

Directive

9180.77

04-21-08

OCHRATOXIN A TESTING

1. PURPOSE

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

2. REPLACEMENT HIGHLIGHTS

This directive is revised to include test instructions for the Charm Sciences ROSA® Ochratoxin Quantitative test kit. This directive supersedes FGIS Directive 9180.77, “Ochratoxin Testing”, dated November 19, 2007.

3. BACKGROUND

Ochratoxin A is a toxin produced by *Aspergillus ochraceus*, and *Penicillium verrucosum*, and is one of the most abundant food-contaminating mycotoxins in the world. *Aspergillus* species proliferate in warm humid conditions, while the *Penicillium verrucosum* species is generally associated with moderate climates.

Historically, Ochratoxin has been found at low levels in corn, wheat, oats, peanuts, and other commodities in the United States. Human exposure occurs mainly through consumption of improperly stored food products, contaminated grains, pork products, and some dried fruits.

No regulatory guidance or tolerance levels for Ochratoxin A have been established by the Food and Drug Administration (FDA) at this time. Future regulatory action for Ochratoxin A in feed and feed ingredients will be decided by FDA on a case by case basis.

Official personnel must adhere to approved test kit conformance limits, and service thresholds (e.g. 5 ppb) requested by applicant.

4. TESTING SERVICES

All official Ochratoxin A testing is performed as prescribed in this directive by authorized employees of the Grain Inspection Packers and Stockyards Administration (GIPSA) or official service providers. Testing is performed as an official criteria factor under the authority of the United States Grain Standards Act (USGSA), as amended.

Individuals who wish official Ochratoxin A testing should contact the nearest FGIS field office or official service provider.

Three types of Ochratoxin A 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. Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

For unit trains, the subplot size for Ochratoxin A testing and for grade analysis may be different. For example, an applicant may request grade analysis on the basis of a subplot containing 2 railcars and request Ochratoxin A analysis on the basis of 5 railcars.

The maximum size subplot for Ochratoxin A testing is 5 railcars for unit trains consisting of less than 200,000 bushels, or less than 50 railcars. 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 for Ochratoxin A on a subplot basis. 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 Ochratoxin A 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 wheat 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:

Grain shipments may be tested on a component sample basis in lieu of the subplot basis under the provisions of the Grain Inspection Handbook, Book III, "Inspection Procedures." Components are combined and averaged to determine the subplot result. Component samples will not be designated as material portions due to Ochratoxin A because the Food and Drug Administration (FDA) has not established action limits at this time. 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

7 CFR 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 Ochratoxin A 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 3 review inspections (reinspection, appeal, Board appeal) may be performed on the original inspection service.

Only 1 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 3 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 Ochratoxin A 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 "**OCHRATOXIN A 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 Ochratoxin A 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 "**OCHRATOXIN A 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	Conformance Limit
	Quantitative
OchraTest™ (VICAM)	5 - 100 ppb
ROSA® Ochratoxin test (CHARM)	5 – 100 ppb

The following table lists the Ochratoxin A 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	
	OchraTest™ (VICAM)	ROSA® Ochratoxin (CHARM)
Wheat	X	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. 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.

9. 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.

Ochratoxin A 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 one of two kinds of space for FGIS personnel to perform onsite Ochratoxin A 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 in Washington D.C..

10. 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 Directive, 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 Ochratoxin A supervision must occur only when the testing area is considered safe.

Interested persons are restricted from entering the Ochratoxin A testing area during testing unless accompanied by official personnel, and must comply with all 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 1 liquid gallon.

- k. Limit the total amount of flammable solvent (including waste) in the laboratory to 2 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 waste or provide other suitable waste disposal procedures consistent with existing laws that do not create a hazard to the community.

11. 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.

12. SAMPLE SIZE

A sample of approximately 1000-grams, **with dockage and stones** removed, is required for Ochratoxin A testing and file sample. 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 Ochratoxin A 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	1000-grams
Railcars	1000-grams
Barges/Sublots	1000-grams
Submitted Samples	1000-grams (recommended)

NOTE: A minimum sample size of 1000-grams is required for composite type samples (e.g., a single sample representing multiple carriers). A 1000-gram sample size is also recommended for submitted samples.

For submitted samples that are 1000-grams or more, a minimum of 1000-grams must be ground for testing purposes.

13. SAMPLE PREPARATIONa. Sub-portions.

Grind the entire sample obtained for Ochratoxin A 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 (reinspection, appeal, and Board Appeal). 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 as required for the Ochratoxin A local 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.)

14. 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.

Ochratoxin A samples that contain an excessive amount of moisture content (above 20%) are problematic to the Ochratoxin A grinding and testing procedures. High moisture samples do not grind to a suitable particle size therefore affecting the accuracy of the test results. Therefore, official personnel must ensure that high moisture 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 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 in 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.

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 sample and regrind at the fine setting. Do not grind high moisture samples on the fine grind setting unless the procedures above are followed (coarse grind then fine grind).

(2) Grinding the Sample.

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

If the grinder does not provide an adjustment for obtaining a 500-gram sub-portion as stated in section 13 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 sample with the grind lever set at the fine selection. 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 sample 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 – 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.

15. 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:

$$\% \text{ Fines} = \text{weight from step (4)} \div \text{the weight from step (2)} \times 100.$$

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.

16. **VICAM OCHRATEST™ TEST KIT**

The extraction solution and other materials used with the OchraTest™ 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 OchraTest™ 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 monthly or more frequently, if needed.

(3) OchraTest Elution Solution.

This solution is supplied prepared, ready-to-use in a 50 ml amber bottle.

b. Fluorometer Calibration.

An FGIS-approved fluorometer is used to determine the Ochratoxin A 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 (60 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).
- (5) Check the calibration by testing the yellow vial.

	<u>Series 4</u>	<u>Series 4EX</u>
Red	30	30
Green	-1.3	-1.3
Yellow	14 ± 2	14 ± 2

- (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, and 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 16 b).

After calibrating the working set, remove the reference set from storage and test the 3 vials as described in section 16 b. The difference in readings of the two sets should not exceed the following limits:

<u>Red</u>	<u>Yellow</u>	<u>Green</u>
30 ± 2 ppb	14 ± 2 ppb	0 ± 2 ppb

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 PBS solution, distilled/deionized water, and OchraTest elution solution must be tested for background fluorescence before use. After calibrating the fluorometer, perform the following tests in the "Real Time Mode".

- (1) Press "OPTIONS" button until "Real Time Test" appears on the screen. Press "ENTER".

- (2) Place 2.0 ml of PBS into a clean cuvette. Place the cuvette in the calibrated fluorometer. The displayed reading should be less than 0.5 ppb. If the reading is greater than 0.5 ppb, replace the PBS solution.
- (3) Dispense 1.5 ml of the OchraTest elution solution into a clean cuvette. Place the cuvette in the calibrated fluorometer. The displayed reading should be less than 0.5 ppb. If the reading is greater than 0.5 ppb, replace the solution.
- (4) Place 2.0 ml of distilled/deionized water into a clean cuvette. Place the cuvette in the calibrated fluorometer. The displayed reading should be less than 0.5 ppb. If the reading is greater than 0.5 ppb, replace the distilled/deionized water.
- (5) Press “OPTIONS” button until “OchraTest” appears on the screen. Press “ENTER”.

e. Test Procedures.

(1) Extraction.

- (a) Place 50-grams of ground sample into blender jar.
- (b) Add 100 ml of the 80/20 methanol/water extraction solution.
- (c) Cover jar and blend at high speed for 1 minute.
- (d) Remove the cover and pour the extract into fluted filter paper.
- (e) Collect the filtrate in a clean vessel 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 PBS solution and mix thoroughly.
- (c) Filter the diluted extract through a glass microfibre filter (Vicom 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 OchraTest™ Affinity Column procedure.

(3) Affinity Column.

- (a) Attach the column to a syringe barrel and pass 10 ml of the diluted extract completely through the immunoaffinity column at a rate of about 1 – 2 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) Fill the syringe barrel with 10 ml of PBS solution.
- (c) Pass the PBS solution through the column at a rate of 1 – 2 drops per second.
- (d) Fill the syringe barrel with 10 ml of distilled/deionized water.
- (e) Pass the water through the column at a rate of 1 – 2 drops per second.
- (f) Dispense 1.5 ml of OchraTest elution solution into the syringe barrel.
- (g) Apply a steady pressure to elute/pass the solution through the column and collect all of the eluate in the cuvette. Maintain pressure to collect the methanol at a rate of approximately 1 drop per second.
- (h) Mix well (about 5 seconds) and **immediately** place the cuvette in a calibrated fluorometer.

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

60 seconds (1 minute) after placing the cuvette into the fluorometer the Ochratoxin A concentration will be displayed. Record the digital readout (Series 4 and 4EX) as total ppb.

When test results indicate that Ochratoxin A is present at a level of 5 ppb or less, certify the results as "equal to or less than 5 ppb."

Test results between 6 ppb and 100 ppb are reported on the work record and certified to the nearest whole ppb.

Test results over 100 ppb are reported on the work record and certified as exceeding 100 ppb unless a supplemental analysis is performed. If test results exceed 100 ppb and a supplemental analysis is performed report both results (> 100 ppb and the supplemental test result) on the work record. Certify only the supplemental analysis test result on the certificate.

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

(5) Cleaning Labware.

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

(6) 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.

(7) Equipment and Supplies Required to Perform Test.

- (a) OchraTest™ affinity column.
- (b) Glass cuvette.
- (c) HPLC grade methanol.
- (d) Glass microfibre filter paper – (Vicam part# 31955).
- (e) Distilled, reverse osmosis, or deionized water.
- (f) Fluted filter paper (Vicam part# 31240).
- (g) Phosphate buffered saline (PBS) (10X concentrate, Vicam part# G1113).
- (h) OchraTest™ calibration standards (Vicam part# 33020).
- (i) OchraTest™ elution solution (Vicam part# 32016).

- (j) Blender with stainless steel container.
- (k) Adjustable pipettor, 20-200 µl.
- (l) Pipette tips, 1 ml.
- (m) Balance.
- (n) Sample grinder.
- (o) Timer.
- (p) Fluorometer – Vicam Series 4, or Series 4EX.
- (q) Pump assembly stand.

f. Storage Conditions.

- (1) Store OchraTest™ eluting solution and columns at room temperature.
- (2) Do not put the OchraTest™ eluting solution in a 50 ml bottle dispenser. **Keep the OchraTest™ eluting solution in its original bottle.**

17. CHARM SCIENCES, ROSA® OCHRATOXIN (QUANTITATIVE) TEST KIT

The extraction solution and other materials used in the Charm Sciences ROSA® Ochratoxin (Quantitative) 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 ROSA® Ochratoxin (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 OCHRA 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 (17e).

Note: Negative Control must read less than or equal to 1 ppb.

(2) Positive Control.

Reconstitute/prepare the Positive Control by adding 6.0 ml of OCHRA Dilution Buffer followed by 600 µl of 70 percent methanol to the Ochratoxin Positive Control. Mix thoroughly, and 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 (17e). **The Positive Control must read between 2 - 6 ppb.**

NOTE: OCHRA Positive Control is supplied dry. Store refrigerated at 32 - 45°F.

(3) Equipment Preparation.

- (a) Incubator temperature should be at 113° F (strip indicator should be green at 113° F or before use).

(b) Incubator must be clean and level.

(4) OCHRA Dilution Buffer.

Predispense 1.0 ml of OCHRA Dilution Buffer into a micro-centrifuge tube for each sample to be tested. Allow to reach room temperature (64-86° F) before use and store unused portions at 32-45°F.

(5) Test Strips.

(a) Remove ROSA® moisture resistant container from the refrigerator and allow it to reach room temperature to limit condensation.

(b) Remove only the number of strips to be used and return container to 32-45°F storage. Strips are stable at room temperature for at least 12 hours.

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 clarified extract is now ready for testing.

Prepare additional sample extracts (up to 4 for quad incubator) following steps 1-3 as stated above in section (17c).

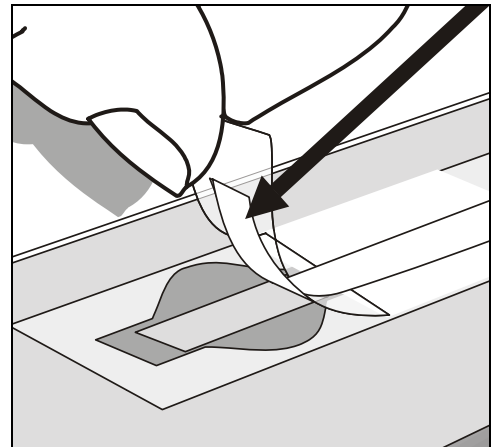
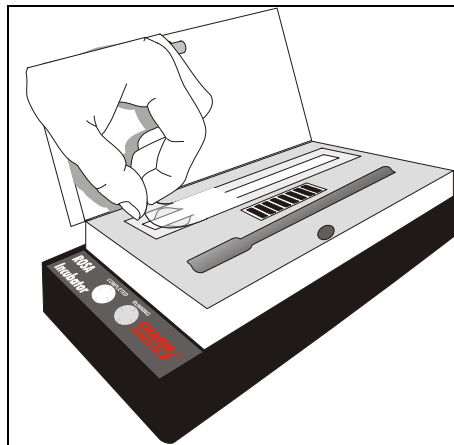
d. Test Procedures.

(1) Sample Preparation.

- (a) Pipette 100 μ l of filtered clarified sample extract to a predisposed (1.0 ml OCHRA Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. Repeat for additional samples.
- (b) Label the test strip to identify sample.
- (c) Filter each sample by drawing into a 1 ml syringe and passing sample through a Minisart RC15 syringe filter. This is the Diluted Extract.

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

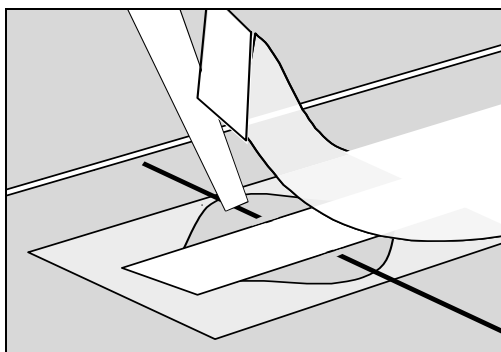
(3) While holding the strip flat on the incubator, use tab to peel tape back to the indicated line exposing the sample pad. Avoid bending back the white wick and sponge under the tape.



e. Sample Analysis Procedures.

(1) Pipette 300 μ l (+/- 15 μ l) 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.

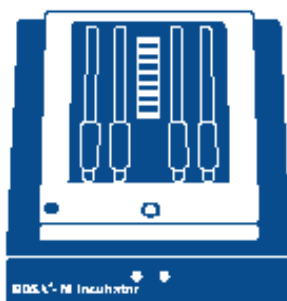
NOTE: Pipette very slowly.



- (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 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.

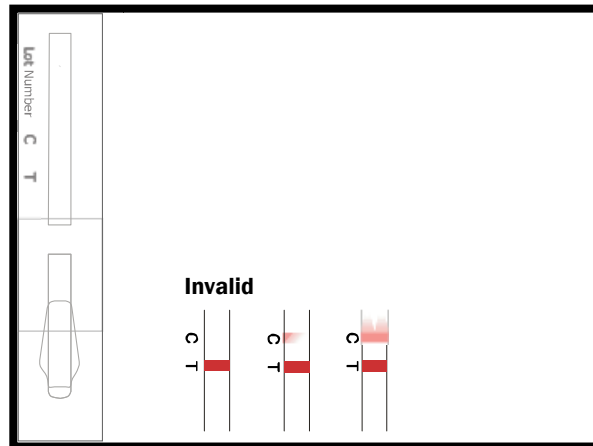


LF-INC4-10-45D: Quad incubator, 10-minute timer with display, set for 45° C for Test Strips.

- (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 a Control Line (C) is missing, smeared, uneven, or if either Test Line is uneven. Additionally, a test is also invalid if the diluted extract is obscuring either the Control (C) or Test Line (T) or if the beads do not flow past the Test and Control Lines. Any strip that does not develop a Control Line should be discarded. A second preparation of the extract (using a fresh dilution) should be made and tested using another strip.



g. Interpreting the Lateral Flow Test Strip using the ROSA-M Reader.

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

LF-ROSA READER-M: ROSA-M Reader supplied with calibrators.



- (2) Read the result on OCHRA Channel (2-Line Mode) using the appropriate **MATRIX 00** on the ROSA-M Reader. If desired, enter **Sample No.** and/or **Operator Name**. Press **ENTER** to read.
- (3) **READING:** The number displayed is the concentration of ochratoxin (ppb) in the sample. A “+” sign on a READING value indicates that the concentration of the sample is beyond the defined scale. For MATRIX 00, a +0012 ppb READING indicates a value greater than 12 ppb.
 - (a) For samples reading 12+ ppb on reader **MATRIX 00**, add 300 µl of diluted extract from step 17d. (1)(c) to a predispensed (1.0 ml OCHRA Dilution Buffer) micro-centrifuge tube, cap, mix and label. This is the **Second Diluted Extract** for quantitation between 10 and 100 ppb using **MATRIX 01**. This extract does not need to be filtered.

h. Equipment and supplies.

- (1) Materials Supplied in Test Kits.

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

- (a) LF-OCHRA-G-20:
 - 1 Container of 20 Ochratoxin test strips.
 - 2 Ochratoxin Positive Control.
 - 3 OCHRA Dilution Buffer.
- (b) LF-OCHRA-G-100:
 - 1 Container of 100 Ochratoxin test strips.
 - 2 Ochratoxin Positive Control.
 - 3 OCHRA Dilution Buffer.
- (c) LF-OCHRA-G-500:
 - 1 5 containers of 100 Ochratoxin test strips.

2 5 Ochratoxin Positive Controls.

3 5 OCHRA Dilution Buffer.

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

- (a) Methanol - ACS grade or better.
- (b) Distilled or Deionized Water.
- (c) 1 ml Pipettor.
- (d) 300 μ l Pipettor.
- (e) 100 μ l Pipettor.
- (f) 100 ml Graduated Cylinder.
- (g) 1000 ml Graduated Cylinder.
- (h) Micro-centrifuge Tube Rack.

(3) Disposable Supplies.

- (a) 200-1000 μ l Pipette Tips.
- (b) 20-200 μ l Pipette Tips.
- (c) 1.5 ml Micro-centrifuge Tubes.
- (d) Whirlpak Bags or equivalent.
- (e) Extraction containers (8oz min).
- (f) Blender.
- (g) Large Weigh Dishes.
- (h) 1 ml Syringes.
- (i) Sartorius Minisart RC 15 Syringe Filters (20/ Box).

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

- (a) 110/220V: Mini-Centrifuge.
- (b) 3 ml Disposable Transfer Pipettes.
- (c) Filter Funnel.
- (d) Filter Paper (Whatman 2V or equivalent).
- (e) Rock-IT Shaker.

i. Storage Conditions.

(1) Test Strips.

- (a) Store refrigerated at 32-45°F in a tightly closed moisture 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) OCHRA Dilution Buffer:
 - 1 Use at room temperature.
 - 2 Micro-centrifuge tubes can be predispensed with 1.0 ml of OCHRA Dilution Buffer.

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

j. Supplemental Analysis Procedures

There is no supplemental analysis procedure for the Charm Sciences ROSA® test kit. If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit.

18. CERTIFICATION

a. General.

Wheat is tested for Ochratoxin A under the authority of the USGSA. Under the USGSA, Ochratoxin A results are recorded on the pan ticket, worksheet, or loading log and certified on an FGIS certificate.

Certify Ochratoxin A test results on grain in accordance with 7 CFR 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 Ochratoxin A when both are determined on the same lot.

7 CFR 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 nearest whole PPB.

When test results indicate that Ochratoxin A is present at a level of 5 ppb or less, certify the results as "Ochratoxin A equal to or less than 5 ppb."

Test results greater than the conformance limit are reported on the work record and certified as exceeding 100 ppb unless a supplemental analysis is performed. If test results exceed 100 ppb and a supplemental analysis is performed report both results (> 100 ppb and the supplemental test result) on the work record. When a supplemental analysis is performed, only certify the final result of the supplemental analysis to the nearest whole ppb.

For example: An Ochratoxin A test result of 150 ppb obtained using a Ochratoxin A test kit with a conformance range of 5 - 100 ppb would result in the following certification statement, "Ochratoxin A exceeds 100 ppb." If a supplemental analysis was performed and the result was 175 ppb certify the result as "Ochratoxin A 175 ppb."

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 Ochratoxin A 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 Ochratoxin A 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.

Ochratoxin A 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 Ochratoxin A 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 Ochratoxin A.

- (1) When Ochratoxin A results are less than or equal to 5 ppb:

“Ochratoxin A equal to or less than 5 ppb.”

- (2) Certify Ochratoxin A test results between 6 ppb and the conformance limit (e.g., 100 ppb) to the nearest whole number in ppb.

"Ochratoxin A (result rounded to the nearest whole number) ppb."

- (3) When test results are greater than the conformance limit (e.g., 100 ppb).

"Ochratoxin A exceeds (enter conformance limit) ppb."

Note: When a supplemental analysis is performed, only certify the final result to the nearest whole ppb.

- (4) Board Appeals performed by the High Performance Liquid Chromatography (HPLC) method are certified to the tenth ppb.

"Ochratoxin A (record actual results to the nearest tenth) ppb.
Results based on HPLC Reference Method."

NOTE: If subplot results are combined and averaged and the lot average is equal to 0 ppb, but an individual subplot result exceeds 0 ppb, then the statement "Ochratoxin A equal to or less than 5 ppb" must be used.

STANDARD CERTIFICATION QUANTITATIVE							
Test Kit Range	Test Result (ppb)	Certify as:	Test Result (ppb)	Certify as:	Test Result (ppb)	Certify as:	
5 - 100 ppb	5 or less	≤ 5 ppb	6 - 100	Nearest whole ppb	101 or more	> 100 ppb	

e. 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 ppb result to parts per million (ppm) using an approved statement. To convert ppb to ppm, divide the ppb result by 1000.

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

- (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:

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

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

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

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

"Sublot sample results: Ochratoxin A (insert result) ppb."

"Composite sample result: Ochratoxin A (insert result) ppb."

- (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 Ochratoxin A result is negative." OR "Negative Ochratoxin A."

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

f. 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/ John Giler

John Giler, Director
Field Management Division