Mycotoxin Handbook

Understanding, Detecting, and Managing Mycotoxins in Feed and Grain



NEOGEN®





Contents

01 Frequently Asked Questions 04

02 Major Mycotoxins 10

03 Technical Information 22

04 Mycotoxin Tests 28

05 Tech Team Top Tips 38

06 Resources 43

01

Frequently Asked Questions







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 are dangerous to only specific animal species, and still others, such as penicillin, are 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 (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.



Understanding PPM and PPB

One part per million (ppm) 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 four grains out of the pound, you have removed 1 ppm.
- One part per billion (ppb) is even smaller — one-thousandth of a ppm. For example, 1 ppb is equivalent to one drop of water in a 10,000-gallon swimming pool.



02

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**
U.S.	All foods except milk		20 ppb	
	Milk			0.5 ppb
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	

Food				
		Maximum Level		
Region	**Commodity**	**B1**	**Total**	**M1**
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, turmeric, dried red pepper, dried paprika, and spice products containing these	10 ppb	15 ppb	
	Wheat flour, dried fruits	10 ppb	15 ppb	
	Confectioneries (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**
U.S.	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 cattle, 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

Feed			
		Maximum Level	
Region	**Commodity**	**B1**	**Total**
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 poultry 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	
China	Corn, peanut meal, cottonseed meal, rapeseed meal	50 ppb	
	Soybean 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

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**
U.S.	Finished wheat products for consumption by humans	1,000 ppb (1.0 ppm)
Canada	Uncleaned soft wheat for use in non-staple foods	2.0 mg/kg (ppm)
	Uncleaned soft wheat for use in baby foods	1.0 mg/kg (ppm)

Food		
Region	**Commodity**	**Maximum Level**
EU	Bread, pastries, biscuits, cereal snacks, and breakfast cereals	500 ppb (0.5 ppm)
	Cereals for human consumption — cereal flour, bran, and germ	750 ppb (0.75 ppm)
	Pasta (dried)	750 ppb (0.75 ppm)
	Milling fractions of maize with particle size >500 micron	750 ppb (0.75 ppm)
	Milling fractions of maize with particle size <500 micron	1,250 ppb (1.25 ppm)
	Processed cereal-based food for babies	200 ppb (0.2 ppm)
	Unprocessed cereals other than wheat durum, oats, and maize	1,250 ppb (1.25 ppm)
	Unprocessed durum wheat, oats, and maize not intended for wet milling	1,750 ppb (1.75 ppm)
Japan	Wheat and wheat products	1,100 ppb (1.1 ppm)
China	Wheat and wheat products	1,000 ppb (1.0 ppm)
	Corn and corn products	1,000 ppb (1.0 ppm)
	Barley and barley products	1,000 ppb (1.0 ppm)
Indonesia	Corn and its products	1,000 ppb (1.0 ppm)
	Wheat and its products	1,000 ppb (1.0 ppm)
	Wheat flour and its products (pastry, bakery, biscuits, snacks)	500 ppb (0.5 ppm)
	Pasta and noodles	750 ppb (0.75 ppm)
	Wheat based breast-milk substitute products	200 ppb (0.2 ppm)

Feed		
Region	**Commodity**	**Maximum Level**
U.S.	Grains and grain by-products for ruminating beef and feedlot cattle older than four months, chickens	10,000 ppb (10 ppm)
	Grain and grain by-products for swine	5,000 ppb (5 ppm)
	Grain and grain by-products for other animals	5,000 ppb (5 ppm)
EU	Cereals and cereal products with the exception of maize by-products	8,000 ppb (8 ppm)
	Maize by-products	12,000 ppb (12 ppm)
	Complementary and complete feeding stuffs	5,000 ppb (5 ppm)
	Complementary and complete feeding stuffs for pigs	900 ppb (0.9 ppm)
	Complementary and complete feeding stuffs for calves (<4 months), lambs, and kids	2,000 ppb (2 ppm)
Japan	Formula feed (cows over three months after birth)	4,000,000 ppb (4,000 ppm)
	Formula feed (except for cows over three months after birth)	1,000,000 ppb (1,000 ppm)
China	Complementary and complete feeding stuffs for swine	<1,000 ppb (<1 ppm)
	Complementary and complete feeding stuffs for calves	<1,000 ppb (<1 ppm)
	Complementary and complete feeding stuffs for lactating animals	<1,000 ppb (<1 ppm)
	Complementary and complete feeding stuffs for cattle	<5,000 ppb (<5 ppm)
	Complementary and complete feeding stuffs for poultry	<5,000 ppb (<5 ppm)

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)**
U.S.	Degermed dry milled corn products (i.e., flaking grits, corn meal, corn flour)	2,000 ppb (2.0 ppm)	
	Cleaned corn used for popcorn	3,000 ppb (3.0 ppm)	
	Whole or partially degermed dry milled corn products; dry milled corn bran; cleaned corn used for mass production	4,000 ppb (4.0 ppm)	
EU	Milling fractions of maize with particle size >500 micron		1,400 ppb (1.4 ppm)
	Milling fractions of maize with particle size <500 micron		2,000 ppb (2.0 ppm)
	Maize intended for human consumption		1,000 ppb (1.0 ppm)
	Maize snacks, maize based breakfast cereals		800 ppb (0.8 ppm)
	Processed maize-based foods for babies		200 ppb (0.2 ppm)
	Unprocessed maize not intended for wet milling		4,000 ppb (4.0 ppm)
Indonesia	Corn and corn as a raw material	2,000 ppb (2.0 ppm)	
	Corn food products (flakes, popcorn, corn chips)	1,000 ppb (1.0 ppm)	
Korea	Grain products and cereals (containing >50% corn, corn processed products, corn powder)	1,000 ppb (1.0 ppm)	
	Processed corn products for popcorn	1,000 ppb (1.0 ppm)	
	Confectioneries (containing >50% corn)	1,000 ppb (1.0 ppm)	
	Corn	4,000 ppb (4.0 ppm)	
	Corn processed food (grinding, cutting, etc.) corn powder	2,000 ppb (2.0 ppm)	

Feed			
Region	**Commodity**	**Guidance Level (B1, B2, B3)**	**Maximum Level (B1+B2)**
U.S.	Corn and corn by-products for equids and rabbits	5,000 ppb (5 ppm)	
	Corn and corn by-products for swine and catfish	20,000 ppb (20 ppm)	
	Corn and corn by-products for breeding ruminants, breeding poultry, and breeding mink	30,000 ppb (30 ppm)	
	Poultry raised for slaughter	100,000 ppb (100 ppm)	
	All other species of livestock and pet animals	10,000 ppb (10 ppm)	
EU	Maize and maize-based products		60,000 ppb (60 ppm)
	Complementary and complete feeding stuffs for pigs, horses, rabbits, and pet animals		5,000 ppb (5 ppm)
	Complementary and complete feeding stuffs for fish		10,000 ppb (10 ppm)
	Complementary and complete feeding stuffs for poultry, calves (<4 months), lambs, and kids		20,000 ppb (20 ppm)
	Complementary and complete feeding stuffs for adult ruminants (>4 months) and mink		50,000 ppb (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 (FDA), many agree that levels between 10–20 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 0.50–80 ppb in food and up to 250 ppb in feed.

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, turmeric	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	250 ppb
	Complementary and complete feeding stuffs for pigs	50 ppb
	Complementary and complete feeding stuffs for poultry	100 ppb
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 children	15 ppb

Feed		
Region	**Commodity**	**Maximum Level**
EU	Oat milling products (husks)	2,000 ppb
	Other cereal products	500 ppb

Feed		
Region	**Commodity**	**Maximum Level**
EU	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	<1,000 ppb
	Complementary and complete feeding stuff for poultry	<1,000 ppb

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 particle 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
	Confectioneries	50 ppb
	Baby food for infants and young children	20 ppb
China	Wheat, corn, and their products	60 ppb

Feed		
Region	**Commodity**	**Maximum Level**
EU	Cereal and cereal products with the exception of maize by-products	2,000 ppb
	Maize by-products	3,000 ppb
	Complementary and complete feeding stuffs for piglets, young sows, puppies, kittens, dogs, and cats for reproduction	100 ppb
	Complementary and complete feeding stuffs for adult dogs and cats other than for reproduction	200 ppb
	Complementary and complete feeding stuffs for sows and fattening pigs	250 ppb
	Complementary and complete feeding stuffs for calves, dairy cattle, sheep, and goats	500 ppb
Japan	Formula feed	1,000 ppb
China	Complementary and complete feeding stuffs, corn	<500 ppb



03

Technical Information



Confirmation Methods

High-performance liquid chromatography (HPLC) is the preferred instrument based confirmation method for mycotoxins. Testing requires a skilled technician, a validated test method, and appropriate equipment. Gas chromatography (GC), thin layer chromatography (TLC), and liquid chromatography-mass spectrometer (LC-MS) are popular confirmation methods.

Commodity Validation/Verification Lists

Neogen test kits have been validated and/or verified on a variety of commodities — from the susceptible to the obscure — based on customer requests. Please contact a Neogen representative or visit neogen.com 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 recheck 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 recheck 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 Reveal® and Reveal Mycotoxin Aqueous Extraction (MAX) tests are the easiest available for those who require only a simple yes/no result, providing screening results in as little as two minutes. Reveal Q+, Reveal Q+ EndPoint, Reveal Q+ MAX, and Veratox® can provide screening results, or results in exact ppm or ppb, in just minutes. Each requires only a minimal amount of training and equipment.

Qualitative Tests

Reveal: A qualitative lateral flow assay using a competitive immunoassay format intended for the visual screening presence of toxin against set thresholds.

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

Quantitative Tests

Reveal Q+: These quantitative lateral flow devices provide an easy-to-use rapid test that delivers accurate, dependable results.

Reveal Q+ EndPoint: These quantitative lateral flow devices provide a solution for higher volume testing.

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.

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 ppm or ppb.

Veratox HS: These high sensitivity (HS) quantitative ELISA tests are used when detection of lower levels of mycotoxins are required.

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



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

Water-based Extraction

Neogen has test kits available that use a water-based extraction method. The MAX line of kits simply require distilled or deionized water to be added to the extraction packets, offering an easy-to-use and environmentally friendly extraction method. The common extraction protocol allows you to test for multiple mycotoxins from a single sample. MAX packets are provided in each kit to test each sample, or can be purchased in a bulk format to prepare a larger volume, ready-to-use solution. Please contact a Neogen representative for a complete list of tests.



04

How Do Neogen's Mycotoxin Tests Work?

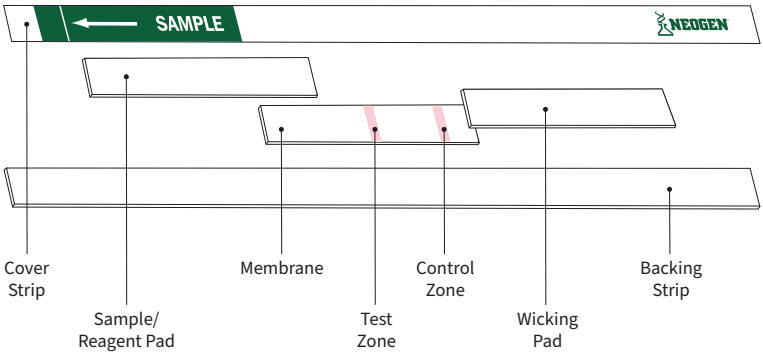




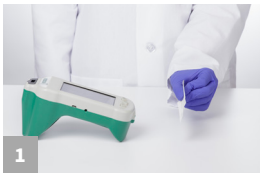
Lateral Flow Solutions

Reveal®, Reveal® MAX, Reveal® Q+, Reveal® Q+ EndPoint, and Reveal® Q+ MAX

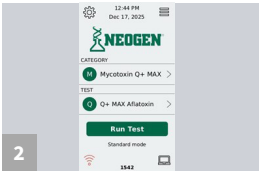
Neogen's Reveal tests for the detection of mycotoxins are lateral flow assays based on a competitive immunoassay format. The extract is wicked through a reagent zone, which contains antibodies specific for the target mycotoxin conjugated to colloidal gold particles. If the target mycotoxin is present, 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+, Reveal Q+ EndPoint, and Reveal Q+ MAX tests, a reader is utilized to convert the line densities into a quantitative result displayed in ppm or ppb.



Example Test Procedure: Neogen® Reveal® Q+ MAX for Aflatoxin (Endpoint)



Prepare by entering the QR code into the Raptor® Solo Diagnostic Reader.



Select test type: Mycotoxin Q+ MAX. Select test: Q+ MAX Aflatoxin. Press run test. On the next screen, select lot.



Obtain a representative sample. Grind and weigh out a 10 g sample.



Add the contents of one MAX 1 packet to the sample, and then 50 mL of distilled or deionized water. Alternatively, add 50 mL of prepared bulk MAX 1 solution to the sample.



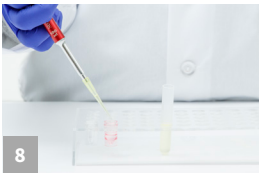
Shake vigorously for three minutes, or blend for one minute.



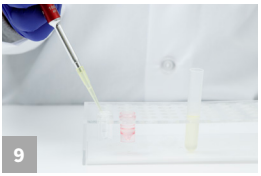
Settle, then filter or use a microcentrifuge and spin for 30–60 seconds.



Add 100 µL of sample diluent to the red dilution cup.



Add 100 µL of sample extraction to the red dilution cup, and mix up and down five times.



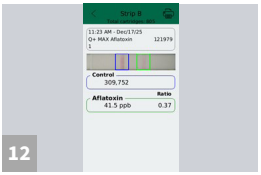
Transfer 100 µL from the red dilution cup into the clear sample cup.



Set a timer for six minutes. Place a new Reveal Q+ MAX for Aflatoxin strip into the clear sample cup and start the timer.



Remove promptly at six minutes and interpret results using the Raptor Solo reader.



Results are displayed on the unit. Samples greater than 50 ppb must be diluted and retested. To read the next test strip, press change at the bottom of the screen and enter the next sample ID. Press accept and insert cartridge containing the next test strip.

Download the complete kit instructions at neogen.com

Example Test Procedure: Reveal® Q+ MAX for Fumonisin (Vortex)



Prepare by entering the QR code into the Raptor reader.



Obtain a representative sample. Grind and weigh out a 10 g sample.



Add the contents of 1 MAX 1 packet to the sample, and then 50 mL of distilled or deionized water. Alternatively, add 50 mL of prepared bulk MAX 1 solution to the sample.



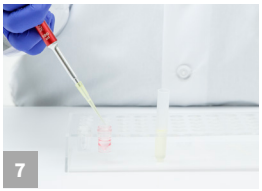
Shake vigorously for 3 minutes, or blend for 1 minute.



Settle, then filter or use a microcentrifuge and spin for 30-60 seconds.



Add 1000 uL of sample diluent to the red dilution cup.



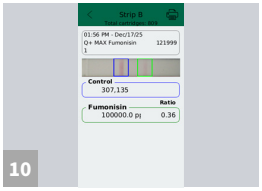
Add 100 uL of sample extraction to the red dilution cup, and mix up and down 5 times.



Insert a test strip into the Raptor Vortex cartridge. Insert the cartridge into the Raptor reader. Enter a sample ID if desired.



400 uL of the sample from the red dilution cup to the back of the Raptor Vortex cartridge. The reader will start automatically.



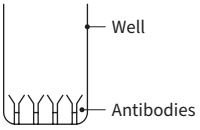
The results will be displayed on the screen after the 5-minute testing period is complete. Note: Samples greater than 5 ppm must be diluted and retested.

ELISA Assays

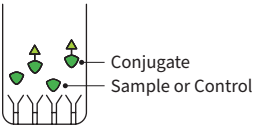
Veratox

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.

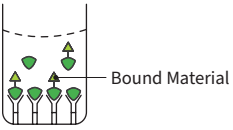
A substrate, which changes color in the presence of the conjugate, is then added to the wells. During an incubation, blue color develops in proportion to the amount of conjugate versus target mycotoxin in the wells. The more conjugate bound, the more blue color that develops, indicating less mycotoxin 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.



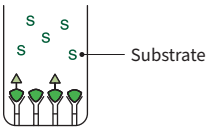
Microwells are coated with antibodies specific to the target substance.



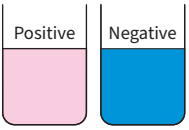
Conjugate competes with target substance/controls for antibody binding sites.



Conjugate and target substance/controls remain bound in wells.



Substrate is added to produce a color change. Red stop is added to stop the reaction.



Results can be interpreted visually for qualitative testing or measured with a reader for quantitative testing. A darker blue color indicates a lower amount of the target substance is present.

Example Test Procedure: Veratox MAX for Aflatoxin



Obtain a sample and grind it. Weigh out 10 g sample, add one MAX 2 packet. Add 50 mL distilled or deionied water.



Shake for three minutes. Allow to settle.



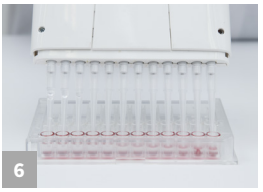
Filter using syringe.



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.



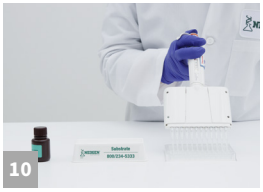
Dump liquid from antibody wells.



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.

Reveal, Reveal MAX, Reveal Q+, Reveal Q+ MAX Quick Reference Guide				
SKU	**Test**	**Extraction**	**Ratio**	**Incubations (Minutes)**
700002474 I 8016	Reveal® for Aflatoxin	10 g sample, 20 mL of 65% ethanol. Shake vigorously or blend for one minute.	1:5	3
700002476 I 8018	Reveal® MAX for Aflatoxin	10 g sample, one MAX 1 aqueous extraction packet + 50 mL deionized water or 50 mL of prepared bulk MAX solution. Shake vigorously for three minutes or blend for one minute.	1:5	3
700002494 I 8075	Reveal® Q+ for Aflatoxin EndPoint	10 g sample, 50 mL of 65% ethanol. Shake vigorously for three minutes or blend for one minute.	1.5	6
700002497 I 8085	Reveal® Q+ for Aflatoxin	10 g sample, 50 mL of 65% ethanol. Shake vigorously for three minutes or blend for one minute.	1:5	6
700002499 I 8088	Reveal® Q+ MAX Aflatoxin	10 g sample, one MAX 1 aqueous extraction packet + 50 mL deionized water or 50 mL of prepared bulk MAX solution. Shake vigorously for three minutes or blend for one minute.	1:5	5
700002536 I 8375	Reveal® Q+ for DON Endpoint	10 g sample, 100 mL distilled or deionized water. Shake vigorously for three minutes, settle, and filter.	1:10	3
700002539 I 8385	Reveal® Q+ for DON	10 g sample in 100 mL of DI or distilled water. Shake three minutes, settle, and filter.	1:10	3
700002541 I 8388	Reveal® Q+ MAX for DON	10 g sample, one MAX 1 aqueous extraction packet + 50 mL deionized water or 50 mL of prepared bulk MAX solution. Shake vigorously for three minutes or blend for one minute.	1:5	3
700002625 I 8885	Reveal® Q+ for Fumonisin	10 g sample in 50 mL of 65% ethanol. Shake three minutes, settle, and filter.	1:5	6
700007795 | 8888	Reveal® Q+ MAX for Fumonisin	10 g sample, one MAX 1 aqueous extraction packet + 50 mL deionized water or 50 mL of prepared bulk MAX solution. Shake vigorously for three minutes or blend for one minute.	1:5	5
700002612 I 8685	Reveal® Q+ for Ochratoxin	10 g sample in 40 mL of 70% methanol. Shake three minutes, settle, and filter.	1:5	9
700002614 I 8688	Reveal® Q+ MAX for Ochratoxin	10 g sample, one MAX 1 aqueous extraction packet + 50 mL deionized water or 50 mL of prepared bulk MAX solution. Shake vigorously for three minutes or blend for one minute.	1:5	5
700002527 I 8285	Reveal® Q+ for T-2/HT-2	10 g sample in 100 mL of distilled water. Shake three minutes, settle, and filter.	1:10	6
700002528 I 8288	Reveal® Q+ MAX for T2/HT2	10 g sample, one MAX 1 aqueous extraction packet + 50 mL deionized water or 50 mL of prepared bulk MAX solution. Shake vigorously for three minutes or blend for one minute.	1:5	5
700002515 I 8185	Reveal® Q+ for Zearalenone	Corn: 10 g in 30 mL of 65% ethanol. Wheat: 10 g in 50 mL of 65% ethanol. Corn/Wheat: Shake vigorously for three minutes or blend for one minute.	Corn 1:3 Wheat 1:5	6
700002516 I 8188	Reveal® Q+ MAX for Zearalenone	10 g sample, one MAX 1 aqueous extraction packet + 50 mL deionized water or 50 mL of prepared bulk MAX solution. Shake vigorously for three minutes or blend for one minute.	1:5	5
700003767 I RQAFM1	Reveal® Q+ for Aflatoxin M1	Mix milk prior to adding the sample to the cartridge.	N/A	5
700003768 I RQAFM1HS	Reveal® Q+ HS for Aflatoxin M1	Mix milk prior to adding the sample to the cartridge.	N/A	5

Veratox Quick Reference Guide					
SKU	**Test**	**Extraction**	**Ratio**	**Controls**	**Incubations (Minutes)**
700002479 I 8030	Veratox® for Aflatoxin	10 g sample, 50 mL of 70% MeOH. Shake vigorously for three minutes.	1:5	0, 5, 15, 50 (ppb)	2/3
700002480 I 8031	Veratox® HS for Aflatoxin	25 g sample, 125 mL of 70% MeOH. Blend for two minutes.	1:5	0, 1, 2, 4, 8 (ppb)	10/10
700002478 I 8019	Veratox® for Aflatoxin M1	Dried milk powders: 10g + 100 mL deionized water, extract on shaker for 30 minutes. Centrifuge for ten minutes, and collect supernatant.	1:10	0, 5, 15, 30, 60, 100 (ppt)	20/10/15
700002531 I 8331	Veratox® for DON 5/5	10 g sample, 100 mL of DI or distilled water. Shake vigorously for three minutes.	1:10	0, 0.5, 1, 2, 6 (ppm)	5/5
700002532 I 8331NE	Veratox® for DON 5/5 NE	10 g sample, 100 mL of DI or distilled water. Shake vigorously for three minutes.	1:10	0.25, 0.5, 1, 2 (ppm)	5/5
700002534 I 8332	Veratox® for DON 2/3	10 g sample, 100 mL of DI or distilled water. Shake vigorously for three minutes.	1:10	0, 0.5, 1, 2, 6 (ppm)	2/3
700002618 I 8830	Veratox® for Fumonisin	10 g sample, 50 mL of 70% MeOH. Shake vigorously for three minutes.	1:5	0, 1, 2, 4, 6 (ppm)	10/10
700002620 I 8832	Veratox® for Fumonisin 5/10	10 g sample, 50 mL of 70% MeOH. Shake vigorously for three minutes.	1:5	0, 0.5,1, 3, 6 (ppm)	5/10
700002609 I 8610	Veratox® for Ochratoxin	10 g sample, 40 mL of 50% MeOH. Shake for five minutes (wheat, barley, and rye samples must be extracted in 70% MeOH).	1:4	0, 2, 5, 10, 25 (ppb)	10/10
700002610 I 8632	Veratox® for Ochratoxin Grain	10 g sample, 40 mL of 50% MeOH. Shake for three minutes.	1:4	0, 2, 5, 10, 25 (ppb)	10/10
700002525 I 8230	Veratox® for T-2/HT-2 Toxins	10 g sample, 50 mL of 70% MeOH. Shake vigorously for three minutes.	1:5	0, 25, 50, 100, 250 (ppb)	5/5
700002513 I 8110	Veratox® for Zearalenone	10 g sample, 50 mL of 70% MeOH. Shake vigorously for three minutes.	1:5	0, 25, 75, 150, 500 (ppb)	5/5
700002482 I 8032	Veratox® MAX for Total Aflatoxin HS	10 g sample, one MAX 2 aqueous extraction packet, 50 mL deionized water. Shake vigorously for three minutes.	1:5	0, 1, 3, 5, 10 (ppb)	15/15
700002483 I 8035	Veratox® MAX for Total Aflatoxin	10 g sample, one MAX 2 aqueous extraction packet, 50 mL deionized water, shake vigorously for three minutes.	1:5	0, 5, 15, 50 ppb	5/5
700002514 I 8135	Veratox® MAX for Zearalenone	10g sample, one MAX 2 aqueous extraction packet, 50 mL deionized water. Shake vigorously for three minutes.	1:5	0, 25, 75, 150, 500	10/5
700002478 I 8019	Veratox® for Aflatoxin M1	Centrifuge milk sample for 10 minutes at 350 rpm, collect supernatant to test	N/A	0, 5, 15, 30, 60, 100	20/10/15

Please visit Neogen.com for equipment needed by test kit





05

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 United States Department of Agriculture (USDA) has set a grind size so that 95% of a material goes through a 20-mesh sieve. The material should have the consistency of instant coffee.

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

MRM stands for 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?

We recommend performing the analysis within four hours of extraction, as mycotoxins in a sample aren't soluble for an infinite period of time. 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 Federal Grain Inspection Service (FGIS) 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.

Official Lot Type Minimum Sample Size (lbs/g)				
Commodity	**Trucks or Containers**	**Railcars**	**Barges, Sublots and Composite Samples**	**Submitted Samples lbs/g**
Aflatoxin	2 lbs/908 g	3 lbs/1,362 g	10 lbs/4,540 g	10 lbs/4,450 g (Recommended)
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 FGIS 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?

FGIS 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 quantitation (often 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 FGIS; www.ams.usda.gov (Agricultural Marketing Service, Federal Grain Inspection Service)
- 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



NEOGEN®

Neogen Corporation, 620 Lesher Place, Lansing, MI 48912 USA
© Neogen Corporation 2026. All rights reserved. Neogen, AccuScan, Raptor, Reveal, and Veratox are registered trademarks of Neogen Corporation.
All other marks mentioned are the property of their respective owners.

FS00697-0826