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 testing instructions for the Vicam FumoniTest 200™ test method. This directive supersedes FGIS Directive 9180.71, dated 12-16-02.

3. BACKGROUND

Fumonisins 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 Furarium Ear Rot, the most common corn disease in the Midwestern United States.

More than ten types of fumonisins have been isolated and characterized. Of these, fumonisin B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3) are the major fumonisins produced in nature. The most prevalent of these mycotoxins in contaminated corn is FB_1, 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 Federal Grain Inspection Service (FGIS) or licensed delegated/designated agency personnel. 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 wanting grains officially tested for fumonisin should contact the nearest FGIS field office or delegated/designated agency.

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 five railcars or fifteen trucks per composite sample).

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

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

The maximum size sublot 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 sublot size is 10 railcars.

(3) Export Shiplots

Export shiplots are analyzed on a sublot 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 sublot 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 sublot basis under the provisions of Book III, Inspection Procedures. Components are combined and averaged to determine the sublot result. Component samples will not be designated as material portions due to fumonisin because the Food and Drug Administration (FDA) has not established action limits at this time. Acceptable quality will be based on the sublot result as compared to the contracted "maximum" specification.

c. Warehouse Sample-Lot Inspection Service.

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

5. REVIEW INSPECTIONS

Sections 800.125 and 800.135 of the USGSA 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 sublot 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 sublot 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 most timely 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 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	Conformance Limit
RIDASCREEN® Fast Fumonisin (r-Biopharm Inc)	5 PPM
Veratox Quantitative Fumonisin (Neogen)	5 PPM
Myco✓ Fumonisin (Strategic Diagnostics Inc.)	5 PPM
FumoniTest 200™ (Vicam)	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™
Corn	X	X	X	X
Sorghum	X			X
Wheat		X		
Corn Meal	X	X	X	X
Corn Gluten Meal	X			
Corn Germ Meal	X		X	X
Corn/Soy Blend	X	X		X
Popcorn		X		X
Rough Rice		X		
Flaking Corn Grits				X

7. DISCLAIMER CLAUSE

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

8. FOOD AND DRUG ADMINISTRATION ACTION

The FDA has stated that, currently, there is no direct evidence that fumonisins 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 leukoencephalomalicia, also known as "Blind Staggers".

The recommended maximum levels for fumonisins 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 fumonisins 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.

Human Foods	
Product	Total Fumonisins (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

Animal Feeds	
Corn and corn by-products intended for:	Total Fumonisins (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 FGIS 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 FGIS 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 FGIS 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)-approved or EPA-certified waste disposal company and make arrangements for removal of chemical wastes 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, 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, 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.

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

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.

14. SAMPLE PREPARATION

a. Subportions.

Grind the entire sample obtained for fumonisin testing and prepare two 500-gram subportions from the ground sample, 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, divide (using a boerner divider) out a portion of 50 grams for fumonisin testing and weigh on an FGIS-approved type scale with a minimum division size of 0.1 gram.

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, sublot, 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.

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 moisture content of 20 percent or more 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. 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 a composite sample is required in addition to the sublot-by-sublot 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 sublot 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 of 20 percent or more 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.

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 for 2 minutes.

(5) Filter the extract through the Whatman No. 1 filter.

c. Sample Preparation.

(1) Dilute 100 ul 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° - 86º F) prior to running the test.

(2) Insert a sufficient number of wells into the microwell holder for all 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 ul of standard and prepared sample to separate wells.

(4) Add 50 ul of enzyme conjugate (red-capped bottle) into each well.

(5) Add 50 ul of the fumonisin antibody (black-capped bottle) into each well.

(6) Using a pipettor, mix the wells by pipetting the liquid up and down in the tips 3-4 times.

(7) Incubate for 10 minutes (± 1 minute) at room temperature.

(8) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until 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 µl of substrate/chromagen (white dropper bottle) to each well.

(11) Using a pipettor, mix the substrate/chromagen in the wells by pipetting the liquid up and down in the tips 3-4 times.

(12) Incubate for 5 minutes (± 0.5 minutes) at room temperature. Cover the wells with a paper towel to protect them from light sources.

(13) Add 100 µl of stop solution (yellow or orange dropper bottle) to each well.

(14) Using a pipettor, mix the solutions in the wells by pipetting the liquid up and down in the tips 3-4 times.

(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, 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 "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 fumonisins 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 white dropper bottle of 6 ml Substrate/Chromagen.

(g) 1 yellow or orange dropper bottle of 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° and 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 ul 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 one 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 ul 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 ul 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° - 86º 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 ul 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 ul 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, 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 fumonisins 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) Warning 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 µl 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 3 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 µl 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 µl 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, 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 "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 25ml 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-µl pipette with disposable tips.

(h) 50 -200 µl 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 two 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			
	Series III	Series IV	MF-2000
Red	6.0	6.0	*
Green	-0.50	-0.50	*
Yellow	2.8 ± 0.3	2.8 ± 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 specifcations, contact the Mycotoxin Testing Group at 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 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 3 vials as described in section 20 b. The difference in readings of the two sets should not exceed the following limits:

Red	Yellow	Green
± 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.

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 g 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, refilter 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 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.

(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	x 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-pipet tips, 50 µl (for 20 µl pipettor, 96 per box) Vicam part # 20658)

(13) Micro-pipet 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. SUPPLEMENTAL ANALYSIS PROCEDURES FOR THE RIDASCREEN, VERATOX, AND MYCO✓ 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 section c. "Sample Preparation" for each 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:

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

In this example: True Fumonisin Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

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

22. 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 certificate.

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 certificates may be issued for grade and for fumonisin when both are determined on the same lot.

Sections 800.125 and 800.135 of the regulations under the USGSA 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 certificate. When official grade or official factors and official criteria are reported on the same 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 "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 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 certificates are issued for the fumonisin testing and separate grade 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 sublot samples taken throughout loading are recorded on the loading log. A material portion occurs if the sublot 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 sublot 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) When fumonisin results are less than or equal to 0.5 ppm.

"Fumonisin equal to or less than 0.5 ppm."

(2) 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."

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

"Fumonisin exceeds (enter conformance limit) ppm."

(4) 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.

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

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

OPTIONAL CERTIFICATION								
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 (µg/kg). Use the following equivalents to determine mg/kg or µg /kg:

ppm = mg/kg ppb = µg /kg

"(Actual ppm result) ppb is equivalent to (converted mg/kg or µg /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 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 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."

/s/ David Orr

David Orr, Director
Field Management Division