver cancer cases in Africa, making liver cancer the number one cause of cancer mortality in Africa. Africa's share of the world groundnut trade has dwindled to a mere 4% from high of 77% in the 1960's at least partly due to difficulty to meet aflatoxin standards of major importing countries. We should also heed the mounting evidence that aflatoxin is associated with childhood stunting and with immune-system suppression.

The challenge is complex and deserves coordinated efforts. The German Federal Ministry for Economic Cooperation and Development (BMZ) and the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) collaborated with the African Union Commission (AUC) to make this publication available. Proper detection is the basis to deal with mycotoxins including aflatoxins which are invisible poisons. Considering the limited laboratory infrastructure and capacity in most parts of developing world, it is important to have rapid, reliable and accessible test methods that can be easily adopted.

In this regard, it is our belief that this publication provides practical information for researchers, food manufacturers, laboratory managers and anyone involved in the management of aflatoxins and mycotoxins in general.



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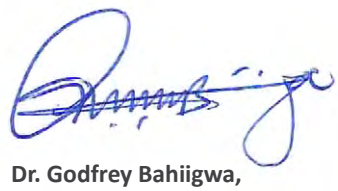

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1. CHALLENGES OF MYCOTOXINS AND THEIR CONTROL

1.1 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 have been identified from fungal cultures. Some of them are observed in foodstuffs and feedstuffs around the world. FAO has estimated that about 25% of global crops are contaminated by molds and thus affected by mycotoxins. The economic losses are estimated to be billions of dollars (FAO, 2004). 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), ochratoxin A (OTA) and zearalenone (ZEA) (Moss, 1991; Steyn, 1995). Among them aflatoxins have a dominant role in terms of incidence in contaminated material.

Contaminations with mold and mycotoxins can occur pre- and/or post-harvest if conditions are poor. Mycotoxins occurrences depend on improper conditions 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 materials as well as in processed feedstuffs and foodstuffs. Due to their stability they are also resistant to high temperature and long-term storage. The common occurrences of mycotoxins in foodstuffs and feedstuffs pose extensive hazard for human and animal health (Hussein and Brasel, 2001; Rai, 2012; Wild and Gong, 2010). The great variability of mycotoxins in molecular structure explains the great variations 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).

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

Table 1: Toxic effect of important mycotoxins

Mycotoxin	Toxicity
Aflatoxin	pulmonary carcinogen, acute aflatoxicosis (Wild and Gong, 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 MA et al., 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 (Food, 2000)

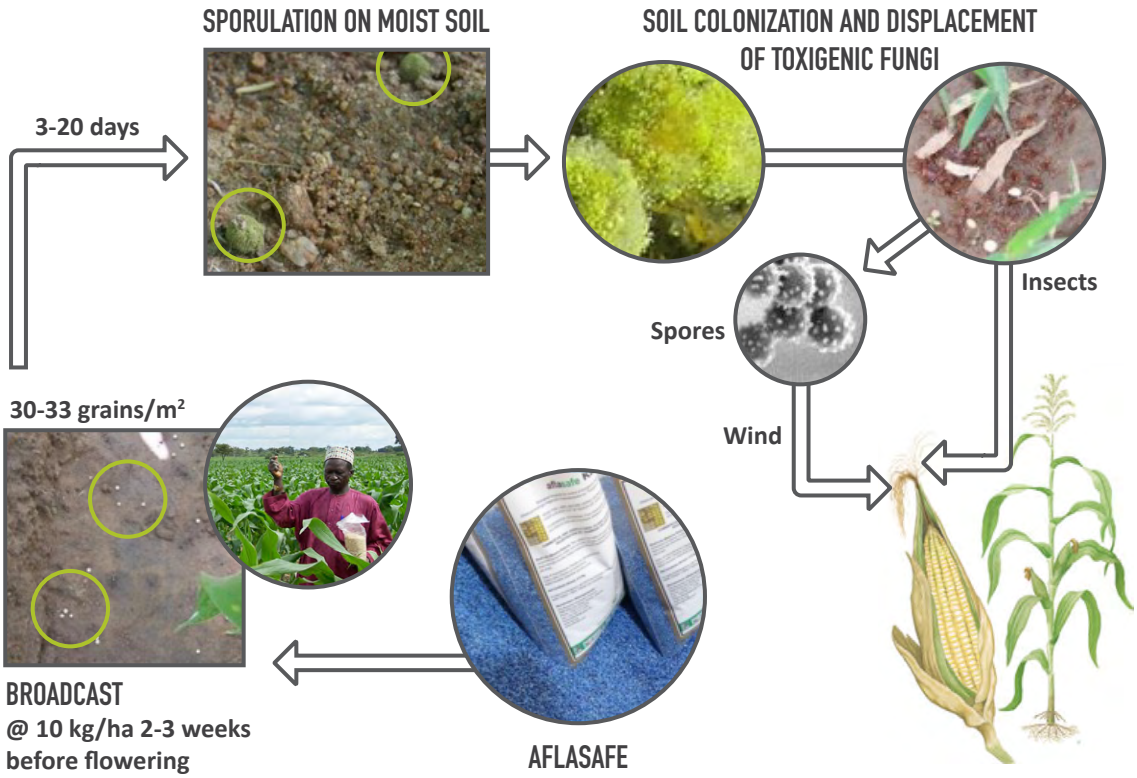
Source: Rai , 2012

1.2 PREVENTION AND REDUCTION OF MYCOTOXIN CONTAMINATION

Preventive methods in cultivation and harvest are possible ways to reduce the challenges 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) and aflasafe™ (www.aflasafe.com). Basically, atoxigenic strains of *Aspergillus flavus* will be applied to the field and crowds out 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 (PJ, 2006). Further studies in the area of such exclusion techniques exist such as Probst et al. (2011) who investigated different isolated strains of *A. flavus* and atoxigenic strains (LOD aflatoxin B_1 < 0,5 ppb), co-inoculated with highly toxic strains. 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. Non-germinating sorghum seed is inoculated with the atoxic strains (Aflasafe™) and thereafter, it should be broadcasted to obtain a more even distribution of the fu ngus across the field. Field tests in Nigeria and Senegal showed 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: How aflasafe™ works in the field.

Courtesy of Ranajit Bandyopadhyay, IITA (www.iita.org, www.aflasafe.com)



Important for the prevention of contaminations with mycotoxins is the fundamental knowledge in genomics, proteomics and metabolomics among others - about the fungus and the mycotoxins. This 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) such as glyoxylase I which is a stress related aflatoxin resistance protein (Bhatnagar et al., 2008b), have emerged by these techniques as promising marker for crop varieties resistant to aflatoxins.

Metabolomic studies complete the understanding of the fungus and the synthesis of mycotoxins during pre- and postharvest. Metabolomic investigations contain metabolites as the results of specific cellular processes in biological organisms.

With such studies (the field of “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 contamination with mycotoxins (Bhatnagar et al., 2008b).

1.3 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 different mycotoxins in food. In Europe limits of 2 ppb (for aflatoxin B1) and 4 ppb (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 5 ppb for aflatoxin B1 and 10 ppb 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 limits. 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 2. An abstract of the mycotoxin regulations in food and feed in the United States is shown in Table 3.

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

1. A maximum level of 10 ppb for total aflatoxins in treenuts (almonds, hazelnuts, pistachios and shelled Brazil nuts) “ready to-eat” (CAC, 2017; IITA, 2015).
2. ML of 15 ppb for total aflatoxins in peanuts and treenuts destined for further processing ” (CAC, 2017; IITA, 2015).
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 1000 ppb for deoxynivalenol in flour, semolina, meal and flakes derived from wheat, maize and barley (CAC, 2015)

7. ML of 200 ppb for deoxynivalenol in 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 5 ppb 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 5 ppb for aflatoxins B1 and 10 ppb for total aflatoxins and 2000 ppb 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

Table 2: Important EU-maximum limits for various mycotoxins

Compound	Commodity	maximum level [ppb]		
		B1	total	M1
Aflatoxins	groundnuts, nuts, dried fruit and processed products there of intended for direct human consumption or use as an ingredient in foodstuffs	2	4	-
	cereals (including buckwheat) and processed products there of intended for direct human consumption or as an ingredient in foodstuffs	2	4	-
	rice, including brown rice (intended for direct human consumption)	2	4	-
	milk (raw milk, milk for the manufacturer of milk based products and heat treated milk)	-	-	0.05
	baby foods and processed cereal, cereal based foods for infants and young children	0.1	-	-
	in feed: all feed materials	20	-	-
Deoxynivalenol	unprocessed cereals (excluding durum wheat, oats and maize)		1,250	
	unprocessed maize		1,750	
	in feed: cereals and cereal products with the exception of maize by-products		8,000*	

	in feed: maize by-products	12,000*
Fumonisins	unprocessed maize	4,000
	maize and maize based foods intended for direct human consumption	1,000
	in feed: maize and maize based products	60,000*
Ochratoxin A	unprocessed cereals	5
	dried vine fruit (currants, raisins and sultanas)	10
	roasted coffee beans and ground roasted coffee.	5
	wine and fruit wine	2
	baby foods and processed cereal based foods for infants and young children	0.5
	in feed: cereal and cereal products	250*
T-2/ HT-2	*unprocessed cereals*	
	- barley (including malting barley) and maize	200*
	cereal grains for direct human consumption	
	- oats	200*
	- maize	100*
	breakfast cereals including formed cereal flakes	75*
Zearalenone	unprocessed cereals other than maize	100
	unprocessed maize	350
	in feed: cereal and cereal products with the exception of maize by-products	2,000*
	in feed: maize by-products	3,000*

**guidance level*

Table 3: Important US-Maximum Limits for various mycotoxins

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*	

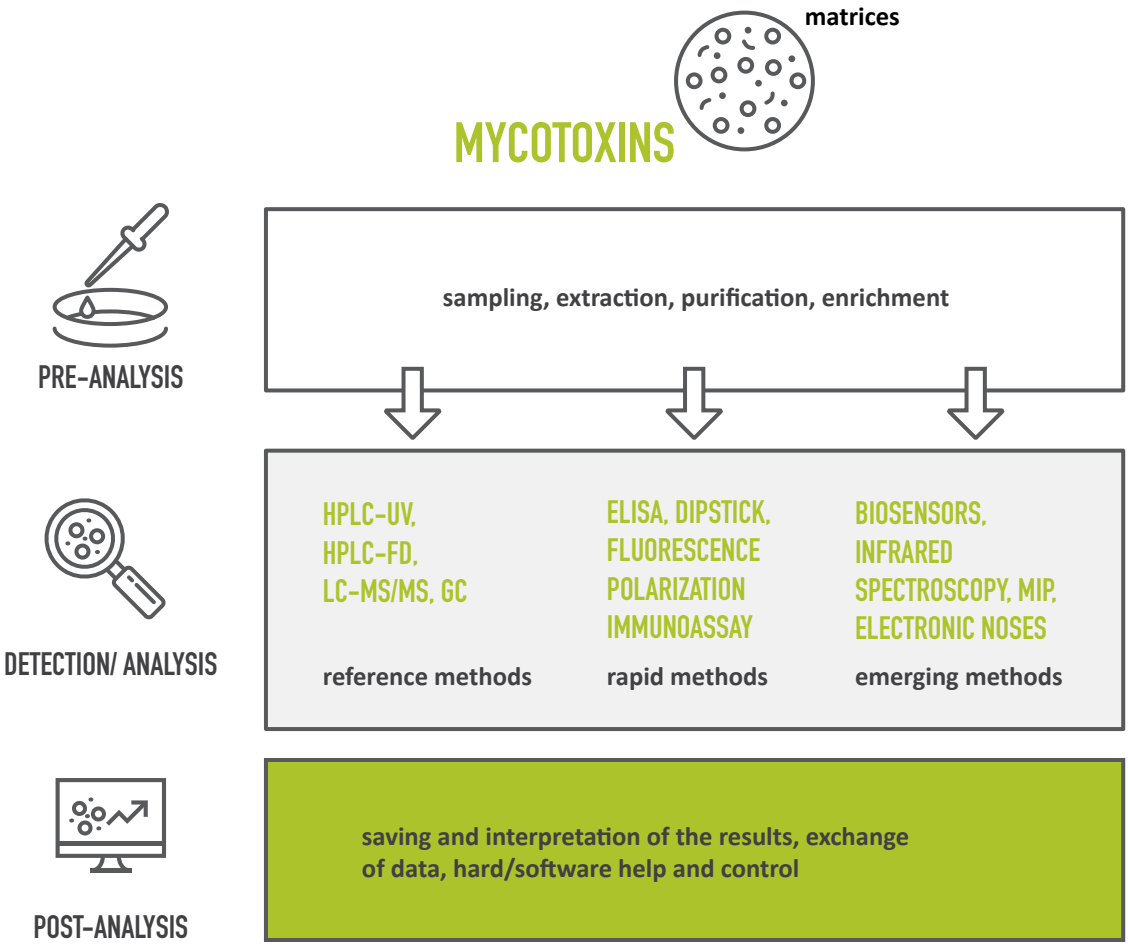
**guidance level*

2. GENERAL STEPS IN THE ANALYSIS OF MYCOTOXINS

Fast, inexpensive, 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, sensitive and simple for non-experts. Most methods involve time consuming preparation, clean-up and purification and this is more or less independent from the method later used for analytical 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).

Test systems for mycotoxins in food and feed commodities involve a multiple-step process (Figure 2). Most methods consist of a pre-analytical step in which the complex sample matrix is homogenized and the mycotoxins are solubilized. The samples are thus extracted, purified and/or enriched. Different analytical approaches are then used for the detection of individual mycotoxins or mixtures of mycotoxins. In a final post-analysis step results are validated, stored or exchanged.

Figure 2: Common sequence of procedures for mycotoxin analysis



2.1 PRE-ANALYSIS

2.1.1 Sampling and sample preparation

The heterogeneous distribution of mycotoxins in cereals, nuts, grains and other commodities takes an important role in the analysis of mycotoxins in food and feed. A number of wheat kernels can show low contamination with mycotoxins but another single kernel from the same unit may show a high contamination. 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 basis for precise determination of the level of contamination of mycotoxins.

A tool that provides support in analysing performance of sampling plans, and determining the most appropriate plan to meet user´s defined objectives, the Mycotoxin Sampling Tool (V 1.1) is available from the FAO website http://www.fstools.org/mycotoxins

Solid products, such as kernels and nuts must be ground to powder form with a defined size to enlarge the surface area and make them accessible for extraction solvents. The equipment needed in the process includes mills, grinder, sieves and filters. Liquids and paste-like solutions must be gently stirred before extraction. Subsamples are then taken for extraction and analysis (Beuchat, 1987; International, 2000).

2.1.2 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, 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.

2.1.2.1 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 4: Strength and weakness of liquid-liquid extraction (LLE).

Strength	purification, decrease interfering substances
weakness	- time consuming
	- extraction effectiveness dependant on the type of matrix or mycotoxin
	- loss of sample because of possible adsorption to the glassware

There are different extraction methods for mycotoxins depending on the type of matrix as well as the chemical properties of the 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 more polar. The extraction of these must be done basically with a combination of water and organic solvents (Holcomb et al., 1992). It is also described, however, that a combination of organic solvents with a small amount of water improves the extraction of aflatoxins. An overview about the solvent of the choice for some mycotoxins is shown in Table 5.

The generally accepted extraction method for the analysis of aflatoxins in peanuts and peanut products, oilseeds and food grains is the CB-method (Contaminants Bureau) (International, 2000). Briefly, in a glass-stoppered flask 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 loaded on a special prepared column. After loading the column with the extract, it should be washed with hexane and anhydrous ether. This fraction would be discarded. Aflatoxin is 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) (International, 2000). The mycotoxin is extracted with a mixture of methanol-water-hexane and separated and extracted three times into chloroform. After evaporation to dryness it should 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).

Table 5: The most effective extraction solvents and alternatives for a number of mycotoxins – reviewed and modified by Biselli (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)

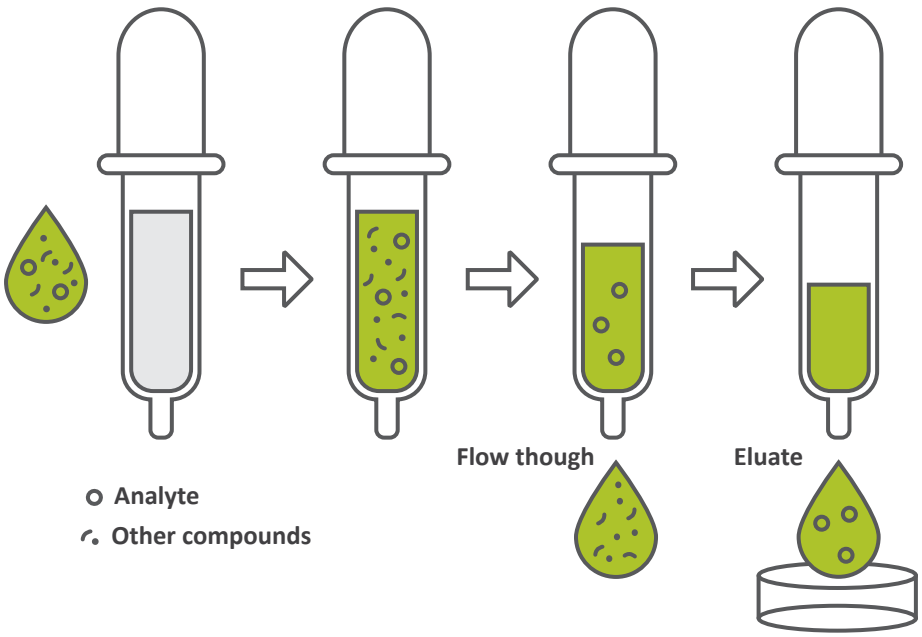
2.1.2.2 Liquid-solid extraction

The liquid-solid extractions methodologies of mycotoxins are usually based on the principle of a chromatographic technique. These are unspecific extraction methods 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 solution is loaded onto the cartridge and rinsed under reduced pressure. Contaminants or the mycotoxin should 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 and specific extraction of mycotoxins from different kinds of commodities. The packing material of the column includes antibodies which specifically bind the analyte and allow the interfering substances to pass feely. The elution of the analyte is achieved with an antibody denaturing solution.

Figure 3: Model for immunoaffinity column (IAC) (Courtesy of Tecna®, manufacturer of mycotoxins ELISA kit - www.tecnalab.com).



An alternative is the SPME (solid phase micro extraction) approach. This is based on either Mycosep™ columns loaded with molecular imprinted polymers (MIPs). Mycosep™ columns keep back the contaminants of a sample but allow the mycotoxins to pass (Egmond, 1986; Pittet, 2005; Turner et al., 2009). MIPs result in the application of imprinting techniques. At first, monomers are co-polymerized with a presented target analyte (imprint molecule). The removal of the imprint molecule reveals a molecular memory for the analyte in shape and size. The special synthetic cavity could be used to separate and to extract analytes from a sample. This extraction method is adapted to the pre-analysis of some mycotoxins such as ochratoxin A (Baggiani et al., 2002; Jodlbauer et al., 2002; Turner et al., 2004; Zhou et al., 2004) or 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 6: Strength and weakness of solid phase extraction (SPE).

Strength	• easy handling, low time expenditure per analysis, no professional/expertise 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

2.2 DETECTION AND QUANTIFICATION OF MYCOTOXINS (ANALYSIS STEP)

1.2 State-of-the-art methods

Reference methods for the quantitative and qualitative determination of mycotoxins are basically chromatographic systems with different detection systems such as HPLC-UV/DAD or LC-MS. They produce high resolution as well as sensitive and reproducible results. They are the accepted methods for any testing related to dispute resolution. The disadvantages of these methods are that they are time consuming, high in costs, sophisticated in equipment and with the need of expert scientific and technical knowledge. In addition, 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 principles and the different applications have been widely published and discussed (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 analyze 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 cleaned-up with immunoaffinity chromatography column (IAC column) followed by reversed phase - HPLC with post column bromiation (LOD: Aflatoxin B1 1 ng/g, total Aflatoxin 2.4 ng/g) (Anklam et al., 2002; Stroka et al., 2000). Improvements in the detection tools of chromatographic techniques led 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 analysis.

However, under less controlled conditions measurement procedures must be reliable and sensitive to be able to control levels set by legislation but also easy and just-in-time for use in the field. Therefore, in the last years investigations in rapid and simple techniques have become increasingly important. Several more sensitive, specific and simple methods for mycotoxin detection are commercially available and are summarized under the term screening methods. These include enzyme-linked immunosorbent assay (ELISA), lateral flow detection (LFD), fluorescence polarization immunoassay (FPI) and thin layer chromatography (TLC). The different methodological approaches again 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).

2.2.2 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 7 giving a first overview.

Table 7: Strength and weakness of enzyme-linked immunosorbent assay (ELISA), lateral flow detection (LFD) or fluorescence polarization immunoassay (FPI).

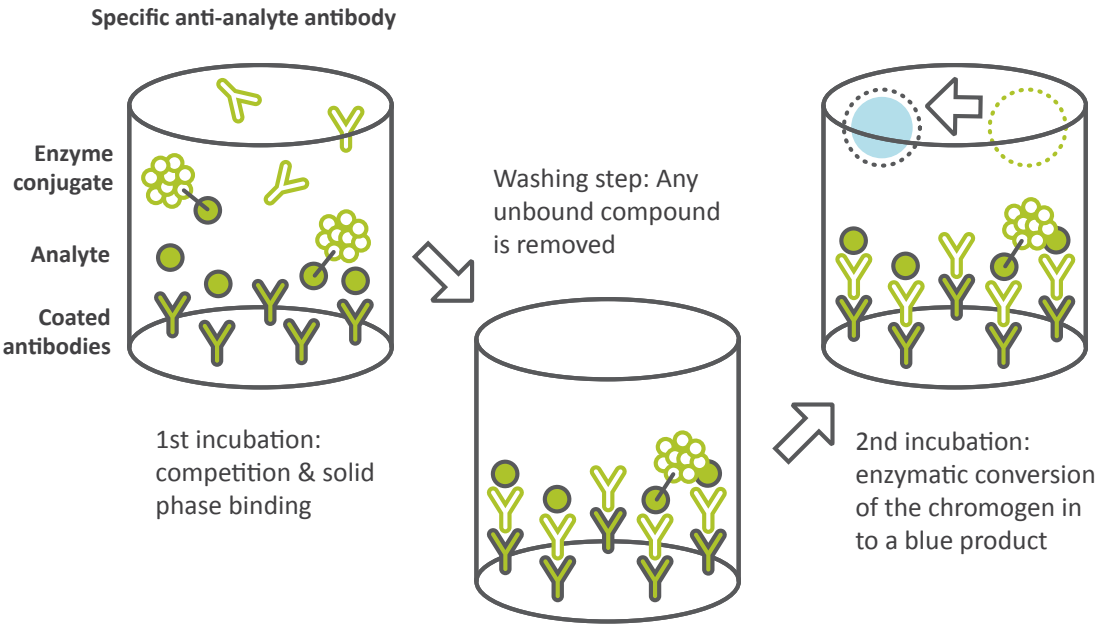
	ELISA	LFD	FPI
Strength	• easy handling • low expenditure at time • sensitive • multiple analysis	• easy handling • rapid • portable • no special • equipment	• easy handling • low time expenditure per sample • sensitive • portable • quantitative and qualitative
weakness	• cross-reactivity • false-positive because of matrix disruptions • high costs	• not qualitative • high 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 determination of mycotoxins in food and feed. The principle is based on the use of antibodies and specific color changes. Different forms of ELISA are commercially available (e.g. single disposable membrane-based test, microtiter 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 4).

Figure 4: Principle of a competitive ELISA to screen mycotoxin (Courtesy of Tecna®, manufacturer of mycotoxins ELISA kit - www.tecnalab.com).

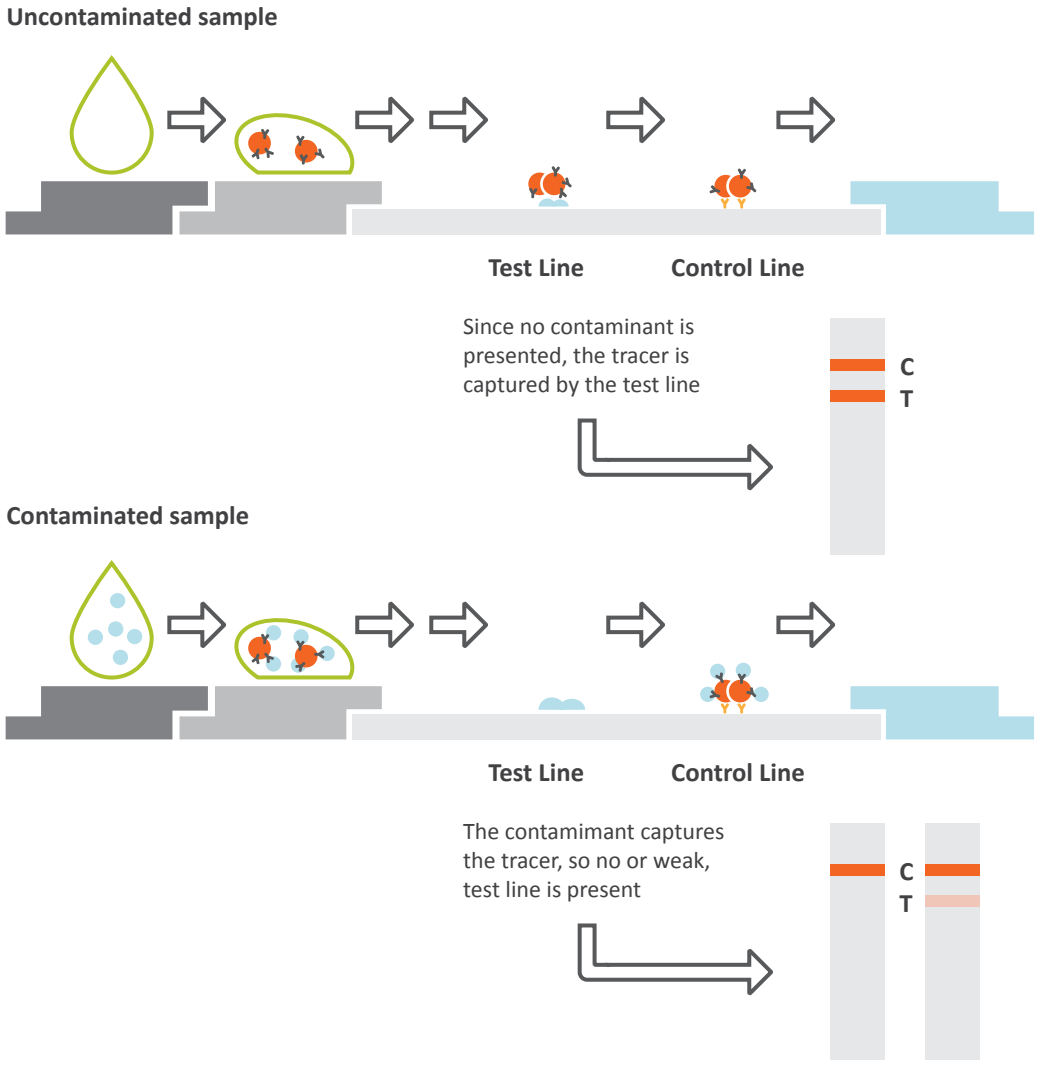


The experiment set-up uses a microtiter plate which is coated with a mycotoxin-specific-immobilized antibody. In the first step, a mycotoxin linked with an enzyme is added to a sample. This mixture should be applied on the microtiter plate. The amount of the mycotoxin-linked enzyme that binds to the 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 will be 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.

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. They are often called "dipstick"-tests. At first a pre-conditioned strip is wetted. Then the extracted sample should be applied and after running, the strip shows the results visually or using a special reader (Figure 5).

Figure 5: Model of a competitive lateral flow detection-dipstick (Courtesy of Tecna®, manufacturer of mycotoxins ELISA kit - www.tecnalab.com).



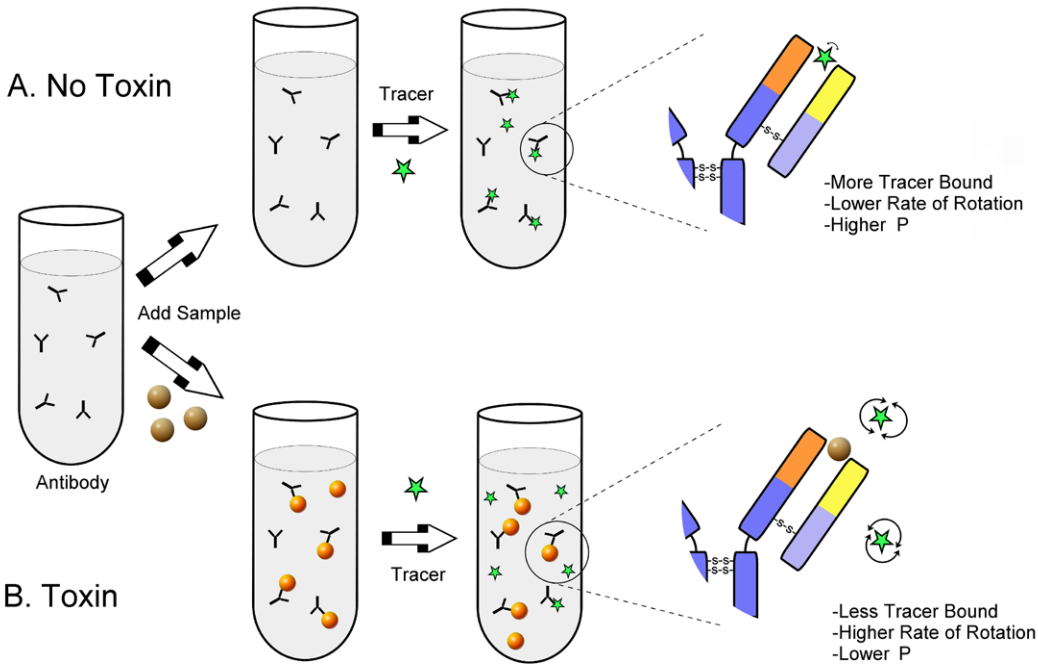
Fluorescence polarization immunoassay (FPI)

This is a newly developed immunoassay based on the indirect measurement of the changes of molecule rotation in a solution (Figure 6). There are only two suppliers of FPI to determine a specific and limited number of mycotoxins.

Basically, a fluorochrome labeled 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 in weight and therefore 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).

The deficiency of such assays is the problem of cross-reactivity which is not completely deleted and hence further research is 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 be eliminated in all immune methods. IACs to clean-up deoxynivalenol (DON) and zearalenone (ZON) has been studies with regard to the cross-reactivity of antibodies for conjugated mycotoxin forms such as glycosides or acetylated forms (Tangni et al., 2010; Versilovskis et al., 2011)

Figure 6: Principle of fluorescence polarization immunoassay (FPI). A: Binding the receptor molecule increases the weight of the fluorescence labeled ligand and slowing its rotation which generates a polarization of light; B: With fewer receptors bound, the rapid rotation of the fluorescence labeled ligand leads to a depolarization of light (Maragos, 2009).



2.2.2.1 Thin layer chromatography (TLC)

The first and well-established method for separation and quantifying mycotoxins is the thin layer chromatography (TLC). TLC provides a less expensive alternative to other LC-based methods. Especially in developing countries it has an important role for surveillance purposes and control of regulatory limit values (Gilbert and Anklam, 2002). Extensive investigations in the field of TLC led to highly sensitive and good separating methods 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 on to 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 one-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 personnel.

Various conditions may 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 purity of the standards, the manner of spotting the plate and the development 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 reaction of sterigmatocystin with aluminum chloride on the plate increases the fluorescence intensity up to 100-fold (Stack and Rodricks, 1971) . Other authors 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. (1998) summarized different detection techniques after TLC: UV-light of long or short wavelength, fluorescence quencher, autoradiography, vaporing of iodine or ammonium or exposition to X-ray (Lin et al., 1998).

Table 8: Detection limits for TLC-methods from www.eurofinsus.com.

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

Table 9: Strength and weakness of thin layer chromatography (TLC).

	TLC
strength	• multiple analysis (Lin et al., 1998; Turner et al., 2009) • cost efficient (Espinosa-Calderón et al., 2011; Turner et al., 2009) • LOD: EU and US P • 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 coloring or enhancing the fluorescence • increase of the costs when IAC is used for purification of the samples

Fluorescence methods

Non-specific fluorescence methods

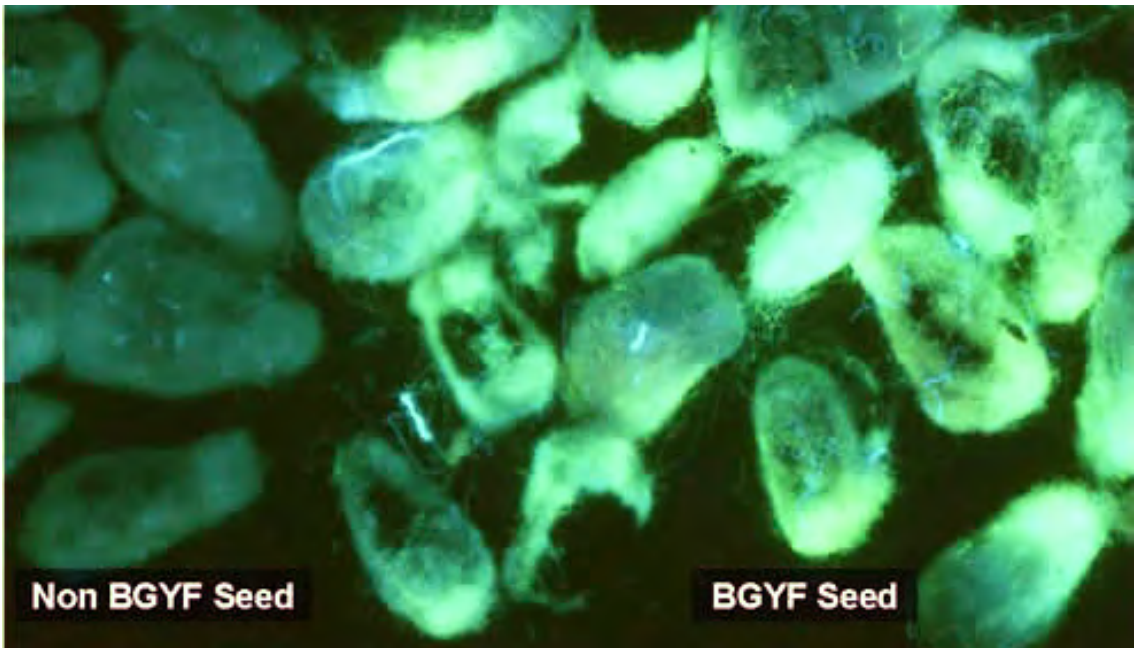
Basic rapid and easy to handle determination methods of mycotoxins commonly use the physicochemical natures of mycotoxins e.g. the ability to stimulate the autofluorescence. Tests like the "black light" test, also called Bright Greenish Yellow Fluorescent (BGYF) test, show mycotoxin producing fungi-infections in samples under UV-light (365 nm) rapidly, indirectly, with low equipment investment. However, these tests are not specific. This analytical principle is used in automated sorting systems e.g. SORTEX from Bühler GmbH (Germany) (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 7). Bühler provides different SORTEX applications depending on the commodity to be analyzed e.g. SORTEX Z+ for rice, grain and beans.

Other machines are the Detox Aflatoxin Laser Sorter from Best and the Nimbus sorting machine from TOMRA. It makes it possible to detect aflatoxins in various grains and combines various lasers for detection.

Pearson et al. (2004) investigated a high-speed dual-wavelength sorting to reduce the aflatoxin and fumonisin contamination in yellow corn. The study was based on a sorting with near-infrared (NIR) reflectance spectra (500-1.700 nm). A reduction of aflatoxin contamination with an average of 82% (level of aflatoxins in corn > 10 ppb) and an average of 38% (level of aflatoxins in corn < 10 ppb) by high-speed sorting (filters at 750 nm and 1,200 nm) has been reported (Pearson et al., 2004). A further study using NIR in optical sorting showed the classification accuracies in post-harvest detection and removal of aflatoxins and fumonisins contaminated maize kernel (Wicklow and Pearson, 2006). Another rapid and visual method described by Atas and coworkers 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 techniques is still limited to screening purposes due to high matrix dependence and the lack of appropriate calibration materials.

Figure 7: 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.



Courtesy Peter J. Cotty Agricultural Research Service, United States Department of Agriculture, School of Plant Sciences, University of Arizona, Tucson http://cals.arizona.edu/research/cottylab/research/epidemiology.html

Fluorescence and column separation

In the early stages scientists introduced a minicolumn technique to detect aflatoxin in peanuts based on the principle of thin layer chromatography (TLC) (Holaday, 1981). Contrary to TLC this method was faster and easier to use. A glass column (Table 11: Holaday "Dip" column) was packed with a 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 was based on visualization under UV-light (e.g. aflatoxins - blue or bluish - green color). The detection limit was 10 ppb.

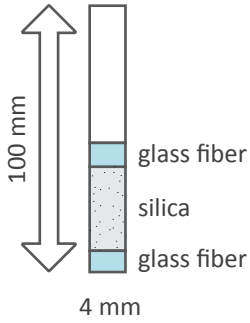
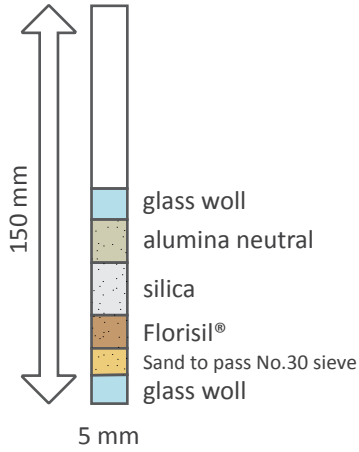
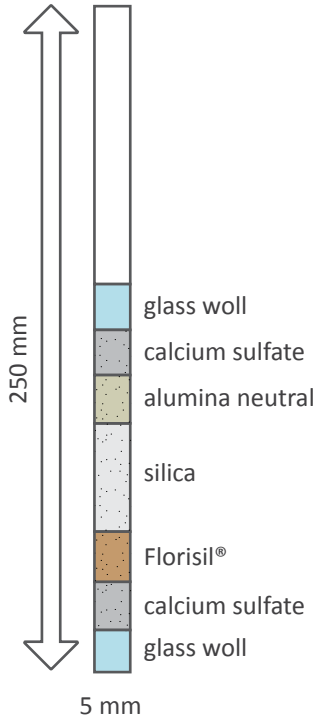
Further developments led to the official technique the Romer minicolumn (Table 11) which is packed with Florisil®. Florisil® is a magnesium silicate (MgO – SiOH 15:85) with a particle size of 150 to 250 µm. It is widely used in analysis of feed and food. Magnesium silicate is also used as filler material and parting agent by the industry (E 553a).

In principle, a small glass column is packed with various layers but generally including magnesium silicate. For example, for sterigmatocystin analysis the minicolumn is stuffed with glass wool and stacked with anhydrous sodium sulphate, neutral alumina, Florisil® and again anhydrous sodium sulphate (Ramakrishna and Bhat, 1990). The column is purged with different organic solvents (e.g. dichlormethane, hexane) under gravity. Thereafter, a methanol-sample solution is rinsed through the minicolumn. The mycotoxin adsorbs to the layer in the column and can be detected under UV-light. The determination is done by comparing the column with a column treated the same way and with a standard only. They are also called "go-no-go" methods because of their semi-quantitative or quantitative but less sensitive determination (Egmond, 1986).

Table 10: 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)

Table 11: Investigations in minicolumn methods to analyze mycotoxins. (Egmond, 1986; Holaday, 1981; International, 2000; Shotwell, 1983).

Holaday "Dip" column (1968)	Velasco column (1972)	Romer minicolumn (official method 975.36 AACC-AOAC method)
detection limit 10 ppb	detection limit 5 ppb	detection limit 5-15 ppb
25 min	> 15 min	-
		
• 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 end of the micro column under UV light	• blue band in the interface of silica-Florisil® under UV light	• blue band at the top of Florisil® under UV light

Fluorescence in solution

Another fluorometric method to analyze 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 one study, a sodium bicarbonate solution-methanol mixture was used for the extraction followed by IAC (Chiavaro et al., 2002). Determination was performed using a xenon-lamp fluorometer from VICAM. The results corresponded well to a reference RP-HPLC-method.

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

Table 12: Strength and weakness of solution fluorometry.

	Solution fluorometry
Strength	rapid, easy handling, inexpensive, sensitive, multiple analysis
Weakness	derivatization, fresh derivatization solution every day, equipment, calibration of the system necessary

Labeling 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 labeling or derivatization strategies are summarized in Table 13.

Table 13: Labeling or derivatization-methods to improve the results of mycotoxin analysis.

Labeling/ derivatization	Contact point	Compound	Reference
1,2-diamino-4,5-dichlorbenze (DDB)	pre-column	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		Aflatoxins, DON, ZON	(Espinosa-Calderón et al., 2011; Francis et al., 1988; Galaverna et al., 2008)
aluminium chloride spraying on TLC-plate and heating	TLC plate	DON, Sterigmatocystin	(Egmond, 1986)
trifluoroacetic acid	pre-column	Aflatoxins	(Egmond, 1986; Espinosa-Calderón et al., 2011)
o-phthalaldehyde	pre-column	Fumonisin	(Shephard et al., 1990)

Derivatization with fluoresceine, 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 labeling not only increases the sensitivity of the test system but also shortens the clean-up procedures and the incubation-time (Lippolis et al., 2011). Other 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 is induced by what is known as KOBRA® cell. Jansen et al. (1987) show a 20-fold increase of the fluorescence intensity of aflatoxin B1 and G1 with post-column iodine derivatization.

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(a-CD), 7 (b-CD), 8 (g-CD) glucose units linked by a-1,4-glycosidic bonds. They are cone-shaped. 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 guest´s solubility, stability, physical and chemical properties. The inexpensive substance is widely used in pharmaceutical products (solubility, stability) and in the textile industry (masking odours) (Galaverna et al., 2008).

The forming of an inclusion complex between different mycotoxins and cyclodextrins leads to an enhancement of the native fluorescence of mycotoxins. This is described as result of the interaction of the coumarin structured mycotoxins and cyclodextrin. The inclusion results not only in changes of the polarity and intermolecular rotation but also in interaction with quenchers (Galaverna et al., 2008). Cucci et al. (2007) described a method to analyze aflatoxin M1 in milk with the use of b-CD. After cyclodextrin was added the detection limit of analysis were decreased from 25 ng/l to 5 ng/l. In addition there was no need to clean-up the samples with IAC before analysis (Cucci et al., 2007).

Maragos et al. (2008) investigate a fluorometric method to detect the non-fluorescence T-2 in maize. They derived 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)-b-cyclodextrin (DIMEB) (Maragos et al., 2008).

Specific fluorescence methods

Another fluorescence method to analyze mycotoxins is the fluorescence polarization immunoassay (FPI) as a rapid screening test. This method is based on the indirect measurement of the changes in molecule rotation of a solution as a function of the size of the molecules. It is one of the methods which are actually pretty much in use in research and it 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. The problem of cross-reactivity is not yet completely solved.

In principle, the fluorochrome labeled 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 in 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.

Table 14: Strength and weakness of fluorescence polarization immunoassay (FPI).

	FPI
Strength	• easy handling • portable
Weakness	• in research • expensive equipment

2.2.3 Other methods in research use

Laser-induced fluorescence (LIF)

Espinosa-Calderón et al. (2011) reviewed publications concerning the laser-induced fluorescence (LIF) as a fluorescence screening method for mycotoxins. The method is based on the detection of the analyte in the mobile phase while passing through the detection window of the LIF-detector. This method enables the analysis of samples with very low concentrations. Because of the high costs for the LIF (laser, special dyes for labeling) however, this method is not widely used.

Near infrared spectroscopy (NIR)

Another method used in practice and in research is the near infrared spectroscopy (NIR). Petterson and Aberg (2003) described the determination of deoxynivalenol in wheat kernel with a wavelength 570 – 1.100 nm and the detection limit was 400 ppt. NIR can be used for the determination of aflatoxins at levels between 200-500 ppb in sample with solid or liquid physical conditions. However, 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 enable 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 is transformed in a utilizable measured variable.

One biosensor method to determine mycotoxins is surface plasmon resonance (SPR) (Gaag et al., 2003; Schnerr et al., 2002; Tudos et al., 2003). Here the measured variable is the change in mass of mycotoxins which are immobilized at a surface of a sensor chip. The mass change results in the attachment of a specific antibody to the mycotoxins. Results have shown to be comparable to LC-MS and the sensor chip can be reused without loss of activity up to 500 times. Such SPR biosensor protocols are described by Puiu et al. (2012) for the direct measurement of albumin-bound AFB1 in blood samples.

DNA-based and aptamer-based biosensors

Dinckaya et al. (2011) published a DNA biosensor-based method to analyze aflatoxin M1 in samples such as in milk. A thiol-modified single stranded DNA (ss-HSDNA) probe was immobilized on a monolayer of cysteamine and gold nanoparticles prepared on gold electrodes. The DNA biosensor particularly 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 is aptamer-based. Aptamers are peptide molecules or DNA or RNA duplex structures that can bind a specific analyte. Chen et al. (2012) 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 highly selective method (only 1 min per measurement) OTA can be determined with a limit of detection of 0.8 ppb (Chen et al., 2012). A similar method, the biosensor DNA-enzyme aptamer was described by

Yang et al. (2012). 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-sensor-based assays are currently under investigation (Prabhakar et al., 2011; Wu et al., 2011).

Electronic nose

A new analytic approach based on biosensors are electronic noses that open a new field 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 detecting volatiles emanating, could distinguish between the presence and the absence of the mycotoxins. The fungal growth and the production of mycotoxins lead to biochemical changes resulting in 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 investigation of Cheli et al. (2009) showed that electronic noses can differentiate between aflatoxins -positive and aflatoxins–negative samples but further quantitative analysis are needed to evaluate the real potential as reliable method in the practical mycotoxins analysis (Cheli et al., 2009).

2.3 POST-ANALYSIS

Companies that market commercially available rapid screening assays usually also provide portable reader, fluorometer or fluorescence polarization reading instruments. Most of the readers allow for primary analysis with the possibility to exchange data or they are linked to PC with special software.

A new way of post-analysis is the use of smart phones for quantification of aflatoxins in the field. The app measures aflatoxins using a phone image of a color-changing test strip. The developer claims the measuring is more accurate than immunoassay tests. The results can be geo-tagged and uploaded to the internet. The cloud application allows secure data storage, information management and compliance reporting [www.mobileassay.com].

Figure 8: Quantification of aflatoxin in the field with a smart phone. Courtesy www.mobileassay.com

3. EXAMPLES FOR COMMERCIALLY AVAILABLE RAPID ANALYSIS TEST SYSTEMS

The following section summarizes some currently used screening test kits for the rapid quantitative analysis of mycotoxins. It does not aim to be comprehensive. Selection of test kits is based on test kits verified in performance by the Grain Inspection, Packers and Stockyards Administration (GIPSA) and the tests are selected based on the specific analytical principle. Test kits verified in performance by GIPSA are based on the analytical principle of ELISA or LFD. Other representative tests reported here are based on the analytical principle of liquid fluorometry or fluorescence polarization assay. All prices given are based on 2012 prices list; consider no volume or other reduction and are meant to serve as reference only. A complete list of currently approved test kits is 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

3.1 TEST KITS BASED ON ELISA OR LFD

3.1.1 Charm Sciences Inc.

(LFD) and enzyme linked immunoassay (ELISA).

Test kits are based on the analytical principle of lateral flow detection (LFD). The WET technology allows extraction from different commodities with a proprietary water based solution without organic solvents.

Table 15: Summarized facts about the rapid analysis test systems from Charm Sciences Inc..

Principle	LFD
equipment	EUR 2,995 (ROSA® EZ-M system)
cost per analysis/ consumables	EUR 7-10
Portability	No
Laboratory	Preparation: yes - Analytics: yes

Table 16: Overview test systems for various mycotoxins from Charm Sciences Inc..

Analyte	Matrix	Method	extraction buffer	Range	[EUR] /100 pcs.
ROSA WET® Aflatoxin Quantitative Test (USDA(GIPSA)-approved)	barley, corn, corn flour, corn germ meal, corn gluten meal, corn me al, corn/ soy blend, Distiller's Dried Grain with Solubles (DDGS), hominy, oats, popcorn, rice bran (defatted), rough rice, sorghum, soybeans, wheat	LFD	Water	Not provided	765
FAST Aflatoxin Quantitative	Corn	LFD	70% methanol	0 – 150 ppb	
BEST Aflatoxin Qualitative	Corn	LFD	non-toxic solution called BEST (composition due to IP not disclosed)	10 ppb, 20 ppb	
Aflatoxin P/N Test Qualitative	Corn	LFD	50% methanol or 70% ethanol	10 ppb, 20 ppb	
ROSA DON P/N Test Quantitative	barley, corn, wheat	LFD	deionized or distilled water	0.5 ppm, 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
ROSA FAST5 Fumonisin Quantitative	barley, corn, flaking corn grits, millet, oats, rough rice, sorghum, wheat	LFD	70% methanol	0 – 6 ppm	
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	
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

3.1.2 EnviroLogix Inc.

Test kits are based on the analytical principle of lateral flow detection (LFD).

Table 17: 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 available
cost per analysis/ consumables	Not available
Portability	No
Laboratory	Preparation: yes - Analytics: yes

Table 18: 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	0.2 – 20 ppm	50% ethanol
Ochratoxin A			
Quantitative	Wheat	0 – 150 ppb	water based

3.1.3 Neogen Corporation

The analytical principle is based on LFD or ELISA. Depending on the mode of detection, visual or reader, results are qualitative or quantitative, respectively.

Table 19: 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

Table 20: Overview test systems from Neogen Corporation for various mycotoxins

Analyte	Method/ time	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, 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, 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, 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, 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, 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, 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, malted barley, oats, wheat products
Fumonisin			
Veratox® Fumonisin *Quantitative*	ELISA/20 min	1 – 6 ppm	corn, barley, DDGS, milo, popcorn, rice, soybeans, wheat
Veratox® Fumonisin 5/10 *quantitative*	ELISA/15 min	0.5 – 6 ppm	corn, barley, DDGS, milo, popcorn, rice, soybeans, wheat
Veratox® Fumonisin HS *Quantitative*	ELISA/15 min	50 - 600 ppb	corn, barley, DDGS, milo, popcorn, rice, soybeans, 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® Ochratoxin Grain *Quantitative*	ELISA/20 min	2 – 25 ppb	corn, barley, other grains
Reveal® Q+ Ochratoxin *quantitative*	LFD/9 min	2 – 20 ppb	grain, 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, 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, wheat products

3.1.4 R-Biopharm AG

Test kits are based on the analytical principle of lateral flow detection (LFD) and enzyme linked immunoassay (ELISA).

Table 21: 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

Table 22: Overview test systems for various mycotoxins from R-Biopharm AG.

Analyte	Method/time	Range	Matrix
Aflatoxin			
AFLACARD®B1 *Qualitative*	LFD/card/10 min	not provided	a wide range of commodities
AFLACARD®total *Qualitative*	LFD/card/10 min	not provided	a wide range of commodities
RIDA®Quick Aflatoxin *quantitative*	LFD	4 – 20 ppb	grain, soy flour, nuts, pistachios, coconut flour, sunflower seeds, figs, dates, 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, oats
RIDASCREEN®FAST DON SC *Quantitative*	ELISA/ 8 min	0.074 ppm	cereals, malt, 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, wort
RIDA®Quick DON *semi-quantitative or quantitative*	LFD/5 min	0.5 ppm, 1.25 ppm	wheat, triticale, 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	not provided	a wide range of commodities
RIDASCREEN® Ochratoxin A 30/15 *Quantitative*	ELISA/5 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, feed
RIDASCREEN®FAST T-2 *Quantitative*	ELISA/15 min	20 ppb	cereals, feed
ZON			
RIDASCREEN®FAST ZON *Quantitative*	ELISA/15 min	17 – 41 ppb	cereal, feed
RIDASCREEN®FAST ZON SC *Quantitative*	ELISA/15 min	5 ppb	Cereals
RIDA®Quick ZON RQS *Quantitative*	LFD/5 min	75 ppb	Corn

3.1.5 Romer Labs®

The test systems are based different analytical principles. Test kits are based on the analytical principle of lateral flow detection (LFD) and enzyme linked immunoassay (ELISA) tests, which are also based on fluorometry.

Table 23: Summarized facts about the rapid analysis test systems from Romer Labs®.

	www.romerlabs.com/en/products/mycotoxins/ in Germany: coring system diagnostics (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

Table 24: Overview ELISA test systems for various mycotoxins from Romer Labs®.

Analyte	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

Table 25: Overview Lateral Flow test systems for various mycotoxins from Romer Labs®.

Analyte	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

Table 26: Overview fluorometric test systems for various mycotoxins from Romer Labs®.

Analyte	Detection limit [ppb]	extraction buffer
Aflatoxin		
FluoroQuant®Afla	3	Not specified
FluoroQuant®Afla Plus	0.5	Acetonitrile
FluoroQuant®Afla Plus	0.6	Methanol
FluoroQuant®Afla IAC (US domestic)	3	Not specified
FluoroQuant®Afla IAC (International)	3	Not specified

3.1.6 VICAM

Test kits are using two different analytical principles such as LFD and fluorometry.

Table 27: 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

The fluorometric test kit is a simple and rapid quantitative method. Sensitive results are obtained within 10 minutes (excluding preparation and extraction).

Table 28: Overview fluorometric test systems for various mycotoxins from VICAM.

Analyte	range	extraction buffer
AflaTest (AFB1, AFB2, AFG1, AFG2 and AFM1) (feeds, food, grains, nuts, dairy product)	0.1 – 300 ppb	salt and methanol/water
AflaB (AFB1, AFB2, AFG1, AFG2 and AFM1)	1 – 300 ppb	salt and methanol/water
Afla M1FL+ (for milk)	12.5 - 200 ppt	
FumoniTest™ (Fumonisin B1, B2 and B3)	0.016 – 10 ppm	salt and methanol/water
OchraTest™ (Ochratoxin A)	0.1 – 100 ppb	methanol/water
ZearalaTest	0.1 – 5 ppm	salt and methanol/ water

Test kits are based on the analytical principle of lateral flow detection (LFD), which give results in or less than 5 minutes (excluding preparation and extraction).

Table 29: 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 ppb	ethanol 70%
DON-V	grain, feed	0 – 5 ppm	water
Fumo-V (Fumonisin)		0 – 5 ppm	ethanol 70%

Additionally, qualitative Tests are available that are a one-step test kit as first step in detection of contaminations. Visual results are obtained in less than 3 minutes (excluding preparation and extraction), non-specialized operators are needed. Aflatoxin (Afla-Check) are detected in the range of 10 to 20 ppb, DON (DON-Check) at 1 ppm. Samples need preparation and extraction prior to analysis.

3.2 TEST KITS BASED ON FLUORESCENCE POLARIZATION IMMUNOASSAYS

3.2.1 Aokin AG

Analysis is based on sample preparation using IAC or SPE (QuickClean) and quantification is based on the analytical principle of fluorescence polarization immunoassay (FPI).

Table 30: 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	Preparation: yes - Analytics: yes

Table 31: Overview test systems for various mycotoxins from Aokin AG.

Analyte	Matrix	Detection limit ppb	range ppb
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

3.2.2 Diachemix Inc.

Table 32: 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 33: Overview test systems fluorescence polarization assay, FPA) for various mycotoxins from Diachemix Inc..

Analyte	Method/time per analysis	Range	Matrix
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 (detection limit 0.17 ppm)	Wheat
Fumonisin FPA qualitative test	FPA 1-2 min	≥ 1 ppm	Corn

3.3 TEST KITS BASED ON FLUOROMETRY

3.3.1 ToxiMet Ltd

The system enables the rapid testing for aflatoxins and ochratoxin. It is sensitive and allows the simultaneous measuring and identification of toxins. The analyte is immobilized on a disposable cartridge and excited with UV light. Fluorescence is detected by a spectrometer to quantify the different mycotoxins. The test is available for aflatoxins (B1, B2, G1, G2, total), and ochratoxin A

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

	http://www.toximet.com/
Principle	Fluorometry
equipment	EUR 7,000 - 22,500 (ToxiQuant)
cost per sample/ consumables	EUR 23
Portability	No
Laboratory	Preparation: yes - Analytics: yes

4. COMPARISON BETWEEN SELECTED IMPORTANT METHODS

Comparison between different analytical methods has to consider the important aspects related to the test principle itself and the conditions in which the system is applied. The general most important question is whether the test is fit for purpose. Do the results need to be quantitative or is qualitative enough? Is an appropriate laboratory environment available to run complex, equipment intensive and operator sensitive test systems? Aspects that help to make such decisions are given in Table 35.

Table 35: Comparison between selected important methods.

Method	HPLC or LC-MS/MS	ELISA	LFD/Dipstick	Fluorescence Polarization Immunoassay (FPI)
	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 color 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	8 - 12 h	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	depending on visualization method	26,000 – 27,000
costs/ sample [EUR]	50 – 180	15	22 – 27	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, trained scientific staff, 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

INFORMATION:

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 SAFETY:

http://www.codexalimentarius.org/
http://www.efsa.europa.eu/en/topics/topic/mycotoxins.htm

ORGANIZATIONS:

http://www.aaccnet.org/Pages/default.aspx
http://www.acs.org/content/acs/en.html
http://aflatoxinpartnership.org
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.

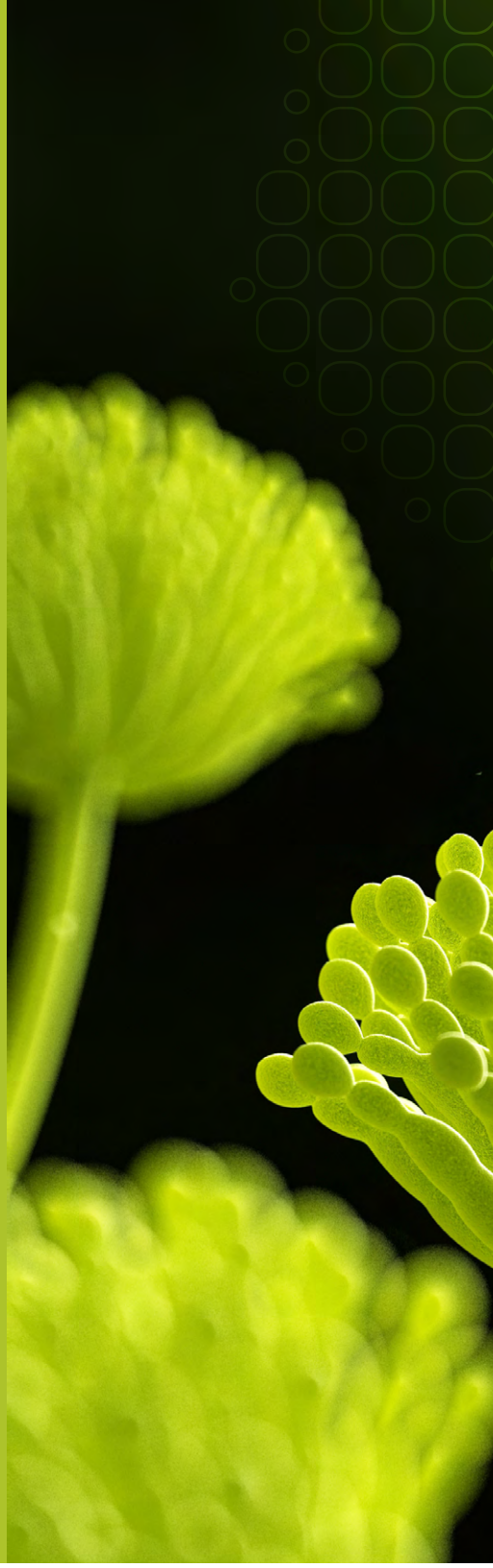
EU-legislation concerning mycotoxins	Compounds
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20100701:EN:PDF	• maximum levels for DON, Afla, ZON, Fumonisin B1, - B2, Ochratoxin A, T2-HT2
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0032:20100302:EN:PDF	• 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

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 ethyl ester	431	Benzene:acetic acid (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



The Partnership for Aflatoxin Control in Africa (PACA) is a collaboration which aims to protect crops, livestock, and people from the effects of aflatoxins. By combating these toxins, PACA will contribute to improving food security, health, and trade across the African continent.

www.aflatoxinpartnership.org

PACA Partnership for Aflatoxin Control in Africa