

# Program Notice

FGIS-PN-14-10

05-23-14

## CHARM SCIENCES ROSA® FAST QUANTITATIVE AFLATOXIN TEST METHOD

### 1. PURPOSE

The purpose of this program notice is to provide new instructions for the Charm Sciences ROSA FAST Aflatoxin Quantitative test method, product number LF-AFQ-FAST. The Grain Inspection, Packers and Stockyards Administration (GIPSA), Technology and Science Division (TSD) recertified/approved this kit with additional commodities and supplemental analysis procedures. The test kit provides quantitative analysis of aflatoxin from 5 – 100 parts per billion (ppb) in various grains and commodities.

### 2. BACKGROUND

GIPSA's Field Management Division (FMD), Policies, Procedures, and Market Analysis Branch (PPMAB), and TSD evaluate new test methods, and improvements to previously approved methods, in order to provide the market with performance-verified rapid mycotoxin test kits.

In order to offer newly approved/revised test methods in a timely manner, this program notice is issued prior to the release of the revised Aflatoxin Handbook. The following test procedures are approved for official use by field offices and official service providers.

### 3. TEST INSTRUCTIONS

#### a. General Information.

The Charm ROSA FAST Aflatoxin Quantitative test kit product number LF-AFQ-FAST, is an immunoreceptor assay utilizing lateral flow test strip technology that provides quantitative results for grains and commodities. The test kit has been performance verified to provide aflatoxin measurements from **5 - 100 parts per billion (ppb)** using diluted extracts in designated testing ranges for corn. Manufacturer submitted data supporting the performance of additional grains/commodities are approved. See Approved Test Kit Information below.

Test procedures to report accurate aflatoxin measurement above the maximum conformance limit of 100 ppb are approved using supplemental (additional diluted sample extracts) analysis procedures for **corn only**.

The extraction solution, and other materials used with this test method necessitates the use of a separate Federal Grain Inspection Service (FGIS) approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in the aflatoxin handbook to ensure a safe and efficient work environment.

Obtain samples according to the instructions in the Grain Inspection Handbook, Book I “Grain Sampling,” and refer to the Aflatoxin Handbook Chapter 3 “Sample Preparation for minimum sample requirement and sample preparation procedures.”

<b>Approved Test Kit Information</b>						
<b>Test Kit Vendor</b>	Charm Sciences Inc. 1-978-687-9200					
<b>Test Kit Name</b>	Charm ROSA FAST Aflatoxin Quantitative Test Method					
<b>Product Number</b>	<b>Conformance Limit</b>		<b>Type of Service</b>		<b>Extraction Solution</b>	<b>Supplemental Analysis</b>
LF-AFQ-FAST	<b>Min</b>	<b>Max</b>	<b>Quan</b>	<b>Qual</b>	70%	Yes
<b>QAC Number</b>	5 ppb	100 ppb	X		Methanol (reagent grade or better)	
AFLRA						
<b>Grain/Commodities Approved for</b>						
corn, barley, basmati rice, brewer’s rice, brown rice, corn flour, corn germ meal, corn gluten meal, corn grits, corn meal, corn screenings, corn/soy blend, distiller’s dried grains, distiller’s dried grains w/ solubles, hominy, milled rice, millet, oats, popcorn, rough rice, rye, sorghum, soybeans, wheat, and wheat flour.						

b. Preparation of Extraction Solution.

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

- (1) Using a graduated cylinder, measure 700 milliliters (ml) of methanol and place it into a clean carboy with spigot.
- (2) Add 300 ml distilled or deionized 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. Mix again before use.

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

c. Preparation of Testing Materials.

**Test Negative and Positive Control weekly to verify performance of equipment and test strips.**

(1) Negative Control.

- (a) To prepare add 100 microliters ( $\mu$ l) of 70 percent methanol (extraction solution) to 1.0 ml of AFQ Dilution Buffer to in a micro-centrifuge tube. Cap, mix, and label.
- (b) To run Negative Control, use 300  $\mu$ l as the test sample and test following Sample Analysis procedures found in section e. (3).

**Note: Negative Control must read less than or equal to 2 ppb.**

(2) Positive Control.

- (a) Aflatoxin B1 Positive Control is supplied dry. Store refrigerated.
- (b) Reconstitute/prepare the Positive Control by adding 300  $\mu$ l of 70 percent methanol (extraction solution) followed by 3.0 ml of AFQ Dilution Buffer. Shake well.
- (c) Allow to stand for 10 minutes at room temperature before use. Mix again before use.
- (d) Reconstituted Positive Control is equivalent to Diluted Extract prepared from 20 ppb aflatoxin B1 in corn.
- (e) Store reconstituted Positive Control refrigerated (0 to 7° C) for up to 1 week or aliquot and freeze within 6 hours of reconstitution at (-15° C) or below for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use.
- (f) Store thawed positive Control refrigerated and use within 24 hours of thawing. DO NOT REFREEZE.
- (g) To run the Positive Control, use 300  $\mu$ l as the test sample and test following Sample Analysis procedures found in section e. (3).

**Positive Control must read between 12 to 28 ppb.**

(3) Equipment Preparation.

ROSA Incubator must be clean, placed on a level surface, and temperature must be at  $45\pm 1^{\circ}\text{C}$ . The temperature indicator should match the ROSA Incubator temperature. ROSA Incubator may take more than 10 minutes to reach proper temperature, depending on the ambient temperature.

(4) AFQ Dilution Buffer.

- (a) Use AFQ Dilution Buffer supplied with the test strips at room temperature ( $18 - 30^{\circ}\text{C}$ ).
- (b) Dispense 1.0 ml of AFQ Dilution Buffer into a micro-centrifuge tube for each sample to be tested. If using dispenser, prime with AFQ Dilution Buffer before dispensing. After dispensing, flush dispenser with deionized or distilled water, then flush out the remaining water until only air comes out. Store flushed dispenser in a clean container.
- (c) Store AFQ Dilution Buffer and predispensed micro-centrifuge tubes refrigerated.

(5) Test Strips.

- (a) Store refrigerated in tightly closed supplied container.
- (b) To open, remove ROSA test strips from moisture resistant foil container and save plastic lid with foil lined foam insert to reseal container. Discard foil seal.
- (c) In high humidity, limit condensation by opening foil container after is warmed to room temperature (20 to 30 minutes from the time the container is removed from refrigerator).
- (d) Inspect desiccant indicator. Beads inside desiccant packets should be blue. Do not use test strips if blue beads have turned purple or pink.
- (e) Remove only the number of test strips to be used in one day. Keep these strips at room temperature for up to 12 hours for daily use. Reseal container tightly using the supplied lid and immediately return to refrigerated storage immediately. Discard any unused test strips.

**Note: Test Calibration Strips daily to verify ROSA-M Reader performance. Calibration Strips must test in the specified ranges. If Calibration Strips or Controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance.**

d. Extraction Procedures.

- (1) Transfer 50 grams of ground sample into a clean extraction container.
- (2) Add 100 ml of (70/30) methanol/water extraction solution.

**Note: Add 150 ml of (70/30) methanol/water solution for Corn Germ Meal, Distiller's Dried Grain, Distiller's Dried Grain with Solubles and Soybeans.**

- (3) Shake vigorously for 1 minute.
- (4) Allow sample to settle for 1 minute to obtain a clear sample extract. If particles are present after settling, centrifuge to clarify extract.
  - **To centrifuge**, transfer (1.0 to 1.5 ml) of sample extract using a transfer pipette to a labeled micro-centrifuge tube and centrifuge for 10 seconds to obtain clarified sample extract.
- (5) Prepare additional sample extracts (up to 4 for quad incubator) following steps (1) – (4).
- (6) Proceed to Test Procedures section for preparation of sample extract(s) for analysis.

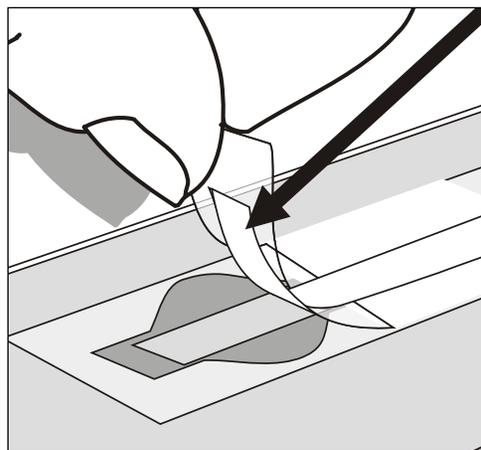
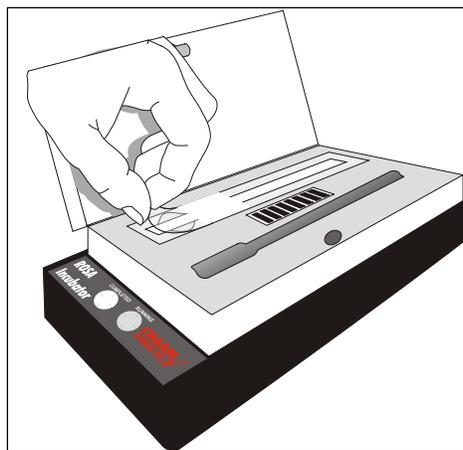
e. Test Procedures.

This test kit uses different testing sensitivity ranges (first and second diluted extracts) for reporting aflatoxin measurements for grains and commodities.

- (1) Sample Preparation of the **First Diluted Extract for 5 to 30 ppb** quantitation.
  - (a) Pipette 100  $\mu$ l of clarified sample extract to a predisposed (1.0 ml AFQ Dilution Buffer) micro-centrifuge tube, cap, mix and label.
  - (b) Repeat for additional samples.

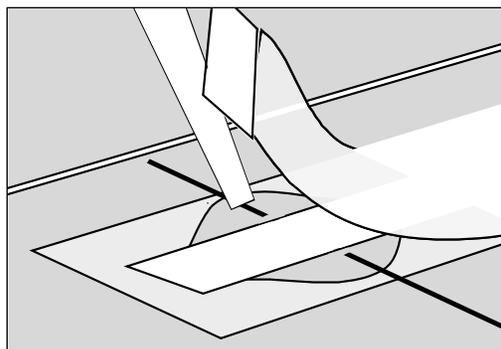
**Note: Filter each diluted extract for barley, corn flour, corn gluten meal, corn grits, corn meal, corn/soy blend, oats, popcorn, rye, wheat, and wheat flour by drawing into a 1 ml syringe and passing through Minisart RC15 syringe filter.**

- (c) Collect filtered Diluted Extract in a clean micro-centrifuge tube, cap, mix, and label.
  - (d) Proceed to Sample Analysis found in section e. (3).
- (2) Sample Preparation of the **Second Diluted Extract** from the First Diluted Extract for 20 to 100 ppb quantitation all grains/commodities.
- (a) Pipette 300  $\mu$ l of the first diluted extract to a predisposed (1.0 ml AFQ Dilution Buffer) micro-centrifuge tube, cap, mix, and label.
  - (b) Repeat for additional samples.
  - (c) Proceed to Sample Analysis section e. (3).
- (3) Sample Analysis.
- (a) Label the test strip to identify sample.
  - (b) Open the incubator lid and place test strip in the ROSA-M Incubator with the flat side facing upward.
  - (c) While holding the strip flat in the ROSA incubator, use tab to **expose sample compartment by peeling tape back to the “Peel to Here” line.** Avoid lifting the test strip and sponge under tape.



- (d) Holding the pipet vertically, slowly pipet **300  $\mu$ l ( $\pm$  15  $\mu$ l)** of the **first, second, supplemental diluted extract, or control** into the sample compartment at the ROSA Incubator line (as shown below).

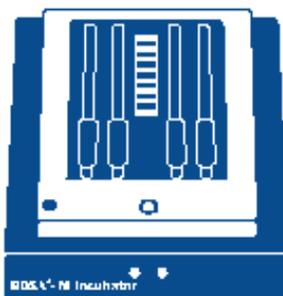
**NOTE: Pipet very slowly.**



- (e) Reseal the tape over the sample pad compartment. When testing multiple samples, complete the peel, pipet, 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.

- (f) Close lid on the incubator and tighten the latch. The timer will automatically start and a red light will illuminate.



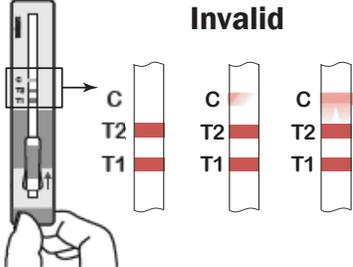
**LF-INC4-3-45D or LF-INC4-5-45D: Quad incubator, 3 or 5-minute timer with display, set for 45° C for Test Strips**

- (g) Incubate for 3 minutes, but not more than 5 minutes for **corn only, and controls**.

**Note: All other approved grain/commodities incubate for 5 minutes, but not more than 7 minutes.**

- (h) After the designated 3 or 5 minute incubation period a beeper will sound, and alternating yellow and red blinking lights will start flashing. This indicates “test complete”.
- (i) Remove strip(s) from the ROSA Incubator. Hold test strip with sample compartment in the down position until interpreted. Do not squeeze sample compartment. Wipe foreign matter (dust, etc.) off test strip.
  - Read test strips within 2 minutes of incubation completion.
  - Lower lid on the ROSA Incubator, but do not latch.

f. Visually Interpreting the Lateral Flow Test Strip.

 <p style="text-align: center;"><b>Invalid</b></p>	<p>The test is <b>INVALID</b> if any of the following are observed:</p> <ul style="list-style-type: none"> <li>• <b>C</b> (Control) line is missing.</li> <li>• <b>T1, T2</b> (Test) or <b>C</b> line is smeared or `uneven.</li> <li>• <b>T1, T2</b> or <b>C</b> line is obscured by diluted extract or Control.</li> <li>• Beads do not flow past <b>T1, T2</b> or <b>C</b> lines.</li> </ul> <p>If test is <b>INVALID</b> re-test the diluted extract or Control.</p> <p><b>DO NOT PUT INVALID TEST STRIPS IN ROSA-M READER</b></p>
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g. Interpreting the Lateral Flow Test Strip using the ROSA-M Reader.

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



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

Read result on **AFLA SL** channel (3-line mode) using the appropriate **MATRIX** on the ROSA-M Reader. If desired, enter **Sample** and/or **Operator**. Press **ENTER** to read.

**NOTE:** Use the following table to determine the appropriate Matrix number. The appropriate **MATRIX** numbers are as follows:

<b>Matrix 00</b>	Assay for analysis of <b>First</b> diluted extract for <b>5 – 30</b> ppb quantitation <b>corn only</b> .
<b>Matrix 01</b>	Assay for analysis of <b>Second</b> diluted extract for <b>20 – 100</b> ppb quantitation <b>corn only</b> .
<b>Matrix 02</b>	Assay for analysis of <b>First</b> diluted extract for <b>5 – 30</b> ppb quantitation for -. barley, basmati rice, brewer’s rice, brown rice corn flour, corn gluten meal, corn grits, corn meal, corn screenings, corn/soy blend, hominy, milled rice, millet, oats, popcorn, rough rice, rye, sorghum, wheat, and wheat flour.
<b>Matrix 03</b>	Assay for analysis of <b>Second</b> diluted extract for <b>20 – 100</b> ppb quantitation. (See <b>Matrix 02 grain/commodities list</b> )
<b>Matrix 04</b>	Assay for analysis of <b>First</b> diluted extract for <b>5 – 30</b> ppb quantitation for corn germ meal, distiller’s dried grains, distiller’s dried grains w/solubles, and soybeans.
<b>Matrix 05</b>	Assay for analysis of <b>Second</b> diluted extract for <b>20 – 100</b> ppb quantitation. (See <b>Matrix 04 grain/commodities list</b> )
<b>Matrix 06</b>	Assay for analysis of <b>Supplemental</b> diluted extract ( <b>corn only</b> ) for 10 to 1100 ppb (Uncorrected Aflatoxin) quantitation.

- (2) **READING:** The number displayed is the concentration of aflatoxin (ppb) in the first and second diluted sample extracts.

A + sign reading on the first diluted sample extract indicates that the concentration of the sample is greater than the sensitivity range of test sample. A Second Diluted Extract should be prepared and analyzed for 20 – 100 ppb quantitation.

For example:

- **First Diluted Extract:** READING of **“+30 ppb”** indicates a value greater than the sensitivity range of the test sample.
- Prepare a **Second Diluted Extract** and run another test strip and READ on the appropriate MATRIX for quantitation from **20 to 100 ppb**, or report as exceeding the sensitivity range of **30 ppb**. See above chart above to select the appropriate MATRIX.
- **Second Diluted Extract READING** of less than **20 ppb** is not within the test range and must be retested using the First Diluted Extract to report quantitative results. See Note:

**Note: Applicants may request qualitative certification in lieu of retesting of results outside the first diluted or second diluted extract test sample sensitivity ranges/concentrations.**

- **Second Diluted Extract READING** greater than **100 ppb** indicates a value greater than the TSD approved maximum conformance limit, and should be reported as exceeding 100 ppb for all grains except **corn**.

To report results above the Second Diluted Extract sensitivity range (**corn only**), a **Supplemental Diluted Sample Extract** must be prepared and tested. See Supplement Analysis section for more information.

h. Reporting and Certifying Test Results.

- (1) Report all results on the pan ticket and the inspection log to the nearest whole ppb.
- (2) Sample results over the upper limit of the First or Second Diluted Extracts are reported as greater than the stated sensitivity range. Supplemental Analysis Testing is approved for (**corn only**) using a Supplemental Diluted Extract.

Sample results over the upper limit of 1100 ppb of the **Supplemental Diluted Extract** are reported as greater than 1100 ppb. Refer to the Certification section of the handbook for more detailed certification procedures.

i. Supplemental Analysis.

The following describes procedures for diluting and testing samples to report aflatoxin results greater than 100 ppb for **corn only** using ROSA FAST Aflatoxin Quantitative Test.

A Supplemental Diluted Extract is prepared by diluting the Second Diluted Extract with AFQ Dilution Buffer and tested on another test strip to determine the Uncorrected Aflatoxin Concentration.

- (1) Prepare Second Diluted Extract according in Test Procedures found in section e. (2).
- (2) Prepare Supplemental Diluted Extract from the second diluted extract to dilute the sample concentration.
  - (a) Pipet 1 ml of AFQ Dilution Buffer into a micro-centrifuge tube.
  - (b) Pipet a designated volume (**see chart below**) of the Second Diluted Extract needed (based on anticipated concentration level) into the micro-centrifuge tube with the AFQ Dilution Buffer cap, mix, and label. This is the Supplemental Diluted Extract.
    - For a testing range of **100 ppb to 430 ppb** add **300 µl** of the Second Diluted Extract.
    - For a testing range of **110 ppb to 1100 ppb** add **100 µl** of the Second Diluted Extract.
  - (c) Determine/record the Dilution Factor used to prepare the dilution required to determine the final Corrected Aflatoxin Concentration. The Dilution Factor (DF) is equal to the sum of the volume of the AFQ Dilution Buffer plus the volume of the Second Diluted Extract divided by the volume of the Second Diluted Extract.

See chart below for examples:

Dilution Factor	AFQ Dilution Buffer	Second Diluted Extract Volume	Divided by the Second Diluted Extract Volume
4.33 =	1.0 ml	<b>(plus)</b> 300 µl (0.3 ml)	<b>(divided by)</b> 0.3 ml
11 =	1.0 ml	<b>(plus)</b> 100 µl (0.1 ml)	<b>(divided by)</b> 0.1 ml

- (d) Follow instruction in Sample Analysis found in section e. (3) for analysis of the Supplemental Diluted Extract using 300 µl of Supplemental Diluted Extract.
- (e) Read results on AFLA SL (3-line mode) with MATRIX 06.

Valid Supplemental Diluted Extract READINGS must be within 10 to 100 ppb detection range.

A reading less than 10 ppb is below the detection range. Prepare another Supplemental Diluted Extract (step (2)) with a lower Dilution Factor, and run another test strip to quantitate. A reading of “+100 ppb” indicates that the concentration of the sample is greater than the test range. Prepare another Supplemental Diluted Extract (step (2)) with a higher Dilution Factor and run another test strip to quantitate.

- (f) The number/result displayed on the ROSA-M Reader is the Uncorrected Aflatoxin Concentration in the sample.
- (g) To covert the Uncorrected Aflatoxin Concentration to the final Corrected Aflatoxin Concentration in the sample. Follow instruction below: Multiply the result displayed on ROSA-M Reader by the Dilution Factor used to prepare the Supplemental Diluted Extract.

For example:

If the Uncorrected Aflatoxin Concentration is 30 ppb (as displayed on the ROSA-M Reader) and the Dilution Factor is 11 the final Corrected Aflatoxin Concentration is 330 ppb (30 ppb x 11 = 330 ppb).

**Note: It is recommended that locations document the volume of second diluted extract and AFQ Dilution Buffer used to determine the DF for informational purposes and quality assurance needs.**

**Note: Supplemental Diluted Extract may initially be used when the aflatoxin concentration is expected to be greater than 100 ppb.**

j. Sanitation.

The sanitation requirements for spillage, labware, and equipment listed are applicable to testing performed in a GIPSA approved laboratory or an alternate testing location depending on test method used.

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

1. Spillage.

Clean all area(s) and materials contaminated by any extraction solution spills. Wipe up the affected area using an absorbent cloth or paper towels, then; wash the area with a warm soap/water solution. Place contaminated cleaning materials in a plastic waste bag, close tightly, and discard with solid hazardous waste material.

2. Labware and Testing Equipment.

Testing methods that use chemicals (methanol, ethanol, and acetonitrile, etc.) must prepare a solution consisting of dishwashing liquid and warm water. Completely submerge all used glassware (i. e. funnels, beakers, etc.) for 5 to 10 minutes, wash thoroughly, then rinse with clean warm water, allow to air dry before reusing. Ensure all testing equipment is free of all associated chemicals and residual sample dust prior to testing.

3. Disposable Materials.

(a) Non-contaminated Disposables:

1. Empty the contents of all chemical solvents from used test kit components (e.g. micro-wells, test columns, test vials, disposable sample cups, pipet tips) into a hazardous waste container.
2. Collect the **completely** emptied test kit components in a garbage bag for landfill disposal.

## (b) Contaminated Disposables:

1. Collect **contaminated** disposable materials (e.g. contaminated gloves, paper towels) with other solid waste in an approved waste container for hazardous waste disposal according to EPA guidelines.
2. Remove all contaminated materials from the laboratory on a daily basis as part of general laboratory/good housekeeping procedures.

## k. Hazardous Waste Disposal.

The liquid portion of any solvent-based extraction kit is considered a hazardous waste because of its ignitability. It cannot be disposed of in the sink or the regular trash. It must be poured into a separate hazardous waste container. This is considered a hazardous waste satellite container.

The waste must be compatible with the container that is used. In other words, the container must be able to hold the waste without deteriorating. In most cases this could be either a glass, plastic or metal container that must be properly labeled

## (1) Liquid Waste:

In some cases the satellite container could be a 55 gallon metal or plastic drum. Locations that generate smaller quantities of waste may use smaller approved containers.

- (a) All satellite containers must be kept closed except when liquid waste is being added.
- (b) When the satellite container is full it must be emptied in the hazardous waste container within 3 days. This hazardous waste container is typically a 55 gallon metal or plastic closed top drum.
- (c) A hazardous waste label **must** be affixed to this drum/container when the first liquid is added to the drum. This is the drum/container that will be shipped to the hazardous waste disposal facility. Since this drum will be used for shipping, it must meet specific DOT regulations.
  - If the drum is new it must be stamped with a 1A1 designation while a plastic drum must be designated a 1H1.

- If using a recycled drum the drum must have a sticker attached indicating the above designations.
- (d) The hazardous waste drum maybe located in another area that is secure:
- Stored inside a secondary containment bin in case of a leak.
  - Visually posted emergency contact information, and accessibility to a phone.

(2) Solid Waste:

Since many of the solid materials (grain residue and filter paper) retain some of the liquid from the extraction and filtering process they are also considered hazardous waste because of their ignitability. They cannot be disposed of in the regular trash, must be placed in a separate container and considered hazardous waste.

The waste must be compatible with the container that is used. Based on the waste the container would have to be either plastic or metal with a wide opening and is usually a smaller container in the immediate area of the waste generation. This container must be labeled as a satellite hazardous waste container

- (a) The satellite container must be kept closed except when someone is adding material to the container.
- (b) In some cases the satellite container could be a 55 gallon metal or plastic drum with an open top.
- (c) When the satellite container is full it must be emptied into hazardous waste container within 3 days.
- (d) This hazardous waste container is typically a 55 gallon open top plastic or metal drum.
- (e) A hazardous waste label **must** be affixed to this drum when the first material is added to the drum. This is the drum that will be shipped to the hazardous waste disposal facility. Since this drum will be used for shipping, it must meet specific DOT regulations.
- If the drum is new it must be stamped with a 1A2 designation while a plastic drum must be designated a 1H2.

- If using a recycled drum the drum must have a sticker attached indicating the above designations.
- (f) The hazardous waste drum maybe located in another area that is secure:
- Stored inside a secondary containment bin in case of a leak.
  - Visually posted emergency contact information, and accessibility to a phone.

**Note: Contact the Policies, Procedures and Market Analysis Branch for more information pertaining to hazardous waste disposal procedures.**

1. Equipment and Supplies.

(1) Materials Supplied in Test Kits.

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

(a) LF-AFQ-FAST-20K:

- 1 1 package containing 20 ROSA strips packed in a moisture resistant container.
- 2 1 Aflatoxin B1 Positive Control.
- 3 1 AFQ Dilution Buffer.

(b) LF-AFQ-FAST-100K:

- 1 1 package containing 100 ROSA strips packed in a moisture resistant container.
- 2 1 Aflatoxin B1 Positive Control.
- 3 1 AFQ Dilution Buffer.

(c) LF-AFQ-FAST-500K:

- 1 5 packages each containing 100 ROSA strips packed in a moisture resistant container.
- 2 5 Aflatoxin B1 Positive Controls.

3 5 AFQ Dilution Buffers.

(2) Materials Required but not Provided.

- (a) Sample grinder.
- (b) Balance.
- (c) Methanol ACS reagent grade or better.
- (d) Deionized or Distilled water.
- (e) Sample extraction containers.
- (f) 1.0 ml pipettor and pipette tips.
- (g) 300 µl pipettor and pipette tips.
- (h) 100 µl pipettor and pipette tips.
- (i) 250 ml graduated cylinder.
- (j) 1.5 ml micro-centrifuge tubes.
- (k) Mini-centrifuge.

(3) Materials required for barley, corn flour, corn gluten meal, corn grits, corn meal, corn/soy blend, oats, popcorn, rye, wheat, and wheat flour.

- (a) 1 ml plastic non-sterile syringes.
- (b) Minisart RC15 syringe filter.

m. Storage Conditions and Precautions.

(1) Storage Conditions.

Store the AFQ-FAST test strips, AFQ Dilution Buffer, and Aflatoxin B1 Positive Control refrigerated at 0 to 7° C.

(2) Precautions.

- (a) Debris on test strips may alter the ROSA-M-Reader optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into the ROSA-M-Reader.

- (b) ROSA Incubator must be clean, level, and temperature must be  $45 \pm 1^{\circ}\text{C}$ . The temperature indicator should match the ROSA Incubator temperature. A daily thermometer check is recommended.
- (c) Keep ROSA Incubator lid lowered, but not latched unless performing a test procedure.
- (d) ROSA Incubator may take 10 minutes to reach proper temperature, depending on ambient temperature.

#### **4. FILING**

Retain a copy of this program notice until the handbook is revised to include the test method stated herein.

#### **5. QUESTIONS**

Please direct any questions or requests concerning this policy to Carl Jackson at (202) 720-8286, or email address [carl.jackson@usda.gov](mailto:carl.jackson@usda.gov) or Patrick McCluskey at 816-659-8403, or email address [patrick.j.mccluskey@usda.gov](mailto:patrick.j.mccluskey@usda.gov).

*/s/ Robert Lijewski*

Robert Lijewski, Director  
Field Management Division