

Directive

9180.71

7-08-09

FUMONISIN TESTING SERVICES

1. PURPOSE

This directive establishes official procedures for determining fumonisin in grain and processed grain products, and certifying the official results.

2. REPLACEMENT HIGHLIGHTS

This directive is revised to include instructions for the Charm Sciences Inc. ROSA® Fumonisin Quantitative test method, Product Number LF-FUMQ. This directive supersedes FGIS Directive 9180.71, dated 12-29-08.

3. BACKGROUND

Fumonisin are environmental toxins produced by molds that grow on agricultural commodities in the field or during storage. *Fusarium moniliforme* is the parent fungi species that causes Fusarium Ear Rot, the most common corn disease in the Midwestern United States.

More than 10 types of fumonisins have been isolated and characterized. Of these, fumonisins B₁ (FB₁), B₂ (FB₂), and B₃ (FB₃) are the major fumonisins produced in nature. The most prevalent of these mycotoxins in contaminated corn is FB₁, which is believed to be the most toxic. Since the fumonisin toxin can grow in corn kernels without any outward signs of mold, testing of the grain is the only positive means of verifying whether fumonisin is present.

4. TESTING SERVICES

All official fumonisin testing is performed as prescribed in this directive by authorized employees of the United States Department of Agriculture (USDA), Grain Inspection, Packers and Stockyards (GIPSA), Federal Grain Inspection Service (FGIS) or official service providers. Testing performed on standardized grains (e.g., corn, wheat) is performed as an official criteria factor under the authority of the United States Grain Standards Act (USGSA), as amended. Testing performed on processed grain products (e.g., corn meal) and other commodities is provided under the authority of the Agricultural Marketing Act (AMA) of 1946, as amended.

Individuals who wish official fumonisin testing should contact the nearest FGIS field office or official service providers.

Three types of fumonisin testing services are available as follows:

a. Submitted Sample Service.

Analysis based on a sample submitted by the applicant for service.

b. Official Sample-Lot Service.

Analysis based on an official sample obtained and analyzed by official personnel.

(1) Single Lot Inspection.

Samples may be obtained and tested on either an individual carrier basis or a composite sample basis (maximum of 5 railcars or 15 trucks per composite sample).

(2) Unit Train Inspection under the CuSum Loading Plan.

Unit trains are analyzed on a subplot basis for corn and on a composite basis for other grains. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

For unit trains, the subplot size for fumonisin testing and for grade analysis may be different. For example, an applicant may request grade analysis on the basis of a subplot containing two cars and request fumonisin analysis on the basis of five cars.

The maximum size subplot for fumonisin testing is 5 railcars for unit trains consisting of less than 200,000 bushels, or less than 50 cars. For unit trains consisting of 200,000 bushels or more, or 50 railcars or more, the maximum subplot size is 10 railcars.

(3) Export Shiplots.

Export shiplots are analyzed on a subplot basis for corn and on a composite basis for other grains. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

The testing frequency for shiplot grain will be the same as the sample for grade analysis unless the applicant specifically requests fumonisin analysis on the basis of a component sample.

(4) Supplemental Testing.

Upon request, supplemental testing may be performed as follows:

Composite samples may be analyzed in addition to the subplot test for corn shiplots or unit trains.

(5) Alternate Testing.

Upon request, alternate testing methods may be used, provided that the minimum testing requirements are met. Examples of alternate testing are as follows:

- (a) Sublot testing may be used instead of composite sample analysis for grains routinely tested on a composite basis.
- (b) Grain shipments may be tested on a component sample basis in lieu of the subplot basis under the provisions of Book III, Inspection Procedures. Components are combined and averaged to determine the subplot result. Acceptable quality will be based on the subplot result as compared to the contracted "maximum" specification.

c. Warehouseman Sample-Lot Inspection Service.

Analysis based on an official sample (grain only) obtained by a licensed warehouseman sampler and analyzed by official personnel.

5. REVIEW INSPECTIONS

USGSA Title 7, Code of Federal Regulations (CFR) Part 800.125 and 800.135 permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factor and official criteria may be handled separately even though both sets of results are reported on the same certificate.

Review inspection services for fumonisin are provided on either a new sample or the file sample in accordance with the regulations. Board appeal inspection services are limited to the analysis of file samples.

NOTE: Do not consider any excess grain sample as a “new sample” for the basis of testing.

For submitted samples, lots that are certified on an individual carrier basis, and composite samples representing multiple carriers, a maximum of three review inspections (reinspection, appeal, Board appeal) may be performed on the original inspection service.

Only one field review (reinspection or appeal inspection) is permitted for shiplot, unit train, or lash barge material portions when testing is performed on a subplot basis. However, if the applicant requests a review of the entire lot, up to three review levels of service (reinspection, appeal, Board appeal) may be obtained for each subplot included in the lot. Inspection results for each review level shall replace the previous inspection result.

a. Reinspection Service.

The laboratory providing original testing services also provides reinspection services.

b. Appeal Inspection Service.

FGIS field offices provide appeal fumonisin testing services. Field offices not equipped to provide testing will make arrangements with another FGIS office to provide the timeliest service possible.

If samples are sent to a field office for analysis, write the words "**FUMONISIN APPEAL**" in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

c. Board Appeal Inspection Services.

Board appeal inspection services are limited to the file sample and are provided by the Board of Appeals and Review (BAR) in Kansas City. The High Performance Liquid Chromatography (HPLC) method is available for determining fumonisin in board appeal samples. The applicant must specify the HPLC method as the desired determination method. Otherwise, the board appeal inspection will be conducted using the rapid method (test kits).

When sending samples to the BAR, write the words "**FUMONISIN BOARD APPEAL**" in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

6. **APPROVED TEST METHODS**

The methods listed below have been conformance tested to perform within FGIS specifications. Each of the approved test methods has been certified to provide quantitative and qualitative results accurate up to the conformance test level at which they were approved.

Any test results that are above the established conformance limits are reported as

exceeding the conformance limit unless a supplemental analysis is performed.

FGIS APPROVED TEST METHODS			
Method and Test Kit	Product No.	Conformance Limit	
		Quantitative	Qualitative
RIDASCREEN® Fast Fumonisin (r-Biopharm)	R5602	0.5 – 5 PPM	
Veratox Quantitative Fumonisin (Neogen)	8830	0.5 – 5 PPM	
Myco✓ Fumonisin (Strategic Diagnostic Inc.)	7000130	0.5 – 5 PPM	
FumoniTest 200™ (Vicam)	G1029	0.5 – 5 PPM	
Fumonisin Qualitative FPA (Diachemix)	61550		1 PPM
AgraQuant® Total Fumonisin (Romer)	COKAQ3000	0.5 – 5 PPM	
ROSA® Fumonisin (Charm Sciences Inc.)	LF-FUMQ	0.5 – 5 PPM	

The following table lists the fumonisin field test kits and the grains/commodities for which they have been approved. For information concerning the testing of other grains/commodities, contact the Policies and Procedures Branch.

GRAIN/ COMMODITY	TEST METHOD						
	Ridascreen® Fast Fumonisin	Veratox Quantitative Fumonisin	Myco✓ Fumonisin	FumoniTest 200™	Fumonisin Qualitative FPA	Romer AgraQuant® Fumonisin	ROSA® Fumonisin Test
Corn	X	X	X	X	X	X	X
Sorghum	X			X		X	
Wheat		X				X	
Corn Meal	X	X	X	X		X	
Corn Gluten Meal	X						
Corn Germ Meal	X		X	X			
Corn/Soy Blend	X	X		X			
Popcorn		X		X		X	

TEST METHODS	
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GRAIN/ COMMODITY	Ridascreen® Fast Fumonisin	Veratox Quantitative Fumonisin	Myco✓ Fumonisin	FumoniTest 200™	Fumonisin Qualitative FPA	Romer AgraQuant® Fumonisin	ROSA® Fumonisin Test
Rough Rice		X				X	
Flaking Corn Grits				X			
Distillers Dried Grains w/Solubles						X	

7. **DISCLAIMER CLAUSE**

The mention of firm names or trade products does not imply that they are endorsed or recommended by USDA over other firms or similar products not mentioned.

8. **FOOD AND DRUG ADMINISTRATION ACTION**

The Food and Drug Administration (FDA) has stated that, currently, there is no direct evidence that fumonisin can cause adverse health effects in humans. Studies currently available demonstrate only inconclusive associations between fumonisins and human cancer.

However, substantial information exists on the adverse health effects of fumonisins in animals. Ingestion of fumonisin-contaminated corn and corn screenings results in a variety of adverse health effects in livestock, the most frequent being equine leukoencephalomalacia, also known as "Blind Staggers".

The recommended maximum levels for fumonisin in human foods and animal feeds that the FDA considers achievable with the use of good agricultural and good manufacturing practices are listed below. The FDA believes that controlling fumonisin to these recommended levels can reduce exposure to fumonisins that may be found in corn products intended for human and animal consumption.

Fumonisin test results are not reported to the FDA because action limits are not established at this time. Recommended level(s) as follows:

<u>Human Foods</u>	
<u>Product</u>	Total Fumonisin (PPM) (FB ₁ + FB ₂ + FB ₃)
Degermed dry milled corn products	2 ppm
Whole or partially degermed dry milled corn products	4 ppm
Dry milled corn bran	4 ppm
Cleaned corn intended for masa production	4 ppm
Clean corn intended for popcorn	3 ppm

<u>Animal Feeds</u>	
<u>Corn and corn by-products intended for:</u>	Total Fumonisin (PPM) (FB ₁ + FB ₂ + FB ₃)
Equids and rabbits	5 ppm (no more than 20% of diet)
Swine and catfish	20 ppm (no more than 50% of diet)
Breeding ruminants, breeding poultry, breeding mink, (includes lactating dairy cattle and hens laying eggs for human consumption)	30 ppm (no more than 50% of diet)
Ruminants ≥ 3 months old raised for slaughter and mink raised for pelt production	60 ppm (no more than 50% of diet)
Poultry raised for slaughter	100 ppm (no more than 50% of diet)
All other species or classes of livestock and pet animals	10 ppm (no more than 50% of diet)

9. WORK AREA REQUIREMENTS

The work area requirements covered under this section apply to FGIS-occupied space only.

a. Sample Grinding Area.

Samples must be ground in space separate from the analytical space. The field office manager and safety officer must determine whether added ventilation or a dust removal device is needed in the grinding area to remove airborne dust particles. Refer to the GIPSA Safety and Health Office in Washington, D.C. for assistance in determining whether added dust removal equipment (e.g., exhaust fan) is required.

b. Sample Testing Area.

Test methods that involve the use of volatile chemicals (e.g., methanol) must be performed in FGIS-approved laboratory space.

10. FGIS LABORATORY REQUIREMENTS

FGIS-approved laboratories are required for mycotoxin testing that involves the use of hazardous materials (e.g., flammable liquids). The requirements covered under this section apply to FGIS-occupied space that is dedicated for the sole function of mycotoxin testing.

Fumonisin testing methods require the use of flammable liquids and suspected carcinogens. The building owner must permit the use of chemicals (e.g., acetonitrile, methanol) in space used by FGIS. FGIS will provide testing services onsite only in facilities that provide protection to FGIS personnel.

Individual elevators may provide two kinds of space for FGIS personnel to perform onsite fumonisin testing. The space may be located (1) in a building along with other occupants, or (2) in a building devoted exclusively to laboratory space.

In either case, the plan for the intended laboratory space is subject to inspection and approval by FGIS prior to construction. The Safety and Health Office and field office manager will review proposed plans and suggest ways to comply with the requirements.

The following are minimum requirements for FGIS-occupied laboratory space.

a. Location.

Locate the laboratory at least 100 feet from the base of the elevator headhouse. This distance is subject to negotiation when the elevator uses exterior grain legs and/or inclined belts in lieu of interior grain legs, or where the headhouse is equipped with blow-out panels, or the headhouse consists of a lightly covered framework.

Laboratories must meet the following requirements when they are located in a building with other occupants:

- (1) Isolate the laboratory from non-laboratory occupants using a fire barrier having at least a 1-hour fire resistance.
- (2) Provide a fire barrier consisting of floors, ceilings, and interior walls.
- (3) Provide all passageways and other openings that lead to adjacent interior space with self-closing fire doors having a 1-hour fire resistance. Do not block these doors open.
- (4) Separate the space from central heating, ventilation, and air-conditioning using automatic-closing fire dampers in the heating, ventilation, and air-conditioning ducts near the fire-barrier, or provide a separate heating, ventilation, and air-conditioning system in the laboratory.

b. Size.

Dedicate the space strictly for laboratory (chemical) work. Supply adequate space for chemical analysis (minimum of 100 square feet).

c. Electrical System.

Provide the laboratory space with electrical power and lighting meeting the standards of the National Electrical Code. Wiring suitable for Class I location is not required. A three-wire system consisting of an energized wire, a neutral wire, and a grounding conductor is satisfactory. Install overhead lighting fixtures through ceilings that serve as fire barriers. Fixtures suspended below such ceilings are acceptable.

d. Plumbing.

Provide the laboratory space with a basin having hot and cold potable water and a sewer connection.

e. Exhaust System.

The exhaust system must remove chemical vapors from the work area. Normal air conditioning and heating may provide adequate ventilation when performing testing procedures in a building devoted exclusively for laboratory space. Refer to the GIPSA Safety and Health Office in Washington, D.C. for assistance in determining whether added ventilation, such as a fume hood, is needed. If needed, situate the laboratory space so that hoods are vented to the exterior of the building. Fume hood ventilation will require a 6 or 8-inch diameter opening, either vertically through the ceiling and roof or horizontally through an exterior wall. In some cases, a portable hood may be sufficient.

f. Eyewash and Safety Shower Station.

Provide the laboratory space with eyewash equipment (eyewash bottle or permanent faucet-mounted fixture). A permanent, faucet-mounted eyewash fixture is highly recommended.

g. Cautionary Markings.

Provide signs for the laboratory door(s) as follows:

- (1) "Biohazardous Material Present"
- (2) "No Smoking, Eating, or Drinking"
- (3) "Flammable Material Present"
- (4) "Wear Safety Protection"
- (5) "Admittance of Authorized Personnel Only"
- (6) Refrigerator Signs

Provide signs for the refrigerator used for storing test kits, chemicals, or solutions, as follows:

- (a) "Biohazardous Material Present"
- (b) "No Food or Drink to be Stored in this Refrigerator"

For further information concerning the laboratory space requirements, contact the GIPSA Safety and Health Office.

11. SAFETY

FGIS employees must comply with good practices to ensure a safe and efficient work environment. To accomplish this, include the following as part of an overall FGIS laboratory/testing area "Standard Operating Procedure" (SOP). Maintain the SOP, this handbook, and current Material Safety Data Sheets (MSDS) at each laboratory/testing location.

During onsite supervision at agency locations, FGIS employees must assess their personal safety requirements. If personal safety is questionable, FGIS employees must determine if personal protective equipment can be used to correct the safety deficiency at the testing location. If FGIS employees cannot utilize personal protective equipment to provide for a safe work environment, then onsite fumonisin supervision must occur only when the testing area is considered safe.

Interested persons are restricted from entering the fumonisin testing area during testing unless accompanied by official personnel and must observe the health and safety rules while in the area.

FGIS personnel must abide by the following safety practices when performing testing in an FGIS-approved laboratory.

- a. Do not smoke, eat, drink, or chew gum or tobacco in the laboratory.
- b. Wash hands immediately before and after eating, drinking, and smoking.
- c. Wear the following protective equipment: disposable, fire-retardant laboratory coat; disposable, impermeable gloves; safety glasses or splash goggles.
- d. Wear an FGIS-approved disposable mask and hair protection when exposed to airborne grain dust.
- e. Do not store food or drink in the laboratory refrigerator used for storing chemicals, solutions, and test kits.
- f. Do not store masks and hair protectors in the grinding area where they might become contaminated by the dust particles.
- g. Label all bottles and containers according to the Hazard Communication Program and the Chemical Hygiene Plan. In addition, when preparing mixtures of solutions, securely apply a label with the name of the solution, the preparation date, and the preparer's initials written in permanent ink.

- h. Store equipment outside the fume hood in a manner that will not clutter bench tops or obstruct movement.
- i. Prepare all chemical solutions and perform chemical analyses under a working fume hood.
- j. Limit the total quantity of waste chemicals in the laboratory to one liquid gallon.
- k. Limit the total amount of flammable solvent (including waste) in the laboratory to two gallons.
- l. Maintain a current MSDS for each chemical in the laboratory. If each supply of chemicals received does not have an MSDS enclosed, contact the company and request one immediately.
- m. Store flammable solvents in an approved storage cabinet.
- n. Store waste chemicals (e.g., methanol) in impermeable metal containers meeting Underwriters Laboratory approval for Class I liquids. The containers must be capable of maintaining a tight seal and must be labeled "Flammable" or "Biohazardous Material" or both, as applicable.
- o. Contact an Environmental Protection Agency (EPA) or EPA approved-certified waste disposal company and make arrangements for removal of chemical waste, or provide other suitable waste disposal procedures consistent with existing laws that do not create a hazard to the community.

12. SANITATION REQUIREMENTS

The sanitation requirements for spillage, labware, and excess sample extract listed in this section are applicable to testing performed at an FGIS-approved laboratory. Official agencies must adhere to the requirements for cleaning labware and should follow procedures established in their area for the disposal of excess sample extract.

Perform the following procedures only while wearing disposable, impermeable gloves, chemical splash goggles, and a fire-retardant laboratory coat. If hands become contaminated, wash immediately with soap and water.

a. Spillage.

Clean areas and materials contaminated by any extraction solution spills. Wipe up the affected areas using an absorbent cloth or paper towels, and then wash the area with a soap/water solution. Place cleaning materials in a plastic waste bag, close tightly, and discard in a dumpster or landfill disposal site.

b. Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, and then rinse with clean water before reusing.

c. Excess Sample Extract.

All sample extracts containing chemicals such as methanol are treated as hazardous chemicals and are disposed of in the chemical waste container. Refer to the appropriate testing procedures for specific waste disposal instructions.

13. SAMPLE SIZE

The manner in which samples are obtained and processed is an important consideration when testing for mycotoxins. To ensure that the test results accurately reflect the fumonisin concentration present in a lot, samples must be representative of the lot and of sufficient size to compensate for uneven distribution of the contaminant. Obtain samples according to the guidelines in the Grain Inspection Handbook, Book I, "Grain Sampling."

The minimum sample size is based on the type of lot. Applicants may request a sample size larger than the minimum sample size.

<u>Lot Type</u>	<u>Minimum Sample Size</u>
Trucks	2 pounds (approximately 908 grams)
Railcars	3 pounds (approximately 1,362 grams)
Barges/Sublots	10 pounds (approximately 4,540 grams)
Submitted Samples	10 pounds / approximately 4,540 grams (recommended)

NOTE: A minimum sample size of 10 pounds is required for composite type samples (e.g., a single sample representing multiple carriers). A 10-pound sample size is also recommended, but not required, for submitted samples.

Testing locations that receive submitted samples that contain less than the recommended 10-pound sample size must grind the entire sample as submitted. For submitted samples that are 10-pounds or more, a minimum of 10 pounds must be ground for testing purposes.

14. SAMPLE PREPARATION

a. Sub-portions.

Grind the entire sample obtained for fumonisin testing and prepare two 500-gram sub-portions from the ground sample. Prepare a 500-gram work portion for original testing services and a 500-gram file sample portion for review testing. For submitted samples, retain as large a sample as possible.

From the 500-gram work portion, obtain a 50-gram test portion and weigh on an FGIS-approved type scale with a minimum division size of 0.1 gram, using one of the following options.

- (1) Collect the 500 gram sample and divide (using an approved divider) out a 50 gram test portion for analysis. Maintain the balance as a file sample.

Or

- (2) Collect the 500 gram sample in a clean container and stir/mix the sample with a spatula or spoon for about 30 seconds ensuring a homogeneous blend (low to high). Using a spatula or spoon dip out a 50 gram test portion for analysis. Maintain the balance as a file sample.

b. Saving File Samples.

Maintain file samples for all lots/samples that do not meet the contractual specification of the applicant for service or are required for the fumonisin monitoring program.

When applicable, maintain a representative file sample for each lot, subplot, composite, or submitted sample tested. For submitted samples that are less than 500 grams, retain as large a sample as possible. For information concerning file sample retention periods refer to FGIS Directive 9170.13, "Uniform File Sample Retention System".

c. Storing File Samples.

If file samples are required, store each sample in a manner that will maintain the integrity of the sample and prevent possible manipulation or substitution. Place the sample in paper bags or envelopes and label each file sample with the test date and identification. Take precautions to ensure that file sample containers are strong enough to prevent loss of sample integrity when storing samples. Do not store samples near heat, windows, or in direct sunlight. (Store samples in cold storage if available).

15. OPERATION OF GRINDERS

Samples must be ground to a fine particle size that is sufficiently fine enough to obtain a homogeneous blend. Avoid over-grinding or pulverizing a sample because it produces an excessively powdery mix that will slow down the filtration process.

Fumonisin samples that contain an excessive amount of moisture content (above 20%) are problematic to the fumonisin grinding and testing procedures. High moisture corn does not grind to a suitable particle size therefore affecting the accuracy of the test results. Therefore, official personnel must ensure that high moisture corn samples are allowed to **naturally dry** to a moisture level of **20% or less before grinding and testing.**

Grinding must be performed in an area separate from the testing area. Use the Romer Mill - Model 2A, Bunn Grinder, or equivalent to grind the sample.

FGIS employees must follow the manufacturer's safety procedures for operating the grinder and must wear protective equipment (i.e., lab coat, mask, gloves, and hairnet) when grinding samples.

a. Romer Mill.

(1) General Operating Instructions:

The Romer Mill simultaneously grinds and subsamples corn at the rate of approximately 1 pound per minute. An adjustable restrictor door located above the collection chute varies the amount of ground sample allowed into the collection chute. Official personnel must adjust the grinder to obtain the required testing and file portions from the sample.

Adjust the grinder by locating the first line (far left) etched on the restrictor door. Position the door approximately 1/3 of the way between the first and second line. For a 10-pound sample, approximately 500 grams will be collected through the collection chute.

Once the grinder is adjusted to obtain the 500-gram sample, mark the location of the setting. To increase the sample size, move the restrictor door to the left.

Samples with high moisture content may cause the grinder motor to overheat and the breaker switch to release. If this occurs, allow the motor to cool and then set the grind lever to the coarsest setting by turning it counterclockwise. After grinding the remainder of the sample at the coarse setting, switch the setting back to fine. Collect the entire 10-pound portion and regrind at the fine setting.

Do not grind high moisture samples on the fine grind setting.

(2) Grinding the Sample:

Grind the entire 10-pound sample with the grind lever set at the finest range. If composite sample is required in addition to the subplot-by-subplot analysis, adjust portion sizes as needed to obtain an adequate size composite and still maintain individual file samples. Obtain the composite sample from the ground subplot samples.

If grinder does not provide an adjustment for obtaining a 500-gram sub-portion as stated in section 15 a. Official personnel must use an approved divider to reduce the size of the ground portion to the stated 500-gram work/file sample.

Note: DO NOT dip out the 500-gram portion used for work and file samples.

b. Bunn Grinder.

(1) General Operating Instructions:

The Bunn-O-Matic grinds corn at a rate of approximately 2 pounds per minute and has a holding capacity of approximately 3 to 4 pounds when fully closed. Official personnel must grind the entire sample and cut it down (using an FGIS-approved divider) to obtain the required testing and file portions from the sample.

Samples with high moisture content may cause the grinder motor to overheat and the breaker switch to release. If this occurs, allow the motor to cool and then set the grind lever to the coarsest setting.

(2) Grinding Samples:

Grind the entire 10-pound sample with the grind lever set at the fine selection. Add 3 to 4 pounds at a time into the hopper until all 10 pounds are ground. If the grinder is experiencing difficulty (e.g., over-heating, bogging down) at the fine setting, change the setting to coarse. After grinding the remainder of the sample at the coarse setting, switch the setting back to fine. Collect the entire 10-pound portion and regrind at the fine setting.

Obtain the composite sample from the ground subplot samples. Official personnel must use an approved divider to reduce the size of the ground portion to the stated 500-gram work and file samples.

Note: DO NOT dip out the 500 gram portion used for work and file samples.

c. Cleaning Grinders.

A small amount of ground sample will remain in the grinder after the total sample has been ground. To prevent the contamination of subsequent samples, clean the grinder using one of the following cleaning procedures.

(1) If a Vacuum Cleaner is Available:

After a sample has been ground and collected, with the unit turned on, use a vacuum cleaner with an attachment that will fit over the mouth of the chute(s). Place the attachment at the bottom of each chute for about 30 seconds. After cleaning the chute(s), turn the power off and prepare for the next sample.

(2) If a Vacuum Cleaner is Not Available:

Clear the grinder by discarding a small portion (first 10 to 15 grams) of the next sample to be tested.

- (a) Pour the sample into the grinder and turn it on long enough to collect the first 10 to 15 grams.
- (b) Turn the power off, and discard the 10-15 grams ground sample.
- (c) Turn the power back on and finish grinding the sample to collect the remaining subsample for analysis.

16. CHECKING PARTICLE SIZE

a. Procedures for Checking the Performance of the Grinder.

For locations that perform mycotoxin testing on coarse (e.g., corn) and small grains, perform the check using a 100-gram sample portion of corn using the following procedures.

- (1) Grind a sample portion of approximately 100 grams of corn having a moisture content of 14.0 percent or less.
- (2) Weigh the entire portion that was ground.
- (3) Sieve the portion across a standard No. 20 wire woven sieve.

- (4) Weigh the portion that passed through the sieve.
- (5) Determine the percent of fine material, by weight, as follows:

Fines = weight from step (4) divided by the weight from step (2) X 100.

For locations that perform mycotoxin testing on small grains only, perform the check using a 100-gram sample portion of wheat (dockage-free) having a moisture content of 13 percent or less.

b. Optimum Particle Size.

The optimum range for particles of coarse and small grain passing through the No. 20 sieve is between 60 and 75 percent. Whenever the ground particles appear to be too coarse, or the results of a grinder check indicate that less than 50 percent of the ground portion passes through the No. 20 sieve, the grinder should be adjusted or repaired to meet the optimum range requirements.

Grinding apparatuses must be checked periodically to determine whether they are producing a final product that meets the particle size requirements as listed above. Official personnel shall determine the frequency of the checks based on a number of items that include visual observation of the ground product, number of samples ground since last check, and time (number of days) since the last check was performed. Record all particle check results in a convenient location for future reference purposes.

17. RIDASCREEN® FAST FUMONISIN TEST KIT

The extraction solution and other materials used in the RIDASCREEN® FAST Fumonisin test kit necessitate the use of separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this directive to ensure a safe and efficient work environment.

a. Preparation of Extraction Solution.

The extraction solvent used in the RIDASCREEN® FAST Fumonisin test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

- (1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.
- (2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- (3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

b. Extraction Procedures.

- (1) Place a sheet of filter paper (Whatman #1 folded or equivalent) into a clean suitable container.
- (2) Label the collection container with the sample identification.
- (3) Weigh a ground 50-gram portion and place it in a suitable container.
- (4) Add 250 ml of 70 percent methanol solution and blend on high for 2 minutes.
- (5) Filter the extract through the Whatman No. 1 filter.

c. Sample Preparation.

- (1) Dilute 100 µl of the filtered extract with 1.3 ml of distilled or deionized water.
- (2) Proceed to the test procedures.

d. Test Procedures.

- (1) Allow reagents and antibody wells to reach room temperature (65° - 86° F) prior to running the test.
- (2) Insert a sufficient number of wells into the microwell holder for all control standards and samples to be tested. (For example: to test 11 samples, use 16 wells - 5 for the standards and 11 for the test samples).

Test Strip #1

Well #	1	2	3	4	5	6	7	8
Sample	C 0	C .222	C .666	C 2.0	C 6.0	S1	S2	S3

Test Strip #2

Well #	1	2	3	4	5	6	7	8
Sample	S4	S5	S6	S7	S8	S9	S10	S11

Where C 0 is the zero control, C .222 is the 0.222 ppm control, C .666 is the 0.666 ppm control, C 2.0 is the 2.0 ppm control, and C6 is the 6.0 ppm control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

NOTE: Do not run more than 3 strips (19 samples) per set of control standards.

- (3) Using a new pipette tip for each standard and sample, pipette 50 µl of standard and prepared sample to separate wells.
- (4) Add 50 µl of enzyme conjugate (red-capped bottle) into each well.
- (5) Add 50 µl of the fumonisin antibody (black-capped bottle) into each well.

- (6) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for 10-15 seconds without spilling reagents.
- (7) Incubate for 10 minutes (\pm 1 minute) at room temperature.
- (8) Dump the contents of the wells. Turn the wells upside down and tap on a paper towel until all the remaining liquid has been removed.
- (9) Using a wash bottle, fill each well with distilled/deionized water. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).
- (10) Add 100 μ l of substrate/chromogen (brown cap- plastic bottle) to each well.
- (11) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for 10-15 seconds without spilling reagents.
- (12) Incubate for 5 minutes (\pm 0.5 minutes) at room temperature. Cover the wells with a paper towel to protect them from light sources.
- (13) Add 100 μ l of stop solution (yellow cap–brown glass bottle) to each well.
- (14) Mix thoroughly by gently sliding the microwell holder back and forth on a flat surface for 10-15 seconds without spilling reagents.
- (15) Measure absorbance at 450 nm using the Biotek EL 301, Awareness Technology Stat-Fax Model 303 PLUS, or the Hyperion Microreader™ 3 Model 4027-002, Microwell readers. Results must be read within 10 minutes.

e. Reading the Results.

- (1) Biotek EL 301 Microwell Reader:
 - (a) Make sure that the microwell reader is on and allowed to warm up for a minimum of 15 minutes before using.
 - (b) Remove sample carriage and hit "Enter".
 - (c) Insert the W2 filter and hit "Enter".
 - (d) Insert the W1 filter (450 nm) and hit "Enter".

- (e) Hit "Clear" and then "Blank". This will cause the instrument to read air as the blank sample.
- (f) Load antibody-coated wells into sample carriage so that the first control labeled 0 is in position A1.
- (g) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.
- (h) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.
- (i) Slide the carriage to position A2 and press "Read". An absorbance value for A2 will appear. Record the value.
- (j) Repeat step (i) until absorbance values have been obtained for all controls and samples. Record the values.
- (k) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

(2) Stat-Fax Model 303 PLUS Microwell Reader.

- (a) To begin from the "Ready" prompt, press "Menu", key in the test number, and then press "Enter".
 - (b) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, and then press "enter". The carrier will advance into the reader, and it should start to print.
 - (c) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N"?
- Press "Yes" (1/A) to print the graph.
- Press "No" (0) to skip this feature.
- (d) The screen will read, "Accept Curve Y/N"?
- Press "Yes" (1/A) to accept the curve, and proceed to read another strip.

When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended".

Press "No" (0) to end the test.

- (3) Hyperion Microreader™ 3 Model 4027-002 Microwell Reader.
- (a) After the power is turned on the instrument will proceed through a calibration mode then advance to the "Main Menu" setting.
 - (b) When prompted to "Run a test," select yes, select the appropriate test number, then press "Enter".
 - (c) At the "Run XXX test?" prompt select yes, select the number of wells (e.g., 8, 12, 16, 24), then press "Enter".
 - (d) At the "Insert strip" prompt insert the test well strip and press "Y" to continue.
 - (e) The reader will read the optical density of the wells and print a report.
 - (f) After the report is printed a "Continue test" prompt will appear. To continue testing select yes and follow the instrument prompts as indicated above.
 - (g) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

f. Reporting and Certifying Test Results.

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed. (Refer to the Supplemental Analysis section of this directive for detailed procedures).

When test results indicate that fumonisin is present at a level of 0.5 ppm or less, certify the results as "Fumonisin equal to or less than 0.5 ppm".

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of this directive for more detailed certification procedures.

g. Cleaning Labware.

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

h. Waste Disposal.

Transfer sample extract solutions (methanol/water) into a liquid waste container for disposal. Follow SOP, established by the field office, for handling and disposing of hazardous waste.

Discard solid material in the trash can for routine disposal.

i. Equipment and Supplies.

(1) Materials Provided in Test Kits:

- (a) 1 microtiter plate.
- (b) 48 antibody coated wells.
- (c) 5 fumonisin standard solutions of 1.3 ml each; 0, 0.222, 0.666, 2.0, and 6.0 ppm fumonisin in water.
- (d) 1 red-capped bottle of 3 ml peroxidase conjugated fumonisin solution.
- (e) 1 black-capped bottle of 3 ml anti-fumonisin antibody.
- (f) 1 brown plastic bottle with brown cap of 10 ml substrate/chromogen.
- (g) 1 brown glass bottle with yellow cap of 14 ml stop reagent.

(2) Materials Required but not Provided:

- (a) ACS Grade methanol.
- (b) Deionized or distilled water.
- (c) 250-ml graduated cylinder.

- (d) 125-ml container.
- (e) Whatman #1 filter paper or equivalent.
- (f) Sample collection tubes.
- (g) Waring high-speed blender (or equivalent) with a one-liter jar.
- (h) Sample grinder.
- (i) Balance.
- (j) Biotek EL 301, Awareness Technology Stat-Fax Model 303 PLUS, or the Hyperion Microreader™ 3 Model 4027-002, microwell readers equipped with a 450-nm filter.
- (k) RIDA™SOFT Win Software.
- (l) Multi-channel pipettor.
- (m) 10 µl, 100 µl, and 1000 µl pipettor and pipette tips.
- (n) Paper towels, Kaydry paper, or equivalent absorbent material.
- (o) Waste receptacle.
- (p) Timer, 3-channel minimum.
- (q) Waterproof marker, Sharpie, or equivalent.
- (r) Wash bottle.

j. Storage Conditions.

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° - 46° F.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided.

The chromogen is light sensitive, therefore, avoid exposure to direct light.

18. NEOGEN VERATOX FUMONISIN TEST METHOD

The extraction solution and other materials used in the Veratox Fumonisin test kit necessitates the use of separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this directive to ensure a safe and efficient work environment.

a. Preparation of Extraction Solution.

The extraction solvent used in the Veratox Fumonisin test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

- (1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.
- (2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- (3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

b. Extraction Procedures.

- (1) Place a sheet of filter paper (Whatman #1 folded or equivalent) into a clean suitable container.
- (2) Label the collection container with the sample identification.
- (3) Weigh a ground 50-gram portion and place it in a suitable container.
- (4) Add 250 ml of 70 percent methanol solution and blend for 2 minutes.
- (5) Filter the extract through the Whatman No. 1 filter. Collect a minimum of 5 ml of the extract.

c. Sample Preparation.

- (1) Dilute the sample by adding 100 µl of the extract to the pink-labeled, pre-filled sample dilution bottle, and mix well.
- (2) The sample extract is now ready for testing without further preparation.
- (3) Proceed to test analysis steps.

d. Analysis Procedures.

- (1) Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to performing the test (approximately 1 hour).
- (2) Remove one red-marked mixing well for each sample to be tested, plus five red-marked wells to be used for controls. Place the wells in the microwell holder.
- (3) Remove an equal number of antibody-coated wells. Immediately return antibody wells that will not be used to the foil pack with desiccant. Fold down ends of the pack and seal with tape to protect the antibody. Mark one end of the strip so that the wells can be identified after washing.
- (4) Mix each reagent by swirling the reagent bottle prior to use.
- (5) Place 100 µl of conjugate from the blue-labeled bottle in each red-marked mixing well.
- (6) Using a new pipette tip for each, transfer 100 µl of controls and sample extracts into the red-marked mixing wells as shown below:

Well #	1	2	3	4	5	6	7	8	9	10	11	12
Sample	C 0	C 1	C 2	C 4	C 6	S1	S2	S3	S4	S5	S6	S7

Where C 0 is the zero control, C 1 is the 1ppm control, C 2 is the 2 ppm control, C 4 is the 4 ppm control, and C 6 is the 6 ppm control. S1 is sample 1; S2 is sample 2, etc.

NOTE: Do not run more than 19 samples per set of control standards.

- (7) Using a 12-channel pipettor, mix the liquid in the wells by pipetting the liquid up and down in the tips 3-4 times. Transfer 100 μ l to the antibody-coated wells. Mix by sliding the microwell holder back and forth on a flat surface for 10-20 seconds without splashing reagents from the wells. Incubate **10 minutes** at room temperature (64° - 86° F). Discard the red-marked mixing wells.
- (8) With a wash bottle, fill each antibody well with deionized or distilled water and then dump the water out. Repeat this step 5 times, then turn the wells upside down and tap on a paper towel until the remaining water has been removed.
- (9) Pipette the needed volume of substrate from the green-labeled bottle into the green-labeled reagent boat. With new tips on the 12-channel pipettor, prime and pipette 100 μ l of substrate into the wells and mix by sliding back and forth on a flat surface for 10-20 seconds. **Incubate 10 minutes.** Discard the remaining substrate and rinse the reagent boat with water.
- (10) Pour the Red Stop solution from the red-labeled bottle (same volume as prepared for substrate) into the red-labeled reagent boat. Eject the excess substrate from the 12-channel pipettor, prime the tips, and pipette 100 μ l of the Red Stop to each well. Mix by sliding back and forth on a flat surface. Discard the tips.
- (11) Wipe the bottom of microwells with a dry cloth or towel and read in the Awareness Stat-Fax Model 321 microwell reader using a 650 nm-filter. Air bubbles should be eliminated, as they could affect analytical results. Results should be read within 20 minutes of completion of the test.

e. Reading the Results with the Stat-Fax Microwell Model 321 PLUS Reader.

To begin from the "Ready" prompt, press Menu. Key in the test number and then press "Enter". The test number is 6.

- (1) The screen will read, "Set carrier to A, press enter". Place wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, and then press enter. The carrier will advance into the reader, and it should start to print.
- (2) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N"?

- (a) Press "Yes" (1/A) to print the graph.
- (b) Press "No" (0) to skip this feature.
- (3) The screen will read, "Accept Curve Y/N"?
 - (a) Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results will print "Test Ended".
 - (b) Press "No" (0) to end the test.
- (4) Record the results for each sample along with the correlation coefficient, slope, and y-intercept data on the data sheet.

NOTE: If the correlation coefficient is less than 0.98 or if the slope exceeds -2.0 ± 0.5 , the Stat-Fax Reader will print "Invalid Calibration" and no results will be reported. If the slope value consistently reads outside these tolerances, contact Neogen as soon as possible to report these findings.

f. Reporting and Certifying the Results.

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed. (Refer to the Supplemental Analysis section of this directive for detailed procedures).

When test results indicate that fumonisin is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of this directive for more detailed certification procedures.

g. Cleaning Labware.

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

h. Waste Disposal.

Transfer sample extract solutions (methanol/water) into a liquid waste container for disposal. Follow SOP, established by the field office, for handling and disposing of hazardous waste.

Discard solid material in the trash can for routine disposal.

i. Equipment and Supplies.

(1) Materials Provided in Test Kits:

- (a) 48 antibody coated microwells.
- (b) 48 red-marked mixing wells.
- (c) 5 yellow-labeled bottles of 1.5 ml each 0, 1, 2, 4, and 6 ppm fumonisin controls.
- (d) 1 blue-labeled bottle of 7 ml fumonisin-HRP conjugate solution.
- (e) 1 red-labeled bottle of 32 ml Red Stop solution.
- (f) 1 dilution kit that contains 40 dilution bottles pre-filled with 7.9 ml of a 10 percent methanol/water solution.
- (g) 1 green-labeled bottle of 24 ml K-Blue Substrate® solution.

(2) Materials Required but not Provided:

- (a) ACS Grade methanol.
- (b) Deionized or distilled water.
- (c) 250-ml graduated cylinder.
- (d) 125-ml container.
- (e) Whatman #1 filter paper or equivalent.
- (f) Filter funnel.

- (g) Sample collection tubes.
- (h) Waring high-speed blender (or equivalent) with a one-liter jar.
- (i) Sample grinder.
- (j) Balance.
- (k) Stat-Fax Model 321 PLUS Microwell reader with a 650 nm filter.
- (l) 12-channel pipettor.
- (m) 100-ul pipettor and pipette tips.
- (n) Paper towels, Kaydry paper, or equivalent absorbent material.
- (o) Waste receptacle.
- (p) Microwell holder.
- (q) Timer: 3-channel minimum.
- (r) Waterproof marker, Sharpie, or equivalent.
- (s) 250-ml plastic squeeze wash bottle.
- (t) 2 reagent boats for use as reagent containers with multi-channel pipettor.

j. Storage Conditions.

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° and 46° F.

19. MYCO✓ FUMONISIN TEST METHOD

The extraction solution and other materials used in the Myco✓ Fumonisin test kit necessitate the use of separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this directive to ensure a safe and efficient work environment.

a. Preparation of Solutions.

(1) Extraction Solution:

The extraction solvent used in the Myco✓ Fumonisin test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

- (a) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.
- (b) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- (c) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.
- (d) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

(2) Wash Solution.

- (a) Transfer the contents of the Wash Concentrate vial to a 500-ml plastic squeeze bottle and add 475 ml of distilled or deionized water.
- (b) Swirl to mix.

b. Extraction Procedures.

- (1) Place a sheet of filter paper (Whatman #1 folded or equivalent) into a funnel mounted over a clean collection container.

- (2) Label the collection container with the sample identification.
- (3) Transfer 50 grams of ground sample into an extraction mixing jar.
- (4) Add 250 ml of the (70/30) methanol/water extraction solvent.
- (5) Cover the extraction jar and blend on high speed for 2 minutes.
- (6) Allow the extract to stand for 2-3 minutes to allow the slurry to settle.
- (7) Filter a minimum of 15 ml of the extract into the collection container.

c. Sample Preparation.

The sample is ready to process without any dilutions. Proceed to test procedures.

d. Test Procedures.

- (1) Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test.
- (2) Place the appropriate number of red mixing wells and clear test wells into a microwell holder.

NOTE: The maximum number of test samples that can be run at one time is 19. Using a strip of 12 wells, designate 5 wells for the calibrators and the remainder of the wells for test samples.

- (3) Using a pipette, dispense 150 µl of Enzyme Conjugate into each red mixing well.
- (4) Dispense 50 µl of each calibrator and sample into the appropriate red mixing wells using an adjustable or fixed 50 µl pipette.

NOTE: Use a clean pipette tip for each addition.

Well Number	1	2	3	4	5	6	7	8	9	10	11	12
Sample/Control	C0	C1	C2	C4	C6	S1	S2	S3	S4	S5	S6	S7

Where C0 is the zero calibrator, C1 is the 1.0 ppm calibrator, C2 is the 2.0 ppm calibrator, C4 is the 4.0 ppm calibrator, and C6 is the 6.0 ppm calibrator. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

- (5) Using a multi-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips into the mixing wells.
- (6) Using a multi-channel pipette, transfer 100 μ l of each reaction mixture directly into the corresponding clear test wells. Discard the mixing wells into an appropriate waste container.
- (7) Let the reaction mixture incubate for **exactly 5 minutes**. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.
- (8) At the end of the 5-minute incubation period, dump the contents of the wells into an appropriate waste container. Using a 500-ml squeeze bottle containing wash solution, vigorously wash each well by overfilling. Repeat the vigorous wash three more times for a **total of four washes**.
- (9) After the last wash, invert the wells and tap on absorbent paper to remove residual wash solution. Wipe excess liquid from the bottom of the wells.
- (10) Pour substrate solution into a clean reagent reservoir.
- (11) Dispense 100 μ l of substrate solution into each test well using a multi-channel pipette.
- (12) Let the substrate solution incubate for **exactly 5 minutes**. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.
- (13) Pour stop solution into a clean reagent reservoir.
- (14) Dispense 100 μ l of stop solution into each test well using a multi-channel pipette.
- (15) Read and record the optical density of the wells at 650 nm using a Hyperion MicroReader™ 3 model 4027-002, or a Biotek EL 301 Microwell Reader. Make sure that the well bottoms are clean and dry before placing in the reader. Read the test results within 20 minutes of test completion.

e. Reading the Results.

(1) Biotek EL 301 Microwell Reader:

- (a) Make sure that the microwell reader is on and allowed to warm up for a minimum of 15 minutes before using.
- (b) Remove sample carriage and hit "Enter".
- (c) Insert the W2 filter and hit "Enter."
- (d) Insert the W1 filter (650 nm) and hit "Enter".
- (e) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.
- (f) Load antibody-coated wells into sample carriage so that the first control labeled 0 is in position A1.
- (g) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.
- (h) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.
- (i) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.
- (j) Repeat step (i) until absorbance values have been obtained for all controls and samples. Record the values.
- (k) Use the data reduction software provided by SDI to quantify results.

(2) Hyperion MicroReader™ 3 Model 4027-002 Well Reader.

- (a) After the power is turned on the instrument will proceed through a calibration mode then advance to the "Main Menu" setting.
- (b) When prompted to "Run a test", select "yes", select the appropriate test number, then press "Enter".

- (c) At the "Run XXX test?" prompt select "yes", select the number of wells (e.g., 8, 12, 16, and 24) then press "Enter".
- (d) At the "Insert strip" prompt insert the test well strip and press "Y" to continue.
- (e) The reader will read the optical density of the wells and print a report.
- (f) After the report is printed a "Continue test" prompt will appear. To continue testing select "yes" and follow the to the instrument prompts as indicated above.
- (g) Use the data reduction software provided by SDI to quantify results.

f. Reporting and Certifying Test Results.

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed. (Refer to the Supplemental Analysis section of this directive for detailed procedures).

When test results indicate that fumonisin is present at a level of 0.5 ppm or less, certify the results as "Fumonisin equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of this directive for more detailed certification procedures.

g. Cleaning Labware.

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

h. Waste Disposal.

Transfer sample extract solutions (methanol/water) into a liquid waste container for disposal. Follow SOP, established by the field office, for handling and disposing of hazardous waste. Discard solid material in the trash can for routine disposal.

i. Equipment and Supplies.

(1) Materials Supplied in Test Kits:

- (a) 48 antibody-coated microtiter wells (4 strips of 12) in foil pouch.
- (b) 48 red-marked mixing wells in poly bag.
- (c) 5 vials each containing 2 ml of 0, 1.0, 2.0, 4.0, and 6.0 ppm of fumonisin calibrators.
- (d) 1 vial containing 10 ml of Fumonisin-HRP enzyme conjugate.
- (e) 1 vial containing 10 ml of substrate.
- (f) 1 vial containing 10 ml of stop solution.
- (g) 1 vial containing 25 ml of 20X wash concentrate.
- (h) 4 multi-channel pipette reservoirs.

(2) Materials Required but not Provided:

- (a) Methanol - ACS grade or better.
- (b) Deionized or distilled water.
- (c) 100 ml graduated cylinder.
- (d) Whatman #1 filter paper or equivalent.
- (e) Glassware with 125 ml capacity for sample extraction.
- (f) Filter funnel.
- (g) 50 μ l pipette with disposable tips.
- (h) 50 200 μ l multi-channel pipette.
- (i) 500 ml plastic squeeze bottle.

- (j) Blender with mixing jars.
- (k) Balance.
- (l) Sample grinder.
- (m) Hyperion MicroReader™ 3 Model 4027-002, or Biotek EL 301 microwell reader equipped with 650 nm filter.
- (n) Timer.
- (o) Waterproof marker.
- (p) Microwell holder.

j. Storage Conditions.

Store test kits between 36° - 46° F when not in use. Avoid prolonged storage of kits at room temperature. Do not freeze test kits.

Do not use reagents from other SDI fumonisin kits with different lot numbers.

Bring kits up to room temperature 64° - 86° F prior to use.

Do not use kit components beyond their expiration date.

20. FUMONITEST 200™ TEST KIT

The extraction solution and other materials used with the FumoniTest 200™ test kit necessitate the use of separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this directive to ensure a safe and efficient work environment.

a. Preparation of Solutions.

(1) Extraction Solution:

The extraction solvent used in the FumoniTest 200™ test method is a methanol/water (distilled or deionized) mixture consisting of 80 percent methanol (HPLC grade) and 20 percent water.

- (a) Using a graduated cylinder, measure 800 ml of methanol and place it into a clean carboy with spigot.
- (b) Add 200 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- (c) Label the container stating the mixture (80 percent methanol and 20 percent water), date of preparation, and initials of technician who prepared the solution.
- (d) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 8 parts HPLC grade methanol to 2 parts of deionized or distilled water.

(2) Phosphate Buffer Saline (PBS):

Prepare the solution by diluting the 100 ml 10X PBS concentrate with 900 ml of distilled or deionized water. Prepare this solution every 7 days or more frequently, if needed.

(3) 0.1% Tween – 20/2.5% PEG/PBS Wash Buffer:

Prepare the solution by diluting the 200 ml 5X PEG/PBS Wash Buffer concentrate with 800 ml of distilled or deionized water. Prepare this solution every 7 days or more frequently, if needed.

(4) Developer A and B Mixture:

Prepare the developer mixture by adding 20 µl of developer B to the 15 ml bottle of developer A.

Prepare this solution every 2 days.

b. Fluorometer Calibration.

An FGIS-approved fluorometer is used to determine the fumonisin level. To ensure accurate results, calibrate the fluorometer prior to use each day and verify at least once an hour using the **Yellow Vial**.

Turn the fluorometer on with the On/Off switch located on the rear panel. When the fluorometer is turned on, allow it to warm up for 10 minutes before calibrating. Once the fluorometer is turned on, it may be left on until close of business for the day. If the fluorometer is turned off during the day, a 10-minute warm up is required.

After turning the fluorometer on, it will identify itself and perform a set of self-tests. If any error message appears, consult the operator's manual.

Follow the procedures listed below to calibrate the fluorometer.

- (1) Set the date, time, test delay time (240 seconds), and measurement units (ppb).
- (2) Follow the prompts on the fluorometer display to calibrate the unit.
- (3) When prompted to insert a calibration vial, wipe the vial with a clean cloth or paper wipe and insert it into the bottom of the well. Be sure that the vial is fully inserted and touches the bottom of the well.
- (4) Enter the correct calibration value (see table below) for the high calibrator (red vial) and low calibrator (green vial).

Note: This step is applicable to the Series III and Series IV fluorometers only. Calibration values are not entered for the MF-2000 Minifluorometer.

- (5) Check the calibration by testing the yellow vial.

Calibrations (in ppm) for Corn, Corn Meal, Corn/Soy Blend, Corn Germ Meal, Sorghum, Flaking Corn Grits, and Popcorn			
	<u>Series III</u>	<u>Series IV</u>	<u>MF-2000</u>
Red	6.0	6.0	*
Green	-0.50	-0.50	*
Yellow	2.8 \pm 0.3	2.8 \pm 0.3	2.4 – 3.1

*** Note: No values for the red and green calibrators.**

The MF-2000 does not give digital display values. Instead, a series of bar graph lights and the FumoniTest™ overlay are used to read the yellow calibrator value. When the yellow vial is inserted, 10 bar graph lights should illuminate. This corresponds to a value between 2.4 – 3.1 ppm. Use the overlay to determine whether the value of the yellow vial is within FGIS specifications.

- (6) Record the result for the Yellow Vial.
- (7) If the value of the yellow calibration vial is not within FGIS specifications, repeat the calibration process (steps 2 through 4 listed above), then check the yellow vial again. If the reading for the Yellow Vial remains above or below FGIS specifications, contact the Mycotoxin Testing Group at Technical Service Division (TSD).
- (8) When the fluorometer is calibrated, place the standards back in the case and close tightly, and store away from any light source.
- (9) Check the calibration of the fluorometer at least once an hour or before analyzing any test samples if more than 1 hour time has elapsed since the last test using the Yellow Vial.

c. Calibration Standards.

(1) Maintenance:

The standard solutions in the three (3) standard vials (Red, Green, and Yellow) degrade slowly in the presence of light.

Since the plastic case containing the vials passes a small amount of light, it is recommended that both case and vials be stored in a cabinet or drawer away from all light except when calibrating or checking the calibration of the fluorometer.

Maintain two (2) sets of standards (two cases) at each location. Select and identify one set as the working standard, the other as the reference standard to be used to check the working standard every 14 days.

The degradation of the working set will occur gradually over a period of time, so anticipate expiration and requisition a replacement set in advance. (A sudden change in the reading of a vial indicates instrument instability, a cracked vial, or undue exposure of the vial to light).

When one vial of a set expires, replace the entire set. About 2 months before the expected expiration of the working set, obtain a new set of control standards from Vicam. When received, compare fluorometer readings of the new set with those of the existing reference set. If the difference between the two sets exceeds 3 ppb for any of the colors, notify TSD.

(2) Biweekly check of working standards:

Calibrate the fluorometer using the working set as described in "Calibration Procedures" (see section 20 b).

After calibrating the working set, remove the reference set from storage and test the three vials as described in section 20 b. The difference in readings of the two sets should not exceed the following limits:

<u>Red</u>	<u>Yellow</u>	<u>Green</u>
± 0.4 ppm	± 0.2 ppm	± 0.1 ppm

If the difference between the working and reference sets exceeds the tolerances, discard the working set. Begin using the old reference set as the working set, and use the new set as the reference set. Keep a permanent record of all calibration verification data.

d. Solution Testing.

The developer solution, PBS solution, distilled/deionized water, and HPLC grade methanol must be tested for background fluorescence before use. After calibrating the fluorometer, perform the following tests.

- (1) Place 2.0 ml of PBS into a clean cuvette. Place the cuvette in the calibrated fluorometer. The displayed reading should be 0 ppm. If the reading is greater than 0, replace the PBS solution.

- (2) Combine 1.0 ml of the developer mixture and 1.0 ml of HPLC grade methanol in a clean cuvette.
- (3) Place the cuvette in the calibrated fluorometer. The displayed reading should be 0 ppm.
- (4) If the reading is 0 ppm, the developer solution and methanol are OK to use. If the reading is greater than 0 ppm, check each reagent separately to determine which reagent is causing the problem and replace it. To check each reagent separately, use the following procedure.
- (5) Place 2.0 ml of HPLC grade methanol into a clean cuvette. Place the cuvette in the calibrated fluorometer. The displayed reading should be 0 ppm. If the reading is greater than 0, replace the methanol.
- (6) Dispense 2.0 ml of the developer mixture into a clean cuvette. Place the cuvette in the calibrated fluorometer. The digital display reading should be 0 ppm. If the reading is greater than 1.0, replace the developer mixture.
- (7) Place 2.0 ml of distilled/deionized water into a clean cuvette. Place the cuvette in the calibrated fluorometer. The displayed reading should be 0 ppm. If the reading is greater than 0, replace the distilled/deionized water.

e. Test Procedures.

(1) Extraction:

- (a) Place 50 grams of ground sample into blender jar.
- (b) Add 5 grams of analytical, USP grade sodium chloride (NaCl) or food grade un-iodized salt.
- (c) Add 100 ml of the 80/20 methanol/water extraction solution.
- (d) Cover jar and blend at high speed for 1 minute.
- (e) Remove the cover and pour the extract into fluted filter paper.
- (f) Collect the filtrate in a clean beaker labeled with the sample identification.

(2) Sample Preparation:

- (a) Pipette or pour 10.0 ml of the filtered extract into a clean beaker.

- (b) Add 40 ml of the 0.1% Tween - 20/2.5% PEG/PBS Wash Buffer and mix thoroughly.
- (c) Filter the diluted extract through a 1.5 µm microfibre filter (Vicam Cat. # 31955) into a clean beaker or directly into the glass syringe barrel. If filtering directly into the glass syringe barrel use the markings on the side of the barrel to measure 10 ml.
- (d) Immediately proceed with the FumoniTest™ Affinity Column procedure.

Note: If this diluted filtrate turns cloudy, refill using a new glass microfibre filter before proceeding with the analysis.

(3) Affinity Column:

- (a) Attach the column to the washing device (either a syringe barrel or an air pumping station) and pass 10 ml of the diluted extract completely through the immunoaffinity column at a rate of about 1 – 1.5 drops per second until air comes through the column.

Note: Sample analysis using these procedures can be greatly simplified by the use of a small aquarium air pump to provide the needed air pressure for loading, filtering, and washing the various extracts.

- (b) Take the column off the glass syringe barrel and put 1 ml of the 0.1% Tween – 20/2.5% PEG/PBS Wash Buffer into the FumoniTest™ Column.
- (c) Attach the column to the syringe barrel and fill the syringe barrel with 10 ml of 0.1% Tween – 20/2.5% PEG/PBS Wash Buffer.
- (d) Pass the solutions through the column at a rate of 1 – 2 drops per second.
- (e) Take the column off the glass syringe barrel and replace the syringe barrel with a clean syringe barrel.
- (f) Put 1 ml of the PBS solution into the FumoniTest™ Column.
- (g) Attach the column to the syringe barrel and fill the syringe barrel with 10 ml of the PBS solution.

- (h) Pass the solutions through the column at a rate of 1 – 2 drops per second.
- (i) Dispense 1.0 ml of HPLC grade methanol into the column. If a syringe barrel rather than the pumping station is used, detach the column, pipette 1 ml of methanol directly into the column headspace, and replace the column.
- (j) Apply a steady pressure to elute/pass the methanol through the column and collect all of the methanol eluate in the cuvette. Maintain pressure to collect the methanol at a rate of approximately 1 drop per second.
- (k) Using a pipettor with a clean tip, add 1.0 ml of Developer A and B mixture directly to the sample eluate solution in the cuvette and mix well (about 5 seconds).
- (l) **Immediately** place the cuvette in a calibrated fluorometer.

(4) Reading, Recording, and Certifying Test Results:

240 seconds (4 minutes) after placing the cuvette into the fluorometer the fumonisin concentration will be displayed. Record the digital readout (Series III and IV) or corresponding bar graph value (MF-2000) as total ppm.

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed. (Refer to the Supplemental Analysis section of this directive for detailed procedures).

When test results indicate that fumonisin is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm".

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results > 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of this directive for more detailed certification procedures.

(5) Supplemental Analysis:

To determine and report a fumonisin level higher than 5 ppm, the filtered test sample extract must be diluted so that a value between 0.5 ppm and 5.0 ppm is obtained. The final fumonisin concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

- (a) Using a pipettor, add 5 ml (instead of 10 ml) of the filtered diluted extract to the top of the Aflatest column headspace. See section 20 e (3) (a).
- (b) Analyze the filtered extract as a normal sample.
- (c) Multiply the analytical results obtained by 2 to obtain the actual fumonisin concentration. For example, if 3.5 ppm was the sample value obtained using the diluted test sample procedure, the actual concentration in the original sample was 7.0 ppm.

Example:	Diluted test sample extract result	3.5 ppm
	Dilution factor	$\times 2$
	Actual aflatoxin concentration	7.0 ppm

Note: Laboratories may dilute samples as a first step if levels typically observed exceed 5 ppm and the applicant requests certified readings above the conformance limit of the test kit.

f. Cleaning Labware.

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

g. Waste Disposal.

Transfer sample extract solutions (methanol/water) into a liquid waste container for disposal. Follow SOP, established by the field office, for handling and disposing of hazardous waste.

Discard solid material in the trash can for routine disposal.

h. Equipment and Supplies Required to Perform Test.

- (1) FumoniTest™ affinity column (Vicam part # G1029 = 25 per box).
- (2) Glass cuvette (Vicam part # 34000).
- (3) HPLC grade methanol.
- (4) 1.5 µm microfibre filter paper – 11cm (Vicam part # 31955).
- (5) Distilled, reverse osmosis, or deionized water.
- (6) Phosphate buffered saline (PBS) (10X concentrate, Vicam part # G1113).
- (7) 0.1% Tween 20/2.5% PEG/PBS Wash Buffer (5X concentrate, Vicam part #G5014).
- (8) FumoniTest™ calibration standards (Vicam part # 33060).
- (9) Commercial blender with stainless steel container (Vicam part # 20200).
- (10) Micro-pipettor, 1 ml (Vicam part # G4033).
- (11) Micro-pipettor, 20 µl (Vicam part # G4031).
- (12) Micro-pipette tips, 50 µl (for 20 µl pipettor, 96 per box) Vicam part # 20658).
- (13) Micro-pipette tips, 1 ml (96 per box) (Vicam part # 20656).
- (14) FumoniTest™ Developer A-fluorometer, 15 ml (Vicam part # G5005).
- (15) FumoniTest™ Developer B (Vicam part # G5004).
- (16) Balance.
- (17) Sample grinder.
- (18) Timer.
- (19) Fluorometer - Romer model RL-100, Vicam Series III and IV, or Vicam model MF-2000.

i. Storage Conditions.

- (1) Store developers and columns in a refrigerator. Bring to room temperature before using.
- (2) Developers A and B should be stored refrigerated until mixing A and B. After mixing the Developer A and B mixture can be stored at room temperature. Store the Developer A and B mixture in Developer A bottle and keep tightly capped when not in use.

21. DIACHEMIX® FUMONISIN FPA

The DIACHEMIX® FUMONISIN QUALITATIVE FPA test kit (Product #61550) uses fluorescence polarization assay technology that provides qualitative (equal to or less than 1 PPM) results in corn only. Fluorescence polarization assays were first commercialized in the 1970s, and are based on measuring the polarization of light caused by changes in molecular size as a result of antigen-antibody reactions. This test kit uses a Sentry™ 100 reader (field portable) that displays results as a (milliP) mP value. (The mP value refers to the polarization measured by the reader and is pronounced “millipee”).

1. Testing Area.

The extraction solution and other materials used in the DIACHEMIX® FUMONISIN FPA test kit does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS approved laboratory or alternate testing space (i.e. table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this directive to ensure a safe and efficient work environment.

2. Sentry™ 100 Sample Reader Calibration.

The SENTRY™ 100 must be calibrated before its first use, every 4 to 6 months or when there is evidence of a performance problem. The SENTRY™ 100 is calibrated using the Calibration Kit provided with the SENTRY™ 100.

(a) Turn on the SENTRY™ 100.

Wait until the “Diachemix LLC” screen is displayed. Use the up or down arrow to highlight the “Calibrate” line. Press the button associated with the “Select” command on the screen to move to the “Calibrate” screen.



(b) Blank Sample Intensity.

- (1) Use the up or down arrow if needed to highlight the “Blank Int.” line.



- (2) Pipette 1 ml of the Blank Standard sample (buffer) into a clean 10 x 75 mm glass borosilicate tube and label the tube. Insert the tube containing the Blank Standard sample in the chamber and close the top of the chamber.
- (3) Read the buffer blank by pressing the button associated with the “Execute” or “Read” command on the screen. “Please Wait” will appear on the screen.
- (4) Use the up arrow to highlight the “Blank Int.” line.
- (5) Perform another read by again pressing the button associated with the “Read” command on the screen again. “Please Wait” will appear on the screen.
- (6) Repeat steps (4) and (5) twice, or as needed to assure a stable read. A stable read would be a series of consistent results (for example, 660...590...620...630). An unstable read would be a series with a substantial variance in results (for example, 660...590...79,000).

(c) Low Polarization Standard.

- (1) Use the up or down arrow if needed to highlight the “Sample Int.” line.

- (2) Pipette 1 ml of Low Polarization Standard into a clean 10 x 75 mm glass borosilicate tube and label the tube. Insert the tube containing the Low Polarization Standard sample in the chamber and close the top of the chamber.
- (3) Read the Low Polarization Standard by pressing the button associated with the "Read" command on the screen. "Please Wait" will appear on the screen.
- (4) Record the value displayed on the "Sample mP" line on a piece of paper.
- (5) Leave the Low Polarization Standard sample in the chamber with the top of the chamber closed.
- (6) Press the button associated with the "Read" command on the screen and then record the resulting "Sample mP" value. Repeat this step eight more times.
- (7) Average the resulting "Sample mP" values from the last 5 of the 10 reads. (Add the last five values together and divide by five).
- (8) The average for the last 5 "Sample mP" values should be between 22 to 28 mP (25 ± 3 mP).
- (9) If the average for the last 5 "Sample mP" values is not between 22 to 28 mP, use the up or down arrow keys to highlight the "G Factor" (Gain Factor) line on the screen.
- (10) Press the button associated with the "Edit" command on the screen.
- (11) Use the numeric keys to change the "G Factor" value displayed on the screen. The "G Factor" assists in calibrating the internal optics.
- (12) Every one one-thousand of a point change (0.001) in the "G Factor" value inversely adjusts the instrument's milliP values by 1 mP. By raising the "G Factor" value one one-thousand of a point (0.001) the instrument's milliP values are lowered by 1 mP. By lowering the "G Factor" value one one-thousand of a point (0.001) the instrument's milliP values are raised by 1 mP. Please note that the instrument automatically inserts a decimal point when all four numbers are input (for example, entering 1 0 0 2 and pressing the button associated with the "Edit" command on the screen inputs the value '1.002').

- (13) Press the button associated with the “Done” command on the screen to accept the edited “G Factor”.
 - (14) Repeat steps (1) through (13) until the average for the last five “Sample mP” values is between 22 to 28 mP (25 ± 3 mP).
- (d) High Polarization Sample Intensity.
- (1) Remain in the screen titled “Calibrate”, use the up or down arrow if needed to highlight the “Sample Int.” line.
 - (2) Pipette 1 ml of High Polarization Standard into a clean 10 x 75 mm glass borosilicate tube and label the tube. Insert the tube containing the High Polarization Standard sample in the chamber and close the top of the chamber.
 - (3) Read the High Polarization Standard by pressing the button associated with the “Read” command on the screen. “Please Wait” will appear on the screen.
 - (4) Record the value displayed on the “Sample mP” line on a piece of paper.
 - (5) Leave the High Polarization Standard sample in the chamber with the top of the chamber closed.
 - (6) Press the button associated with the “Read” command on the screen and then record the resulting “Sample mP” value. Repeat this step eight more times.
 - (7) Average the resulting “Sample mP” values from the last 5 of the 10 reads. (Add the last five values together and divide by five.) The average for the last 5 “Sample mP” values should be greater than 360 mP. If the values are greater than 360 mP, the instrument has passed this quality control check. Please skip to Step (9).
 - (8) The instrument has failed an important quality control check if the average for the last 5 “Sample mP” values is not greater than 360 mP. If this failure does occur, repeat the High Polarization Standard calibration protocol (Steps 1 through 7 above).

If the instrument fails again, then repeat the full Calibration of the Sentry™ 100. If the High Polarization Standard fails again during the full calibration process, contact Diachemix® Technical Assistance and provide a description of the problem, including the “Sample mP” values from the Calibration process.

- (9) Carefully pour the Buffer, Low Polarization Standard and High Polarization Standard from each sample tube back into its appropriate bottle.
 - (10) Press the button associated with the “Back” command to return to the main screen.
- (e) Preparation of Testing Materials.
- (1) Reagents:

Store all FPA reagents in a refrigerator (36°- 45°F) and bring to room temperature for use.

Use all reagents listed below carefully to prevent contamination:

 - (a) FUM Antibody Solution
 - (b) FUM Kit Control
 - (c) FUM-FP-Tracer
 - (2) Consumables:
 - (a) Use clean glassware for each sample to avoid contamination. Do not use scratched or defective test tubes.
 - (b) Do not handle the lower portion of the glass test tube. Fingerprints can distort the FP value.
- (f) Sentry™ 100 Sample Reader Startup.
- (1) Ensure that the reader is calibrated.
 - (2) Turn on the Sentry™ 100 reader and wait for the screen to display “Run Assay”.

- (3) Press the “Select” button.
- (4) Press the “Down” arrow once.
- (5) Press the “Forward” arrow once.
- (6) Advance to the next screen by pressing the “Down” arrow repeatedly until “Run Assay” is highlighted.
- (7) Press the “Select” button to advance to the “Run Assay Batch” screen.
- (8) Keep reading chamber lid closed unless inserting or removing a tube.

(g) Extraction Procedures.

- (1) Transfer 50 grams of ground sample into a clean extraction container.
- (2) Add 250 ml of the distilled or deionized water.
- (3) Blend for 1 minute or shake for 3 minutes, and allow sample to settle 1 minute to obtain clarified sample extract. Particulates may distort the FP value.

NOTE: If particles are present after settling, filter or centrifuge to clarify sample extract. **To Filter:** filter the extract through Whatman 2V (or equivalent) filter paper into a labeled collection container. **To Centrifuge:** transfer 1.0-1.5 ml of sample extract to a labeled micro-centrifuge tube and centrifuge for 10 seconds. Clarified extract is now ready for testing.

3. Test Procedures.

(a) Sample Preparation.

- (1) Pipette 1 ml (1000 μ l) of Antibody Solution into a clean 10 x 75 mm glass borosilicate tube.
- (2) Add 100 μ l of Control solution to the test tube, mark as “Control” and vortex.

- (3) **NOTE:** To vortex the sample: Place the test tube on a vortex mixer to mix for 5 – 10 seconds. Use caution to prevent spills or contamination.
- (4) Similarly prepare a sample tube for each sample by adding 1 ml of Antibody Solution into each individual test tube and 100 μ l of the sample extract to each test tube. Label each tube as needed and vortex.

(b) Sample Analysis.

- (1) While in the “Run Assay Batch” screen, open the reader chamber lid and insert the Control tube into the instrument. Make sure the test tube is inserted to the bottom stop of the reading chamber and close the chamber lid.
- (2) Press the Down arrow once to select the “Blank Int” line and then press the “Read” button.
- (3) After the instrument reading is complete remove the tube. The reader will automatically advance to the next blank intensity reading.
- (4) Continue by reading the sample tubes one by one similar to the Control tube.
- (5) After reading “Blank Int” for all samples, add 100 μ l of Fum-FP Tracer to the first tube (Control). Vortex thoroughly.
- (6) Go to the first reading by entering the number “1” on the reader screen. Place the Control tube back in the reader.
- (7) Press the “Down” arrow to display “Sample Int.” and press the “Read” button. The instrument will display the mP value briefly and it can be retrieved after all the sample tubes have been read.
- (8) Note: If “Control” tube gives a mP value greater than “170” at room temperature, repeat the control.
- (9) Continue with each sample tube using the same steps as the control tube until all samples have been completed.

- (10) To view the results again press the number “1” and then press the “Forward” arrow to advance to the next sample. View each sample’s results in the order run by pressing the “Forward” arrow key.
- (11) Use the Control mP value to set the cutoff value.
- (12) Record the mP value on the work record for Fumonisin testing. The cutoff mP value is the Control mP value + 20. (Example: If the control mP value equals 150 then the cutoff equals 170). Therefore, all results that exceed the cutoff value are considered Negative and all results less than or equal to cutoff are considered Positive (Example: If the cutoff is 170 and the sample result is 230 then the sample is **Negative**. Sample results 170 or less would be certified as **Positive**).

(c) Quality Control Procedures.

- (1) Run the control and calculate the cutoff at the start of each day. Rerun the control and recalculate the cutoff every 100 samples or if the temperature in the lab changes by five degrees (5° F) or more.
- (2) Centrifuge or filter a sample if particulates are present. Particulates may distort FP values.
- (3) Make sure all test tubes are inserted to the bottom stop of the chamber.
- (4) Keep the Sentry™ 100 reading chamber lid closed unless inserting or removing a test tube.
- (5) Do not press the “DONE” key on the reader unless instructed to do so in the protocol.

(d) Reporting and Certifying Test Results.

- (1) Report Negative results on the pan ticket and inspection log as being equal to or less than 1 ppm (≤ 1 ppm). Report Positive results on the pan ticket and inspection log as being greater than 1 ppm (> 1 ppm).
- (2) Certify Negative results as being equal to or less than 1 ppm (≤ 1 ppm) and Positive results as being greater than 1 ppm (> 1 ppm).

- (3) Refer to the Certification section of the handbook for more detailed certification procedures.

(e) Cleaning Labware.

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, and then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

(f) Waste Disposal.

Discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container or down the drain.

(g) Equipment and Supplies.

(1) Materials Supplied in Test Kits. (Product # 61550):

- (a) FUM-FP Tracer.
(b) FUM Kit Control Standard.
(c) FUM Antibody Solution.



(2) Materials required but not provided:

- (a) Sentry™ 100 Reader.
(b) Sample grinder.
(c) Balance.
(d) Distilled/deionized water.
(e) Sample extraction containers.

- (f) 1.0 ml pipettor and pipette tips.
- (g) 100 µl pipettor and pipette tips.
- (h) 500 ml graduated cylinder.
- (i) 10 x 75 mm test tubes.

(3) Optional Equipment and Supplies:

- (a) Mini-centrifuge.
- (b) Whatman 2V filter paper or equivalent.
- (c) Filter funnel.
- (d) Vortex mixer.
- (e) 1.5 ml micro centrifuge tubes.

(h) Storage Conditions and Precautions.

- (1) Test kits should be refrigerated between 36° - 45° F.
- (2) Test Kits can be stored refrigerated for up to 1 year.
- (3) Check test kit expiration date before use.
- (4) Do not use the test kits beyond the noted expiration date.
- (5) Prolonged exposure to high temperatures may adversely affect the test results. Keep all reagents away from heat or flames.

22. ROMER AGRAQUANT® TOTAL FUMONISIN

The extraction solution and other materials used in the Romer AgraQuant® Total Fumonisin test kit necessitates the use of a separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this directive to ensure a safe and efficient work environment.

a. Preparation of Extraction Solution.

The extraction solvent used in the Romer AgraQuant® Total Fumonisin test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

- (1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.
- (2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- (3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

b. Extraction Procedures.

- (1) Place a sheet of filter paper (Whatman #1 folded or equivalent) into a clean suitable container.
- (2) Label the collection container with the sample identification.
- (3) Weigh a ground 50-gram portion and place it in a suitable container.
- (4) Add 250 ml of 70 percent methanol solution and shake or blend for 3 minutes.
- (5) Filter the extract through the Whatman No. 1 filter.

c. Sample Preparation.

- (1) Dilute 50 µl of the filtered extract with 950 µl of distilled or deionized water.
- (2) Sample is now ready for testing without further preparation.

d. Preparation of Solutions.

- (1) Allow reagents and antibody wells to reach room temperature (65° - 86° F) prior to running the test.
- (2) Measure the required amount of Conjugate from the (green-capped bottle) and dispense into a clean reagent boat.
- (3) Measure the required amount of Substrate from the (blue-capped bottle) and dispense into a clean reagent boat.
- (4) Measure the required amount of Stop Solution from the (red-capped bottle) and dispense into a separate reagent boat.

NOTE: Place a cover (paper towel, cardboard, etc.) on each reagent boat to protect from possible contamination and light sources.

e. Test Procedures.

Insert a sufficient number of green bordered dilution strips into the microwell strip holder for all control standards and samples to be tested. (For example: to test 11 samples, use 16 wells - 5 for the standards and 11 for the test samples).

Insert an equal number of antibody coated microwell strips into the microwell strip holder. Return unused microwell strips to the foil pouch with desiccant packet and reseal the pouch.

Test Strip #1

Well #	1	2	3	4	5	6	7	8
Sample	C 0	C 0.25	C 1.0	C 2.5	C 5.0	S 1	S 2	S 3

Test Strip #2

Well #	1	2	3	4	5	6	7	8
Sample	S 4	S 5	S 6	S 7	S 8	S 9	S 10	S 11

Where C 1 is the 0 ppm control, C 2 is the 0.25 ppm control, C 3 is the 1.0 ppm control, C 4 is the 2.5 ppm control, and C 5 is the 5.0 ppm control. S 1 is sample 1, S 2 is sample 2, S 3 is sample 3, etc.

NOTE: Do not run more than 6 strips (48 samples) per set of control standards, when using an 8 channel pipettor. If a single channel pipettor is used do not run more than 2 strips (16 samples) per set of control standards.

- (1) Using an 8 channel pipette dispense 200 μ l of conjugate into each blue bordered dilution wells.
- (2) Using a single channel pipette add 100 μ l of each control standard and prepared sample to the wells containing the conjugate. Use a new pipette tip for each standard and sample.

NOTE: Make sure the pipette tip is completely empty after dispensing.

- (3) Using an 8 channel pipettor with new tips for each dilution well. Mix each well carefully by pipetting up and down 3 times and immediately transfer 100 μ l from each dilution well into a corresponding Antibody Coated Microwell.
- (4) Incubate for 10 minutes (\pm 1 minute) at room temperature. Do not agitate the well plate to mix as it may cause well to well contamination.
- (5) After incubation dump out the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.
- (6) Using a wash bottle, fill each well with distilled/deionized water. Empty out the contents of the wells again and remove all remaining liquid. Repeat this step 4 more times for a total of 5 washes).
- (7) Using an 8 channel pipettor add 100 μ l of substrate to each well.

- (8) Incubate for 5 minutes (± 0.5 minutes) at room temperature. Cover the wells with a paper towel to protect them from light sources.
- (9) Add 100 μl of stop solution to each well, and immediately measure/read results.
- (10) Measure results using Awareness Technology: Stat-Fax Model 303 PLUS Microwell reader. Results must be read within 10 minutes.

f. Reading results using the Stat-Fax Model 303 PLUS Microwell Reader.

- (1) To begin from the "Ready" prompt, press 'Menu', key in the test number, and then press "Enter".
- (2) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, and then press enter. The carrier will advance into the reader, and it should start to print.
- (3) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N"?

Press "Yes" (1/A) to print the graph.

Press "No" (0) to skip this feature.

- (4) The screen will read, "Accept Curve Y/N"?

Press "Yes" (1/A) to accept the curve and proceed to read another strip.

When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended".

Press "No" (0) to end the test.

g. Reporting and Certifying Test Results.

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed. (Refer to the Supplemental Analysis section of this directive for detailed procedures).

When test results indicate that fumonisin is present at a level of 0.5 ppm or less, certify the results as "Fumonisin equal to or less than 0.5 ppm".

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of this directive for more detailed certification procedures.

h. Cleaning Labware.

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

i. Waste Disposal.

Transfer sample extract solutions (methanol/water) into a liquid waste container for disposal. Follow SOP, established by the field office, for handling and disposing of hazardous waste.

Discard solid material in the trash can for routine disposal.

j. Equipment and Supplies.

(1) Materials Provided in Test Kits:

- (a) 96 antibody coated microwells with holder in a sealed pouch.
- (b) 96 non-coated dilution microwells with blue bordering at base.
- (c) 5 fumonisin standard solutions of 1.5 ml each; 0, 0.25, 1.0, 2.5, and 5.0 ppm fumonisins.
- (d) 1 green-capped bottle of 25 ml fumonisin conjugate.
- (e) 1 blue-capped bottle of 15 ml substrate solution.
- (f) 1 red-capped bottle of 15 ml stop solution.

(2) Materials Required but not Provided:

- (a) Methanol, ACS grade or better.
- (b) Deionized or distilled water.

- (c) 8 channel and single channel pipettors capable of pipetting 100 μ l, and 200 μ l with tips.
- (d) 250-ml graduated cylinder.
- (e) 125-ml container.
- (f) Whatman #1 filter paper or equivalent.
- (g) Sample collection tubes.
- (h) Waring high-speed blender (or equivalent) with a one-liter jar.
- (i) Sample grinder.
- (j) Balance.
- (k) Awareness Technology Stat-Fax Model 303 PLUS, Microwell readers equipped with a 450-nm filter.
- (l) Reagents Boats.
- (m) Filter funnels.
- (n) Paper towels, Kaydry paper, or equivalent absorbent material.
- (o) Waste receptacle.
- (p) Timer: 5 minute capacity.
- (q) Waterproof marker, Sharpie, or equivalent.
- (r) Wash bottle.

k. Storage Conditions and Precautions.

The reagents supplied with the test kit can be used until the expiration date on the test kit label when stored refrigerated at temperatures between 36° and 46° F.

Return any unused microwells to their original foil bag and reseal with tape with the desiccant pack provided.

The substrate is light sensitive, therefore, avoid exposure to light sources.

23. CHARM SCIENCES, ROSA® FUMONISIN (QUANTITATIVE) TEST KIT

The extraction solution and other materials used in the Charm Sciences ROSA® Fumonisin (Quantitative) test kit, necessitates the use of separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this directive to ensure a safe and efficient work environment.

Approved Test Kit Information				
Product Number	Conformance Limits		Type of Service	Grain / Commodities Approved for
	Min	Max		
LF-FUMQ	0.5 ppm	5 ppm	Quantitative-	Corn only

Accurate fumonisin measurements above the maximum conformance limit can be obtained by performing a supplemental analysis involving a diluted extract.

a. Preparation of Extraction Solution.

The extraction solvent used in the ROSA® Fumonisin (Quantitative) test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (reagent grade or better) and 30 percent water.

- (1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.
- (2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- (3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

b. Preparation of Testing Materials.

A Negative and Positive Control should be run periodically (daily, weekly, bi-weekly, or monthly) to verify performance of equipment and test strips based on internal quality assurance standards.

(1) Negative Control:

Add 100 µl of 70 percent methanol solution to 1.0 ml of FUM Dilution Buffer to prepare the Negative Control Diluted Extract. Use 300 µl of the prepared extract as your sample, and test following the Sample Analysis Procedures found in section (23 e. (1).

NOTE: Negative Control must read less than or equal to 100 ppb.

(2) Positive Control:

Reconstitute/prepare the Positive Control by adding 300 µl of 70 percent methanol followed by 3.0 ml of FUM Dilution Buffer to the Fumonisin Positive Control. Mix thoroughly for 30 seconds, then allow to stand for 10 minutes at room temperature before use. Mix again before use.

To run the Positive Control use 300 µl as your diluted extract and test the control following the Sample Analysis Procedures found in section 23 d, (2).

NOTE: The Positive Control must read between 400 - 1000 ppb. FUM Positive Control are supplied dry, and must be stored refrigerated between 32 - 45°F.

(3) Equipment Preparation:

- (a) Place clean incubator on a level surface.
- (b) Incubator temperature should be at $45 \pm 1^\circ \text{C}$ (113° F), strip indicator should be green before use.

(4) FUM Dilution Buffer:

- (a) Allow FUM Dilution Buffer to reach room temperature (64-86° F) before use.
- (b) Predispense 1.0 ml of FUM Dilution Buffer into a micro-centrifuge tube for each sample to be tested.

- (c) Store FUM Dilution Buffer and any unused predispensed tubes at 32 - 45°F.

(5) Test Strips:

- (a) Remove ROSA® test strips from moisture resistant foil container and save plastic lid with foil lined foam insert to reseal container.
- (b) In high humidity, limit condensation by opening foil container after is warmed to room temperature (20 to 30 minutes) from the time the container is removed from refrigerator.)
- (c) Remove only the number of strips to be used and return container to 32-45°F storage. Keep these test strips at room temperature for up to 12 hours, and discard any unused test strips.

NOTE: Inspect desiccant indicator. Beads inside desiccant packet should be blue. Discard test strips if desiccant beads turn white or pink.

c. Extraction Procedures.

- (1) Transfer 50 grams of ground sample into a clean suitable container, (e.g. Whirlpak bag or equivalent, 8oz extraction container, or blender jar).
- (2) Add 100 ml of the (70/30) methanol/water extraction solvent.
- (3) Shake or blend for 2 minutes. Allow sample to settle for 1 minute to obtain a clear sample extract.

NOTE: If particles are present after settling, filter or centrifuge to clarify sample extract. **To Filter:** funnel the extract through Whatman 2V (or equivalent) filter paper into a clean/labeled collection container. **To Centrifuge:** transfer 1.0-1.5 ml of sample extract to a labeled micro-centrifuge tube and centrifuge for 10 seconds. The sample extract is now ready for testing.

- (4) Prepare additional sample extracts (up to 4 for quad incubator) following steps 1-3 as stated above in section (c).

d. Test Procedures.

(1) Diluted Extract Sample Preparation:

(a) **Diluted Extract** – Pipette 100 µl of sample extract to a predisposed (1.0 ml FUM Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the Diluted Extract. Repeat for additional samples.

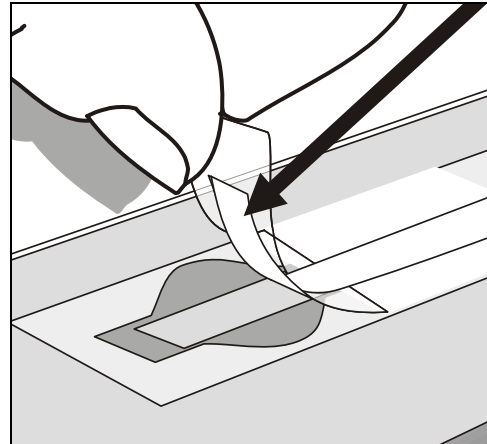
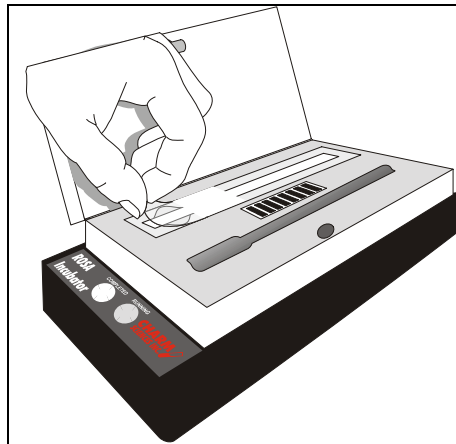
(b) **Second Diluted Extract (0 to 6 ppm)** – is prepared from the Diluted Extract.

1 Pipette 300 µl of Diluted extract to a predisposed (1.0 ml FUM Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the Second Diluted Extract. Repeat for additional samples.

2 Label the test strip to identify sample.

(2) Open the incubator lid and place the test strip in the ROSA® Incubator with the flat side facing upward.

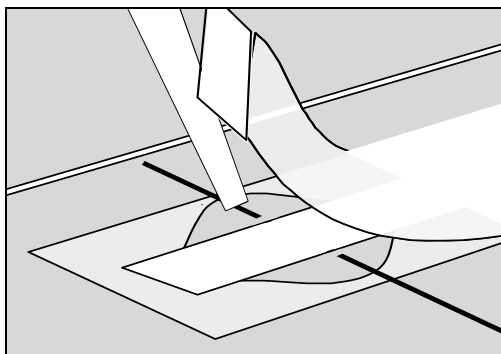
(3) While holding the strip flat on the incubator, use tab to peel tape back to the indicated line exposing the sample pad. Avoid bending back the white wick and sponge under the tape.



e. Sample Analysis Procedures.

(1) Pipette 300 µl (+/- 15µl) of diluted extract, (second diluted extract or control) into the side of the strip sample compartment at the position indicated by the black line on the incubator.

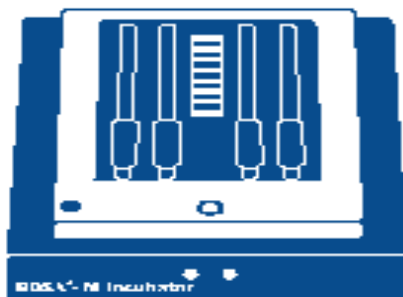
NOTE: Pipette very slowly.



- (2) Reseal the tape over the sample pad compartment. When testing multiple samples, complete the peel, pipette, and reseal steps on each strip before going to the next strip.

NOTE: Add diluted extract, or controls to all strips within 1 minute. If a quad incubator is used, 4 samples can be incubated simultaneously.

- (3) Close lid on the incubator and tighten the latch. The solid red timer light will automatically start when the lid is closed.



ROSA® Incubator with 10-minute timer, and display, set for 45° C (113° F) for Test Strips.

- (4) Incubate for 10 minutes. After the incubation step is complete, a beeper will sound for 2 minutes, and the yellow “test complete” light will begin to flash.
- (5) Remove strip(s) and interpret the results. **Strips must be removed from the incubator and read within 2 minutes of incubation completion.** After strip removal, lower, but do not latch the incubator lid.

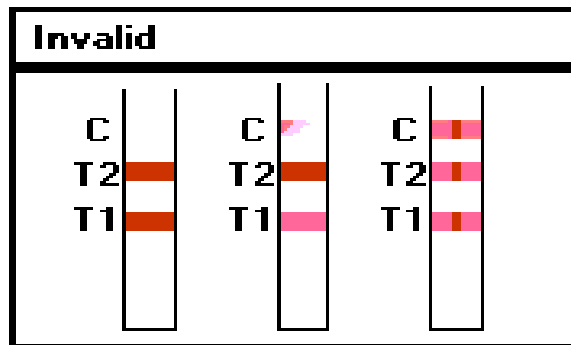
f. Visual Inspection of the Lateral Flow Test Strip.

A test is **invalid** if any of the following is observed:

- (1) Control Line (C) is missing, smeared, uneven.
- (2) Either T1 or T2 (test) lines are uneven.
- (3) Second Diluted Extract or Pos / Neg Control is obscuring the T1, T2, or C lines.
- (4) Beads do not flow past T1, T2, or C lines.

If test is INVALID re-test the Second Diluted Extract or Pos / Neg Control using a fresh dilution, and new test strip.

DO NOT PUT INVALID STRIPS IN ROSA®-INCUBATOR.



g. Interpreting the Lateral Flow Test Strip using the ROSA®-Incubator.

- (1) Insert a clean valid test strip into the ROSA®-Incubator. Slide the strip into the slot, with the sample compartment in the up position, until it stops.

ROSA®-Incubator: is supplied with calibrators.



- (2) Read the result on FUM Channel (3-Line Mode) using the appropriate **MATRIX** on the ROSA®-Incubator. If desired, enter **Sample No.** and/or **Operator Name**. Press **ENTER** to read.
- (3) **READING:** The number displayed is the concentration of fumonisin (ppb or ppm) in the sample. A result in ppb can be converted to ppm by dividing the concentration by 1000 (e.g., 500 ppb = 0.5 ppm). A “+” sign on a READING value indicates that the concentration of the sample is beyond the defined (matrix) scale.
 - (a) The appropriate MATRIX number is MATRIX 01: Assay of Second Diluted Extract (0 – 6 ppm). This extract does not need to be filtered.

h. Reporting and Certifying Test Results.

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed. (Refer to the Supplemental Analysis section (i) for detailed procedures).

When test results indicate that fumonisin is present at a level of 0.5 ppm or less, certify the results as "Fumonisin equal to or less than 0.5 ppm".

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results greater than (>) 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of this directive for more detailed certification procedures.

i. Supplemental Analysis Procedures.

There is a supplemental analysis (Third Diluted Sample) procedure for the Charm Sciences ROSA® Fumonisin test kit.

(1) Third Diluted Extract Sample Preparation.

- (a) **Third Diluted Extract (0 to 60 ppm)** – is prepared from the Second Diluted Extract.
- (b) Pipette 100 µl of **Second Diluted Extract** to a predisposed (1.0 ml FUM Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the Third Diluted Extract. Repeat for additional samples.
- (c) The appropriate MATRIX number is MATRIX 02: Assay of Third Diluted Extract (0 – 60 ppm). This extract does not need to be filtered.

j. Equipment and supplies.

(1) Materials Supplied in Test Kits.

Kits can be purchased that contain 20, 100, or 500 strips and include FUM Positive Control and FUM Dilution Buffer.

(a) LF-FUMQ-20:

- 1 1 Container of 20 Fumonisin test strips.
- 2 1 FUM Positive Control.
- 3 1 FUM Dilution Buffer.

(b) LF-FUMQ-100:

- 1 1 Container of 100 Fumonisin test strips.
- 2 1 FUM Positive Control.
- 3 2 FUM Dilution Buffer.

(c) LF- FUMQ-500:

- 1 5 containers of 100 Fumonisin test strips.
- 2 5 FUM Positive Controls.
- 3 10 FUM Dilution Buffer.

(2) Equipment/Materials required but not included in test kit:

- (a) ROSA®-Incubator.
- (b) Methanol - ACS Reagent grade or better.
- (c) Distilled or Deionized Water.
- (d) 1 ml Pipettor.
- (e) 300 µl Pipettor.
- (f) 100 µl Pipettor.
- (g) 100 ml Graduated Cylinder.
- (h) 1000 ml Graduated Cylinder.
- (i) Micro-centrifuge Tube Rack.
- (j) Filter Funnel.
- (k) Filter Paper (Whatman 2V or equivalent).
- (l) Timer.

(3) Disposable Supplies.

- (a) 200-1000 µl Pipette Tips.
- (b) 20-200 µl Pipette Tips.
- (c) 1.5 ml Micro-centrifuge Tubes.
- (d) Extraction containers (8oz min).

(e) Blender.

(4) Optional Equipment and Supplies (available upon vendor request):

(a) 110/220V: Mini-Centrifuge.

k. Storage Conditions.

(1) Test Strips:

(a) Store refrigerated at 32 - 45°F in a tightly closed moisture resistant container.

(b) Before use remove the test strips storage container and allow it to reach room temperature to limit condensation.

(c) Remove test strips to be used for the day and return the test strips storage container to 32 - 45°F storage.

(d) Strips are stable at room temperature for at least 12 hours.

(e) If the blue desiccant packets in the container turn white or pink, performance test the strips with Negative and Positive Controls before continued use. Discard any strips that indicate invalid test results after performance testing.

(2) Reagents.

(a) Store the Negative & Positive Controls at 32-45°F for up to 1 week, or aliquot and freeze at - 4°F for 2 months.

(b) FUM Dilution Buffer:

1 Use at room temperature.

2 Micro-centrifuge tubes can be predispensed with 1.0 ml of FUM Dilution Buffer.

3 Store bottle and any unused predispensed tubes at 32 -45°F.

24. SUPPLEMENTAL ANALYSIS PROCEDURES FOR THE RIDASCREEN, VERATOX, MYCO✓, AND AGRAQUANT TOTAL TEST KITS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final fumonisin concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the fumonisin result at 9.0 ppm and the conformance limit value is 5 ppm. In order to obtain a true value, dilute 5 ml of the **original extract** with 10 ml of the extraction solution (methanol/distilled or deionized water). This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample.

NOTE: After diluting the sample extract as noted above, start testing procedures from "Sample Preparation" section for the applicable testing method.

Multiply the analytical results obtained by 3 to obtain the actual fumonisin concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True Fumonisin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Fumonisin Result}$$

$$\text{In this example: True Fumonisin Value} = (15 \div 5) \times 3.1 \text{ ppm} \\ = 3 \times 3.1 \text{ ppm} = 9.3 \text{ ppm}$$

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the controls provided with the kits.

25. CERTIFICATION

a. General.

Corn, sorghum, and wheat are tested for fumonisin under the authority of the USGSA. Under the USGSA, fumonisin results are recorded on the pan ticket, worksheet, or loading log and in the remarks section of the inspection certificate.

Certify rice, popcorn, and processed commodities (e.g. corn/soyblend) under the AMA. Certify fumonisin test results on grain in accordance with sections 800.160 through 800.166 of the regulations under the USGSA.

Upon the request of the applicant, separate inspection certificates may be issued for grade and for fumonisin when both are determined on the same lot.

USGSA Title 7, Code of Federal Regulations (CFR) Part 800.125 and 800.135 permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factors and official criteria may be handled separately, even though both sets of results are reported on the same inspection certificate. When official grade or official factors and official criteria are reported on the same inspection certificate, the review inspection certificate shall show a statement indicating that the review results are for official grade, official factors, or official criteria, and that all other results are those of the original, reinspection, or appeal inspection results, whichever is applicable.

b. Standard Reporting and Certification Procedures.

Record the results on the pan ticket and the inspection log to the tenth ppm.

When test results indicate that fumonisin is present at a level of 0.5 ppm or less, certify the results as "Fumonisin equal to or less than 0.5 ppm."

Certify test results that are between 0.6 ppm and the conformance limit (e.g. 5 ppm) to the nearest whole ppm. For example: A fumonisin test result of 5.4 ppm obtained using a fumonisin test kit with a conformance range of 0.5 - 5 ppm would result in the following certification statement "Fumonisin 5 ppm."

Test results greater than the conformance limit are certified as exceeding the conformance limit. For example: A fumonisin test result of 5.5 ppm obtained using a fumonisin test kit with a conformance range of 0.5 - 5 ppm would result in the following certification statement, "Fumonisin exceeds 5 ppm."

c. Certifying Test Results of Single and Combined Lots, Unit Trains, and Shiplots.

(1) Single Lot Inspection Basis for Trucks and Railcars.

Certify each test result on a separate inspection certificate.

(2) Combined Land Carrier Basis for Trucks and Railcars.

If an applicant requests fumonisin testing on a composite basis (up to 5 railcars and 15 trucks) and the inspection for grade on the basis of individual carriers, factor only inspection certificates are issued for the fumonisin testing and separate grade inspection certificates are issued for each carrier.

(3) Composite Sample Testing for Shiplots.

Certify the composite results using the appropriate statement.

(4) Submitted Sample Testing.

Certify the results using the appropriate statement.

(5) Unit Train and Shiplot Inspection under the CuSum Loading Plan.

(a) Recording Test Results.

Fumonisin test results of subplot samples taken throughout loading are recorded on the loading log. A material portion occurs if the subplot result exceeds the limit as specified in the load order.

(b) Certifying Test Results.

Certify the lot based on the mathematical/weighted average (as applicable) of the accepted subplot results using the appropriate statement.

Certify material portions separately.

(c) Material Portions.

If a material portion occurs, the applicant has the option of requesting a review inspection. Review inspection results replace previous results when determining if a material portion exists.

If a material portion designation due to fumonisin is not removed by the review inspection process, the applicant may leave the material portion onboard and receive a separate certificate; return the grain to the elevator; or discharge the material portion along with additional grain in common stowage equivalent to one half the material portion quantity.

d. Approved Statements.

Use one of the applicable statements for certifying fumonisin.

(1) Qualitative Service:

For qualitative service, certify results as being equal to or less than a threshold (e.g., 1 ppm, 5 ppm) or as exceeding the threshold.

"Fumonisin equal to or less than 1 pm."

"Fumonisin exceeds 1 ppm."

(2) Quantitative Service:

(a) When Fumonisin results are less than or equal to 0.5 ppm:

"Fumonisin equal to or less than 0.5 ppm."

(b) Certify fumonisin test results between 0.6 ppm and the conformance limit (e.g., 5 ppm) to the nearest whole number in ppm.

"Fumonisin (result rounded to the nearest whole number) ppm."

(c) When test results are greater than the conformance limit (e.g., 5 ppm).

"Fumonisin exceeds (enter conformance limit) ppm."

(d) Board Appeals performed by the High Performance Liquid Chromatography (HPLC) method are certified to the tenth ppm.

"Fumonisin (record actual results to the nearest tenth) ppm.
Results based on HPLC Reference Method."

e. Optional Statements - Quantitative.

(1) At the request of the applicant, certify test results between 0.6 ppm and the conformance limit to the tenth ppm.

"Fumonisin (result in tenths) ppm."

(2) At the request of the applicant, use the following statement when

fumonisin is not detected (0.0 ppm).

"Fumonisin not detected."

NOTE: If subplot results are combined and averaged and the lot average is equal to 0.0 ppm, but an individual subplot result exceeds 0.0 ppm, then the statement "Fumonisin equal to or less than 0.5 ppm" must be used.

STANDARD CERTIFICATION QUANTITATIVE							
Test Kit Range	Test Result	Certify as:	Test Result	Certify as:	Test Result	Certify as:	
0.5 - 5 ppm	0.5 or less	≤ 0.5 ppm	0.6 - 5.4	Nearest whole ppm	5.5 or more	> 5 ppm	

OPTIONAL CERTIFICATION QUANTITATIVE							
Test Kit Range	Test Result	Certify as:	Test Result	Certify as:	Test Result	Certify as:	
0.5 - 5 ppm	0.0 ppm	Not detected	*	*	*	*	
0.5 - 5 ppm	0.6 - 5.0	Actual tenth ppm	*	*	*	*	

f. Additional Statements.

The statements listed below may be used in addition to the required statements.

- (1) At the request of the applicant, convert and certify the ppm result to parts per billion (ppb) using an approved statement. To convert ppm to ppb, multiply the ppm result by 1000.

"(Actual ppm result) ppm is equivalent to (converted ppb results) ppb."

- (2) At the request of the applicant, convert and certify results in milligrams per kilogram (mg/kg) or micrograms per kilogram ($\mu\text{g}/\text{kg}$). Use the following equivalents to determine mg/kg or $\mu\text{g}/\text{kg}$:

$$\text{ppm} = \text{mg}/\text{kg}$$

$$\text{ppb} = \mu\text{g}/\text{kg}$$

"(Actual ppm result) ppb is equivalent to (converted mg/kg or $\mu\text{g}/\text{kg}$ result)."

- (3) When certifying multiple fumonisin results on the same certificate use the following example as a guideline:

"Sublot sample results: Fumonisin (insert result) ppm."

"Composite sample result: Fumonisin (insert result) ppm."

- (4) Use this statement when the applicant requests the type of test shown on the inspection certificate:

"Results based on (indicate type of test used) method."

- (5) Upon request of the applicant, one of the following statements may precede the applicable results statement when test results are equal to or less than a specified threshold:

"The fumonisin result is negative." OR "Negative fumonisin."

NOTE: These certification statements may be modified as deemed necessary.

g. Reinspection, Appeal, Board Appeal Certificates.

- (1) Results are reported on the same kind of certificate issued for the original service and supersede the previously issued inspection certificate.

Enter the following statement on the reinspection/appeal/board appeal inspection certificate:

"This certificate supersedes Certificate No. (number) dated (date)."

- (2) The superseded certificate is null and void as of the date of the subsequent

(reinspection/appeal/board appeal) certificate.

"The superseded certificate has not been surrendered."

- (3) When a file sample is used, enter the following statement on the reinspection/appeal/board appeal certificate:

"Results based on file sample."

- (4) When reporting more than one official result on the same certificate but at different levels of inspection, explain this condition using one of the following applicable statements:

"(Grade, factor, or official criteria) results based on (new/file) sample. All other results are those of the original inspection service."

"(Grade, factor, or official criteria) results based on the appeal inspection. All other results are those of the (original inspection/reinspection) service."

"(Grade, factor, or official criteria) results based on the Board appeal inspection. All other results are those of the (original inspection/reinspection/appeal inspection) service."

26. QUESTIONS

Direct any questions regarding this directive to the Policies and Procedures Branch at 202-720-0224.

/s/ Robert S. Lijewski

Robert S. Lijewski, Acting Director
Field Management Division