MYCOTOXIN HANDBOOK

Why test for mycotoxins?

NEOGEN®

CONTENTS

Q&A

MYCOTOXINS

What are mycotoxins?

Mycotoxins are toxins produced by organisms categorized as fungi, including mushrooms, yeasts and molds. Fungi of one species or another, or their spores, can be found virtually everywhere. When the growth conditions are right for specific fungi, they will grow very rapidly into colonies, and produce toxins specific to that fungus as a by-product. Growth conditions, which include temperature, humidity, and available organic food sources, can not only affect whether or not a specific fungus will grow, but also the characteristics of the mycotoxin that it may produce.

Mycotoxins can be produced wherever fungi growth conditions exist, for example, in grains preharvest in the field and postharvest in storage. In either case, damage from insects, mishandling and environmental stress can enable the fungi to invade the grains' seeds.

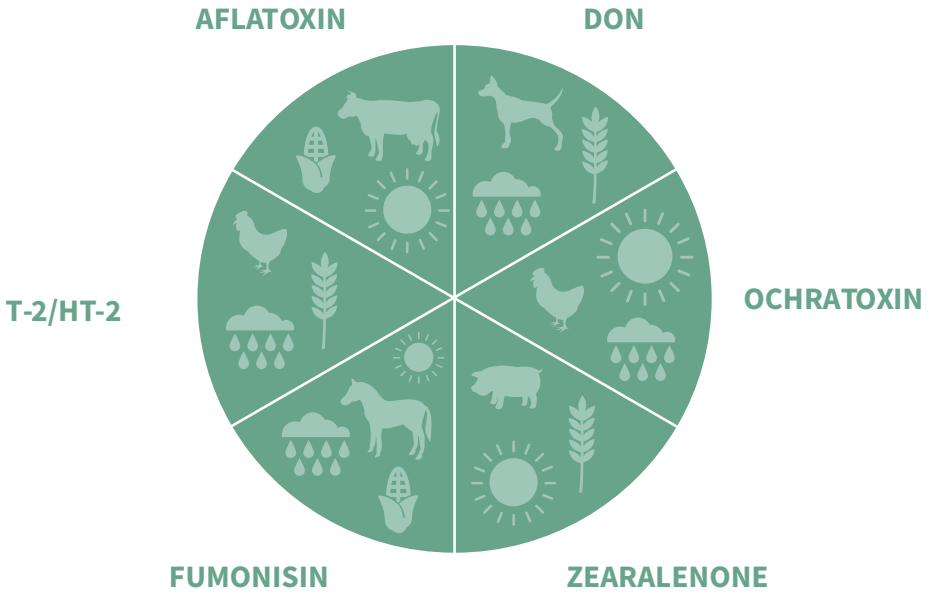
Are mycotoxins harmful?

As their name implies, mycotoxins are generally considered toxic, although not all mycotoxins are demonstrably toxic to every animal that may ingest them. Some mycotoxins, such as aflatoxin, have been shown to be dangerous to both humans and animals, others dangerous to only specific animal species, and still others, such as penicillin, only lethal to other fungi and bacteria.

Extensive mold growth in grains can have other obvious negative effects, such as producing changes in the grains' color, consistency, and smell—which may make the grains undesirable to livestock and as a human food. Mold growth can also rob grains of their fat, protein, and vitamin content, and lead to nutritional deficiencies in livestock.

How many mycotoxins are there?

Researchers have identified thousands of mycotoxins thus far, and continually identify new mycotoxins. Subtypes of numerous mycotoxins have also been identified. Within the identified mycotoxins and their subtypes, a relative few have been determined to pose a significant threat to the health of humans and animals. Those that have been proven to threaten health include aflatoxin, deoxynivalenol (a.k.a., DON or vomitoxin), fumonisin, ochratoxin, T-2/HT-2 toxins, and zearalenone.



Can mycotoxins be killed or otherwise neutralized?

Unlike the fungi that produces them, mycotoxins are chemical substances that are not alive, and cannot be killed. The only known treatment to reduce aflatoxin levels, for example, is ammoniation, which leaves the kernels black and smelling like ammonia. There are no proven treatments to both neutralize a mycotoxin and preserve the integrity of the contaminated commodity. Likewise, extreme heat and freezing do not destroy mycotoxins.

Mycotoxins have also been shown to be resistant to breakdown in an animal's digestive system—meaning that they can be passed along in meat and dairy products.

Commodities known to contain a harmful level of a certain mycotoxin are diverted away from use in products destined to be consumed by animals known to be especially sensitive to that mycotoxin. For example, corn products known to contain harmful amounts of fumonisin, a mycotoxin of special concern to horses and rabbits, would be diverted away from use in horse and rabbit feed.

Do black lights work to detect aflatoxin in corn?

Studies have shown that using black light to detect aflatoxin in corn produces unreliable results. The bright yellow green fluorescence that a black light can produce detects the presence of kojic acid, not aflatoxin. Kojic acid is one of many by-products of *Aspergillus flavus*, one of the two major producers of aflatoxin. But, *Aspergillus flavus* can produce aflatoxin without producing kojic acid, and it can produce kojic acid without producing aflatoxin. In addition, kojic acid can dissipate over time, thus a sample that once "glowed" may not at a later time.

Additionally, another producer of aflatoxin, Aspergillus parasiticus, does not produce kojic acid at all. So while a black light procedure can seem to detect corn contaminated with aflatoxin at times, the procedure is an unreliable indicator for the presence of aflatoxin.

How fine must a sample be ground before testing?

The Federal Grain Inspection Service (FGIS) recommends grinding corn so that 95% passes through a 20 mesh screen, which is about the consistency of fine ground coffee. The sample's particle size is extremely important to subsequent test results. One kernel of corn can hold a very high amount of toxin. Unless kernels are ground and distributed evenly throughout the sample to be tested, variable and inaccurate results can occur. Proper cleaning of equipment between sampling is recommended to prevent cross contamination.

WHAT IS PPM?

"One part per million" is a lot to think about. Here are some facts that put 1 ppm into perspective.

- There are approximately 13,960 kernels of wheat in 1 pound. One kernel in 71 pounds is equal to 1 ppm.
- There are approximately 3,500,000–4,000,000 grains of sand per pound. If you take 4 grains out of the pound you have removed 1 ppm.
- "One part per billion" is 1,000 times smaller than 1 ppm. For example, one second in 32 years is 1 ppb.

MAJOR MYCOTOXINS

The amount of mycotoxin required to produce adverse effects in humans and animals varies by the mycotoxin, and can even vary from animal to animal of the same species. The amount of risk posed by mycotoxins is a combination of the level of contamination of a given commodity, and the total amount of mycotoxins ingested by a specific animal.

AFLATOXIN

Aflatoxin is a toxic and carcinogenic substance produced by certain strains of the molds *Aspergillus flavus* and *A. parasiticus*. There are four principle types of aflatoxin: B1, B2, G1 and G2 in grains. Aflatoxin B1 is the most frequently encountered of the group and the most toxic. The commodities most affected by aflatoxin are corn, peanuts, cottonseed, milo, and the majority of tree nuts. The effects in animals of ingesting excessive amounts of the toxin range from chronic health and performance problems to death. Aflatoxin has been shown to cause liver damage or cancer, decreased milk and egg production, immune suppression and interference with reproductive efficiency.

Regulatory limits for aflatoxin are issued by regional authorities:

Food				
		Maximum Level		
Region	Commodity	B1	Total	M1
US	All foods except milk		20 ppb	
	Milk			0.5 ppb

Food				
		Maximum Level		
Region	Commodity	B1	Total	M1
EU	Cereals, processed	2 ppb	4 ppb	
	Dried fruits to be processed	5 ppb	10 ppb	
	Groundnuts and nuts to be processed	8 ppb	15 ppb	
	Groundnuts, nuts, and dried fruit for direct human consumption	2 ppb	4 ppb	
	Maize to be processed	5 ppb	10 ppb	
	Spices	5 ppb	10 ppb	
	Almonds, pistachios, and apricots for direct human consumption	8 ppb	10 ppb	
	Almonds, pistachios, and apricots to be processed	12 ppb	15 ppb	
	Dietary foods for special medical purposes intended specifically for infants	0.10 ppb		
	Dried fruit for direct human consumption	2 ppb	4 ppb	
	Hazelnuts and Brazil nuts for direct human consumption	5 ppb	10 ppb	
	Hazelnuts and Brazil nuts to be processed	8 ppb	15 ppb	
	Infant formula and follow-on formula, including infant milk and follow-on milk			0.025 ppb
	Processed cereal-based foods and baby foods for infants and young children	0.10 ppb		
	Raw milk, heat-treated milk and milk for the manufacture of milk-based products	2 ppb	4 ppb	0.05 ppb
	Tree nuts for direct human consumption	5 ppb	10 ppb	
Japan	All food		10 ppb	
	Milk			0.5 ppb
Indonesia	Corn and its products	15 ppb	20 ppb	
	Spices	15 ppb	20 ppb	
	Dairy products			0.5 ppb
	Dried milk and related products			5 ppb
Korea	Grain, beans, peanut, nuts and their processed food (grinding, cutting, etc)	10 ppb	15 ppb	
	Processed cereal products and processed bean product	10 ppb	15 ppb	
	Nutmeg, tumeric, dried red pepper, dried paprika and spice products containing these	10 ppb	15 ppb	
	Wheat flour, dried fruits	10 ppb	15 ppb	
	Confectionaries (peanut or nut-containing food)	10 ppb	15 ppb	
	Processed corn products for popcorn	10 ppb	15 ppb	
	Soybean paste, red pepper paste, curry powder	10 ppb	15 ppb	
	Meju	10 ppb	15 ppb	
	Steamed rice	10 ppb	15 ppb	
	Baby foods for infants and young children	0.1 ppb		
	Raw milk and milk before processing			0.5 ppb

Malaysia	Groundnuts, almonds, hazelnuts, pistachios, Brazil nuts (shelled,for further processing)		15 ppb	
	Groundnuts, almonds, hazelnuts, pistachios, Brazil nuts (shelled, ready to eat)		10 ppb	
	Cereal based food for infants and children	0.1 ppb		
	Milk			0.5 ppb
	Infant formula and follow-up formula (ready to drink)			0.025 ppb

Feed			
		Maximum Level	
Region	Commodity	B1	Total
US	Corn and peanut products intended for finishing beef cattle		300 ppb
	Cottonseed meal intended for beef cattle, swine, or poultry		300 ppb
	Corn and peanut products intended for finishing swine of 100 lbs or greater		200 ppb
	Corn and peanut products intended for breeding beef catte, breeding swine, or mature poultry		100 ppb
	Corn, peanut products and other animal feeds and feed ingredients, excluding cottonseed meal, intended for immature animals		20 ppb
	Corn, corn products, cottonseed meal, and other animal feed and feed ingredients		20 ppb
EU	All feed materials	20 ppb	
	Complementary and complete feed	10 ppb	
	Compound feed for dairy cattle and calves, dairy sheep and lambs, dairy goats and kids and young poutry animals	5 ppb	
	Compound feed for cattle, sheep, goats, pigs and poultry not listed above	20 ppb	
Japan	Corn	20 ppb	
	Formula feed for cattle, pig, domestic fowl, quails	20 ppb	
	Formula feed for suckling perion	20 ppb	
	Formula feed for dairy cattle	10 ppb	

Feed			
		Maximum Level	
Region	Commodity	B1	Total
China	Corn, peanut meal, cottonseed meal, rapeseed meal	50 ppb	
	Soybeen meal	30 ppb	
	Complementary, complete and concentrated feeding stuff for piglets	10 ppb	
	Complementary, complete and concentrated feeding stuff for fattening pigs	20 ppb	
	Complementary, complete and concentrated feeding stuff for young broilers, chicks	10 ppb	
	Complementary, complete and concentrated feeding stuff for broilers, layers	20 ppb	
	Complementary, complete and concentrated feeding stuff for young ducks, ducklings	10 ppb	
	Complementary, complete and concentrated feeding stuff for ducks, layers	15 ppb	
	Complementary, complete and concentrated feeding stuff for quails	20 ppb	
	Supplementary feeding stuffs for dairy cattle	10 ppb	
	Supplementary feeding stuffs for beef cattle	50 ppb	
Indonesia	Feed and corn (final products)		50 ppb
	Feed for layer, broiler, and pigs		50 ppb
	Feed for quails		40 ppb
	Feed for ducks		20 ppb

DON

Deoxynivalenol (DON) is most commonly produced by the pink mold *Fusarium graminearum*. DON, a member of the trichothecene family, is produced by fungi living on cereal commodities such as wheat, corn, barley and ensilages. The toxicological effects attributed to DON include: nausea (vomiting), feed refusal, gastroenteritis, diarrhea, immunosuppression and blood disorders.

Regulatory limits for DON are issued by regional authorities:

Food		
Region	Commodity	Maximum Level
US	Finished wheat products for consumption by humans	1,000 ppb
Canada	Uncleaned soft wheat for use in non-staple foods	2.0 mg/kg
	Uncleaned soft wheat for use in baby foods	1.0 mg/kg

Food		
Region	**Commodity**	**Maximum Level**
EU	Bread, pastries, biscuits, cereal snacks and breakfast cereals	500 ppb
	Cereals for human consumption - cereal flour, bran, and germ	750 ppb
	Pasta (dried)	750 ppb
	Milling fractions of maize with particle size > 500 micron	750 ppb
	Milling fractions of maize with particle size < 500 micron	1,250 ppb
	Processed cereal-based food for babies	200 ppb
	Unprocessed cereals other than wheat durum, oats and maize	1,250 ppb
	Unprocessed durum wheat, oats, and maize not intended for wet milling	1,750 ppb
Japan	Wheat and wheat products	1,100 ppb (tentative)
China	Wheat and wheat products	1,000 ppb
	Corn and corn products	1,000 ppb
	Barley and barley products	1,000 ppb
Indonesia	Corn and its products	1,000 ppb
	Wheat and its products	1,000 ppb
	Wheat flour and its products (pastry, bakery, biscuits, snacks)	500 ppb
	Pasta and noodles	750 ppb
	Wheat based breast-milk substitute products	200 ppb

Feed		
Region	**Commodity**	**Maximum Level**
US	Grains and grain by-products for ruminating beef and feedlot cattle older than 4 months, chickens	10 ppm
	Grain and grain by-products for swine	5 ppm
	Grain and grain by-products for other animals	5 ppm
EU	Cereals and cereal products with the exception of maize by-products	8 ppm
	Maize by-products	12 ppm
	Complementary and complete feeding stuffs	5 ppm
	Complementary and complete feeding stuffs for pigs	0.9 ppm
	Complementary and complete feeding stuffs for calves (<4 months), lambs, and kids	2 ppm
Japan	Formula feed (cows over 3 months after birth)	4,000 ppm
	Formula feed (except for cows over 3 months after birth)	1,000 ppm
China	Complementary and complete feeding stuffs for swine	<1 ppm
	Complementary and complete feeding stuffs for calves	<1 ppm
	Complementary and complete feeding stuffs for lactating animals	<1 ppm
	Complementary and complete feeding stuffs for cattle	< 5 ppm
	Complementary and complete feeding stuffs for poultry	< 5 ppm

ERGOT ALKALOIDS

Ergot alkaloids are highly toxic secondary metabolites of the fungi from the *Claviceps* genus, which commonly infect cereals and pasture grasses, particularly rye and wheat. The most common species of this fungus is *Claviceps purpurea*, which affects crops during prolonged cool, cloudy, and wet weather during the flowering period. The fungi replace the developing ovaries of the seed with hard masses of fungal tissue called sclerotia (or ergots). The sclerotia are brown to purple-black and contain the ergot alkaloids.

The sclerotia are harvested with the grain and contaminate grain and animal feed. The sclerotia are visible to the naked eye. However, they may be difficult to detect if they were fragmented or ground up during the grain processing. Ergot is not a storage issue but can be present in stored grains resulting from harvesting infected grain.

Ingestion of ergots in grain and flour can cause illness or death in humans and animals. Diseases caused by ingestion of ergot-contaminated feed or food have been called various names, including ergotism, ergot poisoning, ergot toxicosis, and St. Anthony's fire. There are four forms of ergot toxicosis in animals: gangrenous or cutaneous, hyperthermic, reproductive, and convulsive.

The six ergot alkaloids studied (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, and ergocomine) have been defined by the European Food Safety Authority (EFSA) as being of major importance to human and animal health.

Due to their high toxicity, EU regulations are expected to be published on the maximum limits of these toxins.

FUMONISINS

Discovered in 1989, fumonisins are a family of mycotoxins produced by different species of the mold *Fusarium*. These molds commonly infect corn (in fact, they are considered ubiquitous in corn) and rice, hence the potential for fumonisins to be found in feed and foodstuffs is high. Fumonisins affect various animals differently and have been linked to esophageal cancer in humans. The Environmental Protection Agency classifies fumonisins as Category II-B carcinogens.

Horses are extremely sensitive to low amounts of fumonisin, which can cause leukoencephalomalcia (liquefaction of the brain). In swine, research has shown fumonisin attacks the cardiopulmonary system causing pulmonary edema, as well as liver and pancreatic lesions.

Regulatory limits for fumonisin are issued by regional authorities:

Food			
Region	Commodity	Guidance Level (B1,B2,B3)	Maximum Level (B1 + B2)
US	Degermed dry milled corn products (i.e. flaking grits, corn meal, corn flour)	2,000 ppb	
	Cleaned corn used for popcorn	3,000 ppb	
	Whole or partially degermed dry milled corn products; dry milled corn bran; cleaned corn used for mass production	4,000 ppb	
EU	Milling fractions of maize with particle size > 500 micron		1,400 ppb
	Milling fractions of maize with particle size < 500 micron		2,000 ppb
	Maize intended for human consumption		1,000 ppb
	Maize snacks, maize based breakfast cereals		800 ppb
	Processed maize-based foods for babies		200 ppb
	Unprocessed maize not intended for wet milling		4,000 ppb
Indonesia	Corn and corn as a raw material	2,000 ppb	
	Corn food products (flakes, popcorn, corn chips)	1,000 ppb	
Korea	Grain products & cereals (containing >50% corn, corn processed products, corn powder)	1,000 ppb	
	Processed corn products for popcorn	1,000 ppb	
	Confectioneries (containing > 50% corn)	1,000 ppb	
	Corn	4,000 ppb	
	Corn processed food (grinding,cutting etc) corn powder	2,000 ppb	

Feed			
Region	Commodity	Guidance Level (B1,B2,B3)	Maximum Level (B1 +B2)
US	Corn and corn by-products for equids and rabbits	5 ppm	
	Corn and corn by-products for swine and catfish	20 ppm	
	Corn and corn by-products for breeding ruminants, breeding poutry and breeding mink	30 ppm	
	Poultry raised for slaughter	100 ppm	
	All other species of livestock and pet animals	10 ppm	
EU	Maize and maize-based products		60 ppm
	Complementary and complete feeding stuffs for pigs, horses, rabbits, and pet animals		5 ppm
	Complementary and complete feeding stuffs for fish		10 ppm
	Complementary and complete feeding stuffs for poultry, calves (<4 months), lambs and kids		20 ppm
	Complementary and complete feeding stuffs for adult ruminants (>4 months) and mink		50 ppm

OCHRATOXIN

Ochratoxin, commonly produced by the molds *Aspergillus ochraceus* and *Penicillium viridicatum*, can be found in corn, barley, green coffee and various dried fruits. Ochratoxin may be present in conjunction with aflatoxin, one of the most potent naturally occurring carcinogens. In fact, ochratoxin is a suspected carcinogen.

Ochratoxin affects kidneys in animals exposed to naturally occurring levels of this mycotoxin. Turkeys and other poultry exhibited lower productivity levels during field outbreaks of ochratoxicosis. Symptoms included retarded growth and decreased feed conversion. It has also been known to affect egg production in laying hens.

Although there has been no advisory or regulatory level for ochratoxin issued by the Food and Drug Administration, many agree that levels between 10–20 parts per billion (ppb) for commodities destined for human or animal consumption may cause health problems and economic losses. Some international markets have set regulation limits ranging from 5 to 50 ppb.

Regulatory limits for ochratoxin are issued by regional authorities:

Food		
Region	**Commodity**	**Maximum Level**
EU	Dried vine fruits	10 ppb
	Processed cereals and cereal products	3 ppb
	Roasted coffee beans	5 ppb
	Soluble coffee	10 ppb
	Unprocessed cereals	5 ppb
	Wine, grape juice and grape must	2 ppb
	Dietary foods for special medical purposes intended specifically for infants	0.50 ppb
	Licorice	20 ppb
	Licorice for use in food	80 ppb
	Nutmeg, ginger, tumeric	15 ppb
	Processed cereal-based foods and baby foods for infants and young children	0.50 ppb
	Spices - capsicum, pepper	30 ppb
Malaysia	Cereal-based food for infants and children	0.5 ppb
Korea	Coffee or ground coffee or coffee powder	5 ppb
	Instant coffee or soluble coffee, decaffeinated coffee	10 ppb
China	Grains, beans, and their products	5 ppb
Indonesia	Cereal (rice,corn,sorghum,wheat) and their products	5 ppb
	Spices	20 ppb
	Coffee	5 ppb
	Instant coffee	10 ppb
	Dried raisins	10 ppb
	Grape juice	2 ppb
	Beer	0.2 ppb
	Cereal grain based breast-milk substitute products	0.5 ppb

Feed		
Region	**Commodity**	**Maximum Level**
EU	Cereal and cereal products	0.25 ppm
	Complementary and complete feeding stuffs for pigs	0.05 ppm
	Complementary and complete feeding stuffs for poultry	0.1 ppm
China	Complementary and complete feeding stuffs, corn	< 100 ppb

T-2/HT-2 TOXINS

T-2/HT-2 toxins are trichothecene mycotoxins produced by several species of *Fusarium* molds. As T-2 toxin is readily metabolized to HT-2 toxin, and the toxins have been shown to produce numerous adverse effects on many animals, these two mycotoxins are frequently evaluated together.

Animals affected by the toxins include swine, dairy cattle, poultry, dogs, cats and horses. Effects of the toxins include digestive disorders, hemorrhaging, edema, oral lesions, dermatitis, and blood disorders. Damage caused by the toxins to the digestive track is irreversible. In the most severe cases, these toxins will cause death. T-2 toxin is the principal causal toxin in the human disease alimentary toxic aleukia.

Poultry studies have shown T-2 intoxication has led to a reduction in weight gain and other problems such as beak lesions, poor feathering, motor function impairment and increased susceptibility to *Salmonella* spp.

The best protection against these mycotoxins is monitoring for their presence in feeds and foods. That means testing all along the pathway from initial harvest of grains to the finished product.

Regulatory limits for T-2/HT-2 are issued by regional authorities:

Food		
Region	**Commodity**	**Maximum Level**
EU	Unprocessed cereals: Barley (including malting barley)	200 ppb
	Oats (with husk)	1,000 ppb
	Wheat, rye and other cereals	100 ppb
	Cereal grains for direct human consumption: Oats	200 ppb
	Maize	100 ppb
	Other cereals	50 ppb
	Cereal grains for human consumption: Oat bran and flaked oats	200 ppb
	Cereal bran except oat bran, oat milling products other than oat bran and flaked oats, and maize milling products	100 ppb
	Other cereal milling products	50 ppb
	Breakfast cereals including formed cereal flakes	75 ppb
	Bread, pastries, biscuits, cereal snacks, pasta	25 ppb
	Cereal based foods for infants and young childred	15 ppb

Food		
Region	Commodity	Maximum Level
EU	Oat milling products (husks)	2,000 ppb
	Other cereal products	500 ppb
	Compound feed, with the exception of feed for cats	250 ppb
	Compound feed for cats	50 ppb
China	Complementary and complete feeding stuff for swine	<1 ppm
	Complementary and complete feeding stuff for poultry	<1 ppm

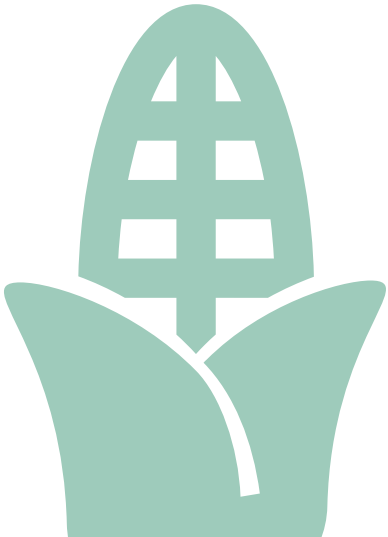
ZEARALENONE

Zearalenone is primarily produced by the mold *Fusarium graminearum*, which also commonly produces DON. Hence, there is evidence that if zearalenone is detected, there is a high probability that other fusarial mycotoxins may be present. Zearalenone is classified as an estrogenic mycotoxin because it frequently causes estrogenic responses in animals. When zearalenone-contaminated feed or grain is eaten by livestock, it can cause a wide variety of reproductive problems. In swine, it causes vulvovaginitis, low birth weights, fetal reabsorption, aborted pregnancies, reduced litter sizes, abnormal estrus and feminization of immature males. The FDA has issued advisory levels for zearalenone at <500 ppb.

Regulatory limits for zearalenone are issued by regional authorities:

Food		
Region	Commodity	Maximum Level
EU	Bread, pastries and biscuits	50 ppb
	Cereals for human consumption - cereal flour, bran and germ	75 ppb
	Refined maize oil	400 ppb
	Milling fractions of maize with particle size > 500 micron	200 ppb
	Milling fractions of maize with partical size < 500 micron	300 ppb
	Processed cereal and maize-based food for babies	20 ppb
	Unprocessed cereals other than maize	100 ppb
	Unprocessed maize not intended for wet milling	350 ppb
	Maize intended for human consumption, maize snacks, maize based breakfast cereals	100 ppb
Korea	Grain and their process foods (grinding,cutting etc)	200 ppb
	Confectionaries	50 ppb
	Baby food for infants and young children	20 ppb
China	Wheat, corn and their products	60 ppb

Food		
Region	Commodity	Maximum Level
EU	Cereal and cereal products with the exception of maize by-products	2 ppm
	Maize by-products	3 ppm
	Complementary and complete feeding stuffs for piglets, young sows, puppies, kittens, dogs and cats for reproduction	0.1 ppm
	Complementary and complete feeding stuffs for adult dogs and cats other than for reproduction	0.2 ppm
	Complementary and complete feeding stuffs for sows and fattening pigs	0.25 ppm
	Complementary and complete feeding stuffs for calves, dairy cattle, sheep and goats	0.5 ppm
Japan	Formula feed	1000 ppb
China	Complementary and complete feeding stuffs, corn	< 500 ppb



TECHNICAL INFORMATION

CONFIRMATION METHODS

High performance liquid chromatography (HPLC) is the preferred instrument based confirmation method formycotoxins. Testing requires a skilled technician, a validated test method, and appropriate equipment. Gas chromatography (GC) and thin layer chromatography (TLC) are also popular confirmation methods.

COMMODITY VALIDATION LISTS

NEOGEN's test kits have been validated on a variety of commodities from the most susceptible to the most obscure based on customer requests. Please contact a NEOGEN representative for the most up-to-date list.

pH ADJUSTMENT PROCEDURE

Commodities to be tested should have a pH of 6.0–8.0. Most raw or unprocessed grains, such as corn or wheat, have a pH between 6.0–8.0 and will not need to be adjusted. To ensure the accuracy of subsequent testing, excessively acidic or alkaline samples should be adjusted using this method:

1. Grind and extract sample per the test kit's written instructions.
2. Filter 5 mL into a clean test tube.
3. Check pH with pH paper or meter.

If acidic (pH is below 6): Adjust the pH with 1N NaOH (sodium hydroxide) to 6.0–8.0. Add one drop of 1N NaOH to the sample extract, vortex or swirl to mix and re-check the pH. If still acidic add another drop and check pH. Continue until the pH is 6.0–8.0

If alkaline (pH is above 8): Adjust the pH with 1N HCl (hydrochloric acid) to 6.0–8.0. Add one drop of 1N HCl to the sample extract, vortex or swirl to mix and re-check the pH. If still alkaline add another drop and check pH. Continue until the pH is 6.0–8.0

4. The sample extract is now ready to test.

SCREENING VS. QUANTIFYING RESULTS

NEOGEN's rapid tests for the detection of mycotoxins are available in multiple formats. NEOGEN's Agri-Screen®,Reveal, and Reveal MAX tests are the easiest available for those who require only a simple yes/no result, providing screening results in as little as 2 minutes. Reveal Q+, Reveal Q+ MAX , Veratox and Neocolumns can provide screening results, or results in exact parts per million or billion, in just minutes. Each requires only a minimal amount of training and equipment.

QUALITATIVE TESTS

Reveal - A qualitative lateral flow assay based on a competitve immunoassay format intended for the visual screening presence of toxin against set thresholds.The AccuScan Gold and Raptor Solo lateral flow test readers provide an easy method to objectively read, store, and analyze results from NEOGEN's Reveal product line.

Reveal MAX - A qualitative lateral flow assay based on a competitive immunoassay format intended for the visual screening presence of toxin against set thresholds The test uses a water based extraction, eliminating the hazardous solvents in your laboratory, and the need for hazardous waste disposal.

QUANTITATIVE TESTS

Reveal Q+ MAX A quantitative lateral flow device that utilizes a common water-based extraction which allows users to test for up to six mycotoxins from one sample.

Reveal Q+ These quantitative lateral flow devices provide an easy-to-use rapid test with unparalleled accuracy.

Veratox These quantitative tests compare up to 19 samples at a time against test controls. Through the use of a microwell reader, the tests provide accurate sample results in parts per million or billion.

Veratox MAX These quatitative ELISA tests use a common water based extraction, eliminating the need for harsh solvents.

NeoColumn These immunoaffinity columns efficiently clean and concentrate the toxins prior to analysis by HPLC, fluorometric reader, or NEOGEN's Veratox test kits.

SAMPLE EXTRACTION

Sample extraction is always performed at a specific ratio of solid sample to liquid extraction solution. Sample sizes can vary from the written instruction as long as the sample to extraction solution ratio remains the same.

Example: 1:5 extraction ratio

Ground Sample	Extraction Solution
5 g	25 mL
10 g	50 mL
50 g	250 mL

More representative results are achieved with a greater sample size. For example the USDA-FGIS recommends using a 50 g sample for aflatoxin testing and blending to extract (50 g + 250 mL). However, using a blender to extract is not always feasible and by using a smaller sample, the process can be sped up and simplified by utilizing disposable extraction cups and supplies.

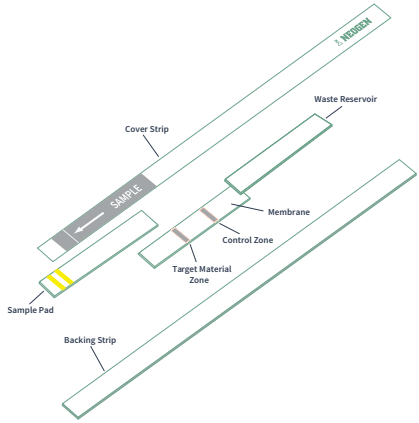
WATER-BASED EXTRACTION

NEOGEN has test kits available that use a water-based extraction method. These kits simply require distilled or deionized water to be added to the extraction packets, offering an easy-to-use and environmentally friendly extraction method. Please contact a NEOGEN representative for a complete list of tests.

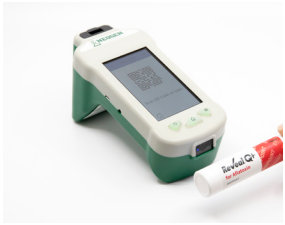
HOW DO NEOGEN'S MYCOTOXIN TESTS WORK?

REVEAL, REVEAL Q+ AND REVEAL Q+ MAX

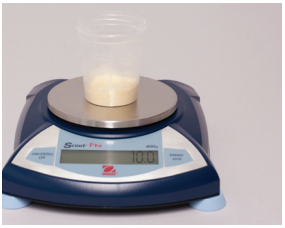
NEOGEN's Reveal tests for the detection of mycotoxins are lateral flow assays based on a competitive immunoassay format. The extract is wicked through a reagent zone, which contains antibodies specific for the target mycotoxin conjugated to colloidal gold particles. If the target mycotoxin is present, it will be captured by the particle-antibody complex. The mycotoxin-labeled antibody complex is then wicked onto a membrane, which contains a zone of mycotoxin. This zone captures any unbound mycotoxin antibody, allowing the particles to concentrate and form a visible line. As the level of mycotoxin in a sample increases, free mycotoxin will bind with the antibody-gold particles. This allows less antibody-gold to be captured in the test zone. Therefore, as the concentration of target mycotoxin in the sample increases, the test line density decreases. The membrane also contains a control line which will always form regardless of the presence of mycotoxin, ensuring the strip is functioning properly. For the Reveal Q+ and Reveal Q+ MAX tests, an AccuScan reader is utilized to convert the line densities into a quantitative result displayed in ppm or ppb.



REVEAL Q+ MAX FOR AFLATOXIN TEST PROCEDURE

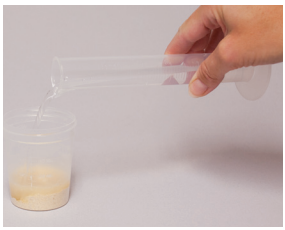


Prepare by entering values into the Raptor or Raptor Solo reader



Obtain a representative sample. Grind and weigh out 10 grams

Add contents of 1 MAX 1 packet to the sample

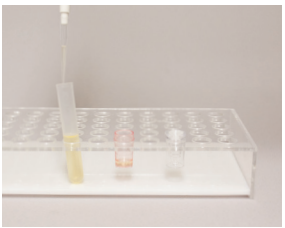


Add 50 mL distilled or deionized water to the sample

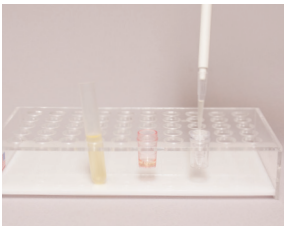
Shake vigorously for 3 minutes, or blend for 1 minute

Allow to settle, then filter

Add 100 µL of sample diluent to the red dilution cup



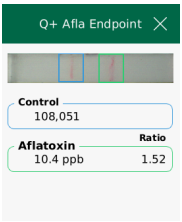
Add 100 µL sample extract to the red dilution cup and mix up and down 5 times



Transfer 100 µL diluted sample extract to sample cup

Place a new Reveal Q+MAX for Aflatoxin strip into the sample cup. Set a timer for 6 minutes

Remove promptly at 6 minutes and interpret results using the Raptor or Raptor Solo reader

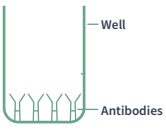


Results shown on reader

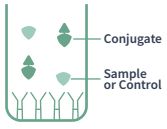
VERATOX

NEOGEN's microwell mycotoxin tests are competitive direct enzyme-linked immunosorbent assays (CD-ELISAs). Each test kit contains antibody-coated microwells with antibodies specific to the kit's target mycotoxin. First, samples and controls are added to their respective mixing wells. Next, an enzyme conjugate (the target mycotoxin chemically linked with an enzyme) is added. The samples/controls and conjugate are mixed and transferred to antibody wells where they compete for the antibody binding sites. The more target substance in the sample, the less conjugate that binds in the wells. After an incubation, the wells are washed to remove all unbound materials.

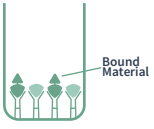
A substrate, which changes color in the presence of the conjugate, is then added to the wells. During an incubation, blue color develops in proportion to the amount of conjugate versus target mycotoxin in the wells. The more conjugate bound, the more blue color that develops, indicating less mycotoxin present. In the Veratox quantitative format, results are obtained by measuring the wells' color change in a microwell reader and comparing the readings against a standard curve.



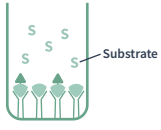
1. Microwells are coated with antibodies specific to the target substance



2. Conjugate competes with target substance/controls for antibody binding sites



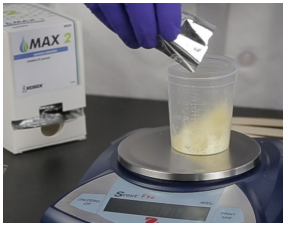
3. Conjugate and target substance/controls remain bound in wells



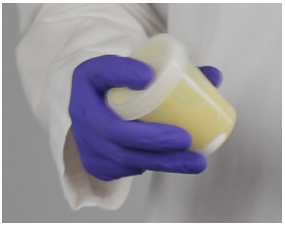
4. Substrate is added to produce a color change

5. Results are read visually or in a reader—the less blue color, or more red, the more target substance detected

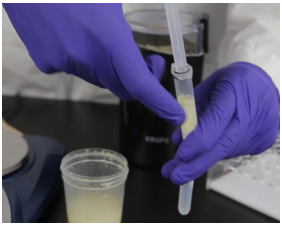
VERATOX FOR AFLATOXIN TEST PROCEDURE



Weigh out 10 g sample, add one MAX 2 packet. Add 50 mL distilled or deionized water.

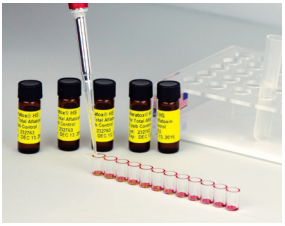


Shake for 3 minutes. Allow to settle.

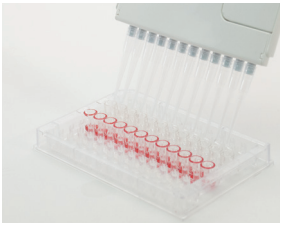


Filter using syringe.

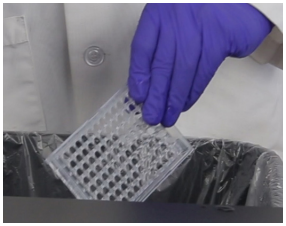
Add 100 µL conjugate to each red marked mixing well.



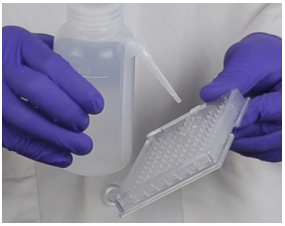
Add 100 µL controls and samples to their respective wells.



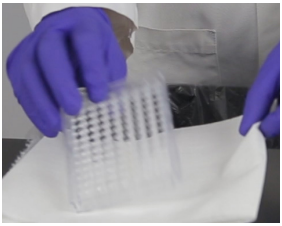
Mix. Transfer 100 µL to antibody wells. Incubate at room temperature for 15 minutes, sliding microwell holder back and forth gently for first 30 seconds.



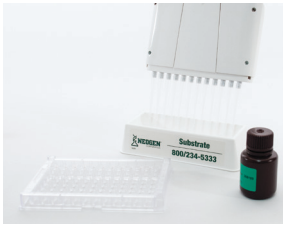
Dump liquid from antibody wells.



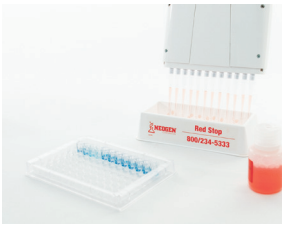
Wash wells with deionized water. Repeat wash step five times.



Tap out water on absorbent towel.



Transfer 100 µL substrate from the reagent boat to the antibody wells. Incubate at room temperature for 15 minutes, sliding microwell holder back and forth gently for first 30 seconds.



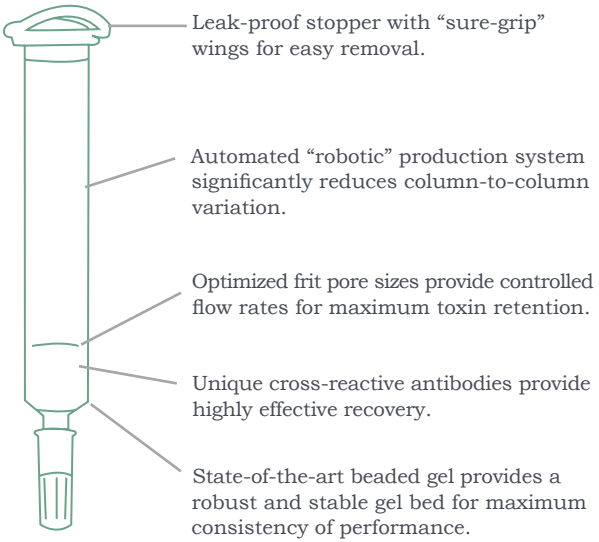
Transfer 100 µL Red Stop from reagent boat to antibody wells.

Read results using a microwell reader with a 650 nm filter. Results should be read within 20 minutes of adding Red Stop.

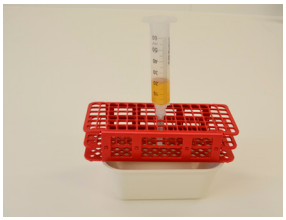
NEOCOLUMN

The NeoColumn test format is a high performance immunoaffinity column designed for the clean-up and concentration of a sample prior to HPLC, GC-MS, ELISA and other analytical methods. Clean-up columns are available for aflatoxin in both narrow and wide bore columns and in the wide bore column for DON, ochratoxin A and zearalenone. These columns deliver highly accurate results and recoveries on a range of validated matrices.

NeoColumn for Aflatoxin DR is an affinity column immunoassay. Aflatoxin is extracted from a ground sample by blending and filtering. Extracted toxin in the filtrate is sampled and diluted with water. The diluted extract is filtered and applied to the column. Positive pressure is used to induce flow through the column allowing the antibody to capture any aflatoxin present. Then the column is washed to remove any non-bound materials. Bound aflatoxin is eluted using 100% methanol and collected in a test tube. Aflatoxin fluorescence is enhanced by the addition of a developer (bromine solution) and read in a calibrated fluorometer, which displays the concentration of aflatoxin.



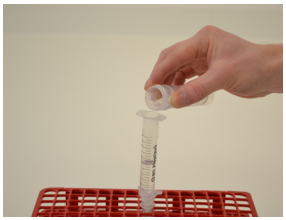
NEOCOLUMN FOR AFLATOXIN WIDE BORE TEST PROCEDURE



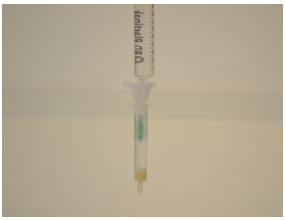
Add 20 mL of prepared extract to the reservoir

Remove the bottom cap of the column to initiate the flow dropwise, allowing entire sample to pass through to top of frit. Replace the bottom cap.

Add 2–3 mL of 25% methanol, and then reattach the reservoir/adapter.



Add 20 mL of 25% methanol.



Remove the bottom cap and allow the wash to flow through the column.

Ensure all liquid is removed from the column.

Slowly elute the bound aflatoxin from the column by passing 2 mL of 50:50 acentonitrile/ HPLC methanol through the column dropwise. Collect eluant into a clean glass vial.

Add 2 mL of HPLC grade water to the column, pushing through dropwise. The total elution volume now will be 4 mL.

Reveal, Reveal Q+, and Reveal Q+ MAX Quick Reference Guide				
Product Number	Test	Extraction	Ratio	Incubations (minutes)
8016	Reveal for Aflatoxin	10g sample, 20mL 65% ethanol, shake vigorously or blend for 1 minute.	1:5	3
8018	Reveal for Aflatoxin	10 g sample, 20 mL of 70% MeOH. Shake vigorously or blend for 1 minute	1:2	3
8085	Reveal Q+ for Aflatoxin	10 g sample, 50 mL of 65% ethanol. Shake vigorously for 3 minutes or blend for 1 minute	1:5	6
8385	Reveal Q+ for DON	10 g sample in 100 mL of DI or distilled water. Shake 3 minutes, settle and filter	1:10	3
8885	Reveal Q+ for Fumonisin	10 g sample in 50 mL of 65% ethanol. Shake 3 minutes, settle and filter	1:5	6
8685	Reveal Q+ for Ochratoxin	10 g sample in 40mL of 70% methanol. Shake 3 minutes, settle and filter	1:5	9
8285	Reveal Q+ for T-2/HT-2	10 g sample in 100 mL of distilled water. Shake 3 minutes, settle and filter	1:10	6
8185	Reveal Q+ for Zearalenone	Corn: 10 g in 30 mL of 65% ethanol Wheat: 10 g in 50 mL of 65% ethanol. Shake vigorously for 3 minutes or blend for 1 minute	Corn 1:3 Wheat 1:5	6
8388	Reveal Q+ MAX DON	10 g sample, (1) MAX 1 aqueous extraction packet, 50 mL deionised water. Shake vigorously for 3 minutes or blend for 1 minute	1:5	5
8688	Reveal Q+ MAX for Ochratoxin	10 g sample, (1) MAX 1 aqueous extraction packet, 50 mL* deionised water. Shake vigorously for 3 minutes or blend for 1 minute	1:5	5
8188	Reveal Q+ MAX for Zearalenone	10 g sample, (1) MAX 1 aqueous extraction packet, 50 mL deionised water. Shake vigorously for 3 minutes or blend for 1 minute	1:5	5
8388	Reveal Q+ MAX for DON	10 g sample, (1) MAX 1 aqueous extraction packet, 50 mL deionised water. Shake vigorously for 3 minutes or blend for 1 minute	1:5	5
8288	Reveal Q+ MAX for T2/HT2	10 g sample, (1) MAX 1 aqueous extraction packet, 50 mL deionised water. Shake vigorously for 3 minutes or blend for 1 minute	1:5	5
8488	Reveal Q+ MAX for Ergot Alkaloids	10 g sample, (1) MAX 1 aqueous extraction packet, 50 mL deionised water. Shake vigorously for 3 minutes or blend for 1 minute	1:5	8

Veratox Quick Reference Guide					
Product Number	Test	Extraction	Ratio	Controls	Incubations (minutes)
8030	Veratox for Aflatoxin	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes.	1:5	0, 5, 15, 50 (ppb)	2/3
8031	Veratox HS for Aflatoxin	25 g sample, 125 mL of 70% MeOH. Blend for 2 minutes.	1:5	0, 1, 2, 4, 8 (ppb)	10/10
8019	Veratox for Aflatoxin M1	Dried milk powders: 10g + 100 mL deionised water, extract on shaker for 30 minutes. Centrifuge for 10 minutes, and collect supernatant.	1:10	0, 5, 15, 30, 60, 100 ppt	45
8331	Veratox for DON 5/5	10 g sample, 100 mL of DI or distilled water. Shake vigorously for 3 minutes.	1:10	0, 0.5, 1, 2, 6 (ppm)	5/5
8331NE	Veratox for DON 5/5 NE.	10 g sample, 100 mL of DI or distilled water. Shake vigorously for 3 minutes.	1:10	0.25, 0.5, 1, 2 (ppm)	5/5
8335	Veratox for DON 2/3	10 g sample, 100 mL of DI or distilled water. Shake vigorously for 3 minutes.	1:10	0, 0.5, 1, 2, 6 (ppm)	2/3
8830	Veratox for Fumonisin 10/10	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes.	1:5	0, 1, 2, 4, 6 (ppm)	10/10
8835	Veratox for Fumonisin 5/10	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes.	1:5	0, 0.5,1, 3, 6 (ppm)	5/10
8610	Veratox for Ochratoxin	10 g sample, 40 mL of 50% MeOH. Shake for 5 minutes (wheat, barley and rye samples must be extracted in 70% MeOH).	1:4	0, 2, 5, 10, 25 (ppb)	10/10
8630	Veratox for Ochratoxin Grain	10 g sample, 40 mL of 50% MeOH. Shake for 3 minutes.	1:4	0, 2, 5, 10, 25 (ppb)	10/10
8230	Veratox for T-2/HT-2 Toxins	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes.	1:5	0, 25, 50, 100, 250 (ppb)	5/5
8110	Veratox for Zearalenone	10 g sample, 50 mL of 70% MeOH. Shake vigorously for 3 minutes.	1:5	0, 25, 75, 150, 500 (ppb)	5/5

Veratox MAX Quick Reference Guide					
Product Number	Test	Extraction	Ratio	Controls	Incubations (minutes)
8032	Veratox MAX for Total Aflatoxin HS	10 g sample, (1) MAX 2 aqueous extraction packet, 50 mL deionised water. Shake vigorously for 3 minutes.	1:5	0, 1, 3, 5, 10 (ppb)	15/15
8035	Veratox MAX for Total Aflatoxin	10 g sample, (1) MAX 2 aqueous extraction packet, 50 mL deionised water, shake vigorously for 3 minutes.	1:5	0, 5, 15, 50 ppb	5/5
8135	Veratox MAX for Zearalenone	10g sample, 1X MAX 2 Aqueous Extraction packet, 50mL deionised water. Shake vigorously for 3 minutes.	1:5	0, 25, 75, 150, 500	10/5

NeoColumn Quick Reference Guide				
Product Number	Test	Limit of Detection	Recovery	Testing Time
8040 Narrow Bore	NeoColumn for Aflatoxin (for HPLC clean-up)	0.1 ppb	>90% each of B_1, B_2, G_1, G_2 for cereal grains and nuts; conditions may vary depending on commodity	25 minutes
8043 Wide Bore	NeoColumn for Aflatoxin (for HPLC clean-up)	0.1 ppb	>90% each of B_1, B_2, G_1, G_2 for cereal grains and nuts; conditions may vary depending on commodity	25 minutes
8047	NeoColumn for Aflatoxin DR (Direct-read & HPLC clean-up)	1 ppb	>90% B_1 >80% B_2, G_1, G_2	5 minutes
8140	NeoColumn for Zearalenone	5 ppb	>90% for maize, wheat, animal feed and breakfast cereals; conditions may vary depending on commodity	20 minutes
8240	NeoColumn for T-2/HT-2 Toxins	125 ppb	≥95%	100 minutes
8340	NeoColumn for DON	0.1 ppm	>85%	25 minutes
8640	NeoColumn for Ochratoxin A	<0.1 ppb	>95% for cereals; conditions may vary depending on commodity	30 minutes

Product No.	Product	Reveal	Reveal Q+/ Reveal Q+ MAX	Veratox	NeoColumn
9401	Grinder	•	•	•	•
9427	Scale	•	•	•	
9428	Extraction container	•	•	•	•
9447 / 9368	Graduated cylinder	•	•	•	•
NA	DI or distilled water	•	•	•	•
9420	Filter syringe or equivalent	•	•	•	•
9421	Sample collection tube		•	•	•
9475	Sample cup rack	•	•		•
9402	Well holder			•	
9400	Wash bottle			•	
9426	Timer	•	•	•	•
9278 / 9272	100 µL pipettor	•	•	•	
9273	12-channel pipettor			•	
9407 / 9410	Pipette tips and rack	•	•	•	
9595	AccuScan Gold Reader	optional	•		
9303	NEOGEN 4700 Microwell Reader			•	
N/A	Plate rotary shaker			•*	
N/A	Vortex			•*	
N/A	Centrifuge			•*	
8089	MAX1 Extraction Packets		•		
8036	MAX2 Extraction Packets			•**	

TECH TEAM TOP TIPS

Is the particle size of a ground sample important?

Yes it is. Mycotoxins are embedded in different grain particles, and need to be released so that they are properly extracted. The USDA has set a grind size so that 95% of a material goes through a mesh sieve. The material should have the consistency of instant coffee.

What does MRM stand for and why should I use it?

MRM: Mycotoxin Reference Material. This is a naturally contaminated sample which has been verified by multiple methods, usually HPLC/LCMS. Technicians can use that sample as if it were an unknown to determine if they are getting the correct result. These types of samples are offered by NEOGEN.

What's the difference between mycotoxin advisory limits and regulatory limits?

Advisory limits are just that: advisory levels regarding mycotoxin limits set by experts within the regulatory community, like the FDA. However, numbers above those limits are not cause for recall alone. Advisory limits differ from regulatory limits, which are also determined by regulatory bodies. If a particular foodstuff is over the regulatory limit, a recall can be instituted. Users should be aware of regulatory limits not just where they operate, but also where they export to.

What is the typical percentage CV of mycotoxin levels from rapid test kits?

10–15% CV or relative standard deviation is the typical %CV.

But remember that test kits contribute to only a portion of the total error contributed during analysis. Method variation also takes into account the technician, instrumentation or equipment itself, as well as sampling variability. These all come together as the total error for the method.

How do we know if an analyst is proficient?

Analysts should participate in a proficiency program. NEOGEN offers programs that provide proficiency testing samples on an annual or quarterly basis. These samples are tested as normal, and their results are reported through an online portal. The analyst's proficiency is then gauged against other users of that test method by determining how closely the analyst came to the correct answer, and the overall variability of the analyst's test results.

How long is a sample valid for before a test is run?

Because the mycotoxins in a sample aren't soluble for an infinite period of time, we recommend the analysis be performed within 4 hours of extraction. Generally this means that if extraction takes place in the morning, analysis should be carried out before lunch. Likewise, if the sample is extracted in the afternoon, perform the analysis before going home. For the same reason, we recommend not saving extracts for extended periods of time to be analyzed later, because the value can slowly decline over time and not be indicative of the actual result.

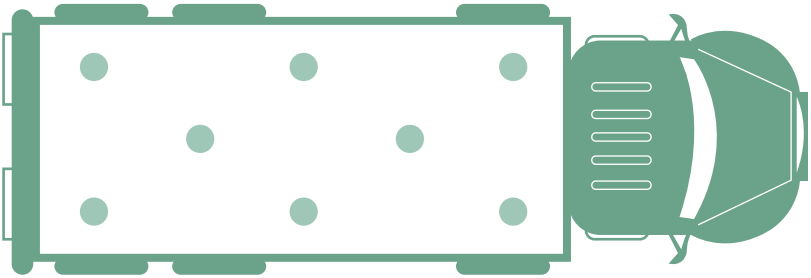
How large a sample should I grind?

You can refer to GIPSA recommendations or your regional grain inspection handbook for specific guidelines depending on the size of the vessel. 2 lbs is typical for trucks and containers, 3 lbs for railcars and 10 lbs for barges and other large vessels. It is recommended that the entire sample be ground together so that it represents the entire lot.

Commodity	Official Lot Type Minimum Sample Size (lbs/grams)			Submitted Samples lbs/g
	Trucks or Containers	Railcars	Barges. Sublots & Composite Samples	
Aflatoxin	2 lbs/908 g	3 lbs/1,362 g	10 lbs/4,540 g	10 lbs/4,450 g (Recommended)
Deoxynivalenol (DON)	200 g 2 lbs/908 g (corn only)	200 g 1000 g (corn only)	200 g 1000 g (corn only)	200 g 1000 g (corn only) (Recommended)
Zearalenone	2 lbs/908 g	3 lbs./1,362 g	3 lbs/1,362 g	10 lbs/4,540 g (Recommended)
Fumonisin	2 lbs./908 g	3 lbs/1,362 g	3 lbs/1,362 g	10 lbs/4,540 g (Recommended)

Why do I get different results on samples taken from the same truck load?

A sample that is tested may not be fully representative of the total load. Always encourage that a standard GIPSA or regional authority sampling plan be adhered to, so that the testing that happens from one lab to the next can be consistent. When comparing labs, its good practice to test from the same ground sample to ensure consistency in analysis.



How many times should I probe a truck of grain to get a representative sample?

GIPSA recommends that a multi-tier probe be used, and that each of those probes have individual compartments within them. Depending on the size of the vessel, the number of compartments can vary.

Why is there a wide range of acceptance for reference material?

There are different sources of variance for particular testing: method itself, technician, equipment, sample, etc. Samples are very well characterized by HPLC methods, but to account for other sources of variance a wider margin of error is given to the individual technician to be able to demonstrate proficiency when using that reference material.

What does LOQ stand for and why is it important?

Limit of quantification (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy following the kit insert protocol. The limit of detection (LOD) is the lowest quantity of a substance that can be distinguished from the absence of that substance (blank value) within a stated confidence limit.

Helpful hints and pipetting techniques

- Swirl, don't shake, all reagents before using. Otherwise, the reagents will foam.
- Always change pipette tips when there is a change in the reagent.
- Veratox test kits should be stored at 2–8°C (35–46°F) but allowed to warm to ambient temperature, 18–30°C (64–86°C) before use to ensure optimum performance.
- Prime pipette tips prior to dispensing all reagents. To prime the tip, draw up the reagent and discharge it back into the same container. Priming the tips coats the inside of the pipette tip so that the volume dispensed will be identical regardless of tip wetting properties.
- When drawing or dispensing reagents, always drag the pipette tip against the container rim to remove liquid on the outside of the tip.
- When dispensing reagents into the microwells, place the tip point against the inside wall of the microwell. This helps draw all of the liquid out of the tip and eliminates drops that form on the end of the pipette tip. In addition, placing the tip against the microwell holds the tip in place as the liquid is dispensed.
- Always check the fluid levels in your tips prior to dispensing to be sure that the same amount is being collected each time (100 µL). If the proper amount was not collected or bubbles are present, refill the tip.
- Most pipettors should be lubricated and calibrated every 12 months.
- If a sample result is greater than the kit's stated range of quantization (many times the kit's highest control), it is not considered a valid result. For accurate results you must dilute and rerun the sample.

RESOURCES

- Canadian Grain Commission; grainscanada.gc.ca
- North American Miller's Association (NAMA) 202/484-2200; namamillers.org
- USDA GIPSA; www.gipsa.usda.gov (Grain Inspection, Packers and Stockyards Administration)
- FAPAS® Central Science Laboratory, Sand Hutton, York, UK; Tel: (+44) 1904 462100; fapas.com
- North Dakota State University, Veterinary Diagnostic Laboratory; 701/231-8307; vdl.ndsu.edu
- AOAC International; aoac.org
- European Commission - Legislation on mycotoxins - ec.europa.eu European Food Safety Authority - Mycotoxins - efsa.europa.eu •