

Program Notice

FGIS-PN-14-13

9-12-2014

CHARM SCIENCES, INC.
ROSA WET-S5 AFLATOXIN QUANTITATIVE TEST METHOD

1. PURPOSE

The purpose of this program notice is to add the recently approved Charm ROSA WET S5 Aflatoxin Quantitative Test kit, product number LF-AFQ-WETS5 to the approved test kit list for official mycotoxin testing. The ROSA WET-S5 Aflatoxin test eliminates the use of organic solvents (methanol, ethanol, etc.) to report/certify aflatoxin results in parts per billion (ppb) in selected grains and commodities.

2. BACKGROUND

The Technology and Science Division (TSD) provides performance evaluations of mycotoxin test kits for manufacturers seeking approval of their product for use in the official inspection system. The Policies, Procedures, and Market Analysis Branch (PPMAB), provides instructions for the use of approved mycotoxin test kits. In an effort to offer official use of the Charm ROSA WET S5 Aflatoxin Quantitative Test in a timelier manner, PPMAB is issuing this program notice prior to the release of the revised Mycotoxin Handbook. The following test procedures are approved for use by official testing locations.

3. TEST INSTRUCTIONS

a. General Information.

The Charm Sciences ROSA WET-S5 Aflatoxin Quantitative Test kit is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology and Water Extraction Technology (WET) that eliminates the use of organic solvents (methanol, ethanol, etc.). WET uses a non-hazardous extraction powder added to the sample followed by water (distilled or deionized) to extract aflatoxins into the aqueous solvent. Aflatoxin interacts with colored beads in the lateral flow test strip, and the color intensity in the test and control zones are then measured by the ROSA-M Reader or Charm EZ-M and displayed in parts per billion (ppb).

Obtain samples according to the instructions in the Grain Inspection Handbook, Book I "Grain Sampling."

| Approved Test Kit Information | | | |
|---------------------------------------|---|------------|------------------------------|
| Test Kit Vendor | Charm Sciences Inc. 978-687-9200 | | |
| Test Kit Name | ROSA WET-S5 Aflatoxin Quantitative Test Method | | |
| Product Number | Conformance Limit | | Type of Service |
| LF-AFQ-WETS5 | Min | Max | Supplemental Analysis |
| FOL Code | 5 ppb | 100 ppb | |
| AFLRD | | | |
| Grain/Commodities Approved for | Corn, barley, corn flour, corn grits, corn meal, corn screenings, corn/soy blend, distillers dried grains with solubles, hominy, oats, rough rice, sorghum, soybeans, and wheat. | | |
| Extraction Method: | <u>Shake vigorously</u> - 50 gram sample with <u>one packet</u> of WET-S Extraction Powder and <u>150 milliliters (mL)</u> of deionized or distilled water for 1.5 minutes. For 50 gram sample of barley, corn/soy blend, distillers dried grains with solubles, and soybeans use <u>250 mL</u> of distilled or deionized water, <u>two packets</u> of WET-S Extraction Powder and shake for 1.5 minutes. | | |
| Test Format | Lateral Flow Strip. | | |
| Detection Method | ROSA-M Reader, Model # LF-ROSAREADER-M-NB, Charm EZ-M Model # LF-ROSA-EZ-M. | | |

b. Preparation of Testing Materials and Equipment.

(1) Test Strips.

- (a) Store refrigerated in tightly closed in supplied container.
- (b) To open, remove and save plastic lid with foil lined foam insert to reseal container. Lift foil tab and peel foil seal off container. Discard foil seal.
- (c) In high humidity, limit condensation by opening container after it has warmed to room temperature (estimated between 20 - 30 minutes from the time the container was removed from the refrigerator).
- (d) Inspect/verify desiccant indicator in test strip container before use. Beads inside desiccant packets should be blue. Do not use test strips if blue beads have turned purple or pink.

- (e) Remove from the container only the number of test strips to be used in one day, document time of removal. Keep these test strips at room temperature during daily use for up to 12 hours. Discard any unused test strips.
- (2) AFQ Dilution Buffer.
- (a) Use AFQ-W Dilution Buffer supplied with each test kit at room temperature (18 to 30° C).
 - (b) Keep AFQ Dilution Buffer at room temperature during daily use for up to 12 hours.
 - (c) Dispense AFQ Dilution Buffer into a clean micro-centrifuge tube and label for each sample to be tested.
 - (d) Store AFQ Dilution Buffer bottle and predispensed micro-centrifuge tubes refrigerated.
- (3) EP Control.
- (a) Reconstitute one packet WET-S Extraction Powder in 150 ml deionized or distilled water by gently swirling until extraction powder is dissolved.
 - (b) Store reconstituted EP Control refrigerated for up to 1 week or aliquot (at least 1.5 ml) to micro-centrifuge tubes, label, 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. Store thawed EP Control refrigerated and use within 24 hours of thawing. Do not refreeze.
 - (c) Use to prepare Negative Control and Positive Control.
- (4) Negative Control.
- (a) Prepare negative control by adding 300 microliters (µL) EP Control to 600 µL of AFQ Dilution Buffer. Cap, mix and label.
 - (b) Mix again before use.
 - (c) Use Negative Control in Sample Analysis section.
- (5) Positive Control.

The Aflatoxin B1 Positive Control is supplied dry. Store refrigerated.

- (a) Reconstitute positive control with 1.0 ml of EP Control followed by 2.0 mL AFQ Dilution Buffer. Shake well. Allow to stand for 10 minutes at room temperature. Mix again before use.
- (b) Store reconstituted Positive Control refrigerated for up to 1 week, or transfer aliquot (at least 0.5 mL) to micro-centrifuge tube, label, and freeze within 6 hours of reconstitution at - 15° C or below for up to 2 months. Thaw frozen Positive Control slowly (overnight in refrigerator or with cool water) and shake well before use.
- (c) Store thawed Positive Control refrigerated and use within 24 hours of thawing. DO NOT REFREEZE.
- (d) Use Positive Control in Sample Analysis section.

(6) WET-S Extraction Powder (Required, Sold Separately).

- (a) Store at room temperature in supplied packet.
- (b) Do not open until ready to use.
- (c) WET-S Extraction Powder is non-hazardous, and may be disposed as normal waste.
- (d) WET-S Extraction Powder is predispensed in packets for a 50 gram test sample. Use WET-S Extraction Powder packet labeled with appropriate sample weight only.

(7) Extraction Solution.

- (a) Obtain distilled or deionized water for extraction.
- (b) Clearly label and store at room temperature in a tightly sealed container.

c. Performance Testing Procedures (Reader Set-up and Test Strips).

- (1) **ROSA-M Reader:** Enter performance mode in ROSA-M Reader by selecting MYCO2 channel in 3-line mode (MYCO2 flashing) and sequentially pressing ESC, 5, ENTER. Follow ROSA-M Reader prompts to test calibration strips (LOWCAL and HIGHCAL) and controls (NEGCONTROL and POSCONTROL).

- b. **Charm EZ-M:** Enter performance mode in Charm EZ-M by selecting Perf. Mon. from the Main Menu, followed by Perf. Test. Follow Charm EZ-M prompts to test calibration strips (LO CAL and HI CAL) and controls (NEG CTRL and POS CTRL). Select AFQ-WETS5 from the TESTS list if prompted.
- c. Test calibration strips daily to verify ROSA-M Reader or Charm EZ-M performance. Calibration strips must test in specified ranges printed on the calibration strips.
- d. Test Negative and Positive Controls weekly to verify performance of equipment and test strips. Valid Control Ranges are:
 - Negative Control: Less than 3 ppb.
 - Positive Control (range): 12 to 28 ppb.

If Calibration Strips or Controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance. Notify your monitoring field office, Domestic Inspection Operations Office (DIOO), or Technology and Science Division (TSD) with any documented information for quality control purposes.

- d. Equipment Preparation (ROSA Incubator only).
 - (1) ROSA Incubator must be clean and level.
 - (2) ROSA Incubator must be 45+/- 1° C. The temperature indicator should match incubator temperature. A daily thermometer check is recommended.
 - (3) Keep ROSA Incubator lid lowered, but not latched, unless performing a test procedure.
 - (4) Incubators may take more than 10 minutes to reach proper temperature depending on ambient temperature.

Note: The Charm EZ-M Incubator is not approved for official use:
- e. Extraction Procedures for: Corn, corn flour, corn grits, corn meal, corn screenings, hominy, oats, rough rice, sorghum, and wheat.
 - (1) Grind/mill sample so that 60 to 75 percent passes through a 20 mesh sieve.

- (2) Mix thoroughly and transfer **50 grams** (+/- 0.2) portion of ground sample into a clean extraction container.
- (3) Add contents of **one (1)** packet WET-S Extraction Powder for a 50 gram ground sample portion.
- (4) Add 150 mL distilled or deionized water.
- (5) Shake vigorously for 1.5 minutes by hand.
- (6) Allow sample mixture to settle for 1 minute to obtain **settled** sample extract. Must be used within the next 30 minutes.
- (7) Transfer 1 to 1.5 mL settled sample extract using a transfer pipet into a clean micro-centrifuge tube (about $\frac{3}{4}$ full) and label, and centrifuge in mini-centrifuge for at least 10 seconds.
- (8) This is the **clarified** sample extract. Must be used within 2 hours.
- (9) Pipet 600 μ L of AFQ Dilution Buffer into a clean micro-centrifuge tube.
- (10) Pipet 300 μ L of centrifuged/clarified sample extract into the micro-centrifuge tube containing 600 μ L AFQ Dilution Buffer, cap, mix (shake vigorously or vortex), and label.
- (11) This is the **Diluted** Sample Extract.

Repeat for additional samples (up to four (4) samples for each quad ROSA Incubator.

- (12) Proceed to Sample Analysis section.

f. Extraction Procedures for: Barley, corn/soy blend, distiller dried grains with solubles, and soybeans.

- (1) Grind/mill sample so that 60 to 75 percent passes through a 20 mesh sieve.
- (2) Mix thoroughly and transfer **50 grams** (+/- 0.2) portion of ground sample into a clean extraction container.
- (3) Add contents of two (2) packet WET-S Extraction Powder for a 50 gram ground sample portion.
- (4) Add 250 mL distilled or deionized water.

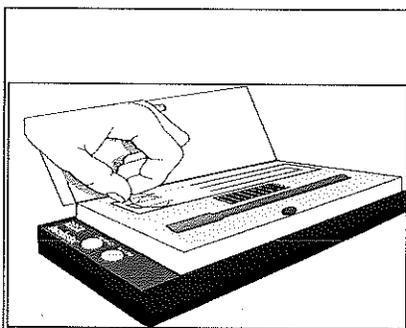
- (5) Shake vigorously for 1.5 minutes by hand.
- (6) Allow sample mixture to settle at least 1 minute to obtain **settled** sample extract. Must be used within the next 30 minutes.
- (7) Transfer 1 to 1.5 mL settled sample extract using a transfer pipet into a clean micro-centrifuge tube (about $\frac{3}{4}$ full) and label, and centrifuge in Mini-centrifuge for at least 10 seconds.
- (8) This is the **clarified** sample extract; and must be used within 2 hours.
- (9) Pipet 400 μL of AFQ Dilution Buffer into a clean micro-centrifuge tube.
- (10) Pipet 500 μL of centrifuged/clarified sample extract into the micro-centrifuge tube containing 400 μL AFQ Dilution Buffer, cap, mix (shake vigorously or vortex), and label.
- (11) This is the **Diluted** Sample Extract.

Repeat for additional samples (up to four (4) samples for each quad ROSA Incubator.

- (12) Proceed to Sample Analysis section.

g. Sample Analysis.

Check that ROSA Incubator temperature is $45 \pm 1^\circ\text{C}$. Use AFQ-WETS5 test strips. Re-shape dented sample compartments to fit into ROSA Incubator.

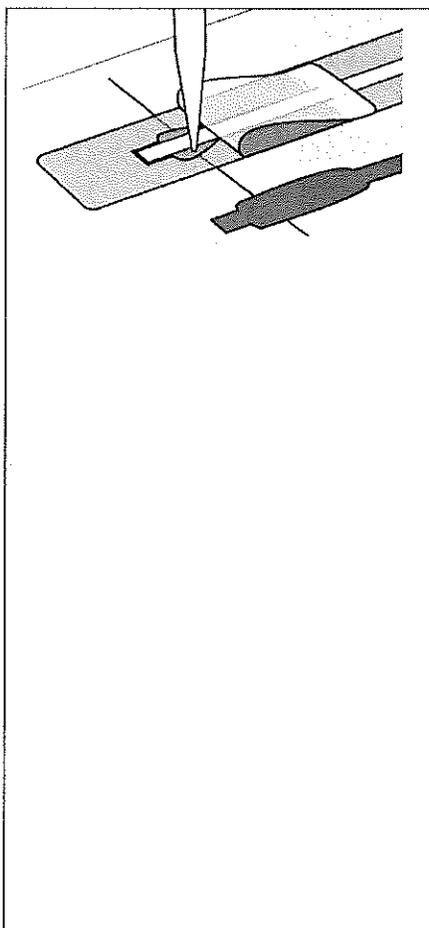


Step 1 • Label test strip(s) with sample identification. Avoid crushing sample compartment.

Step 2 • Place test strip in ROSA Incubator with the flat side facing upward.

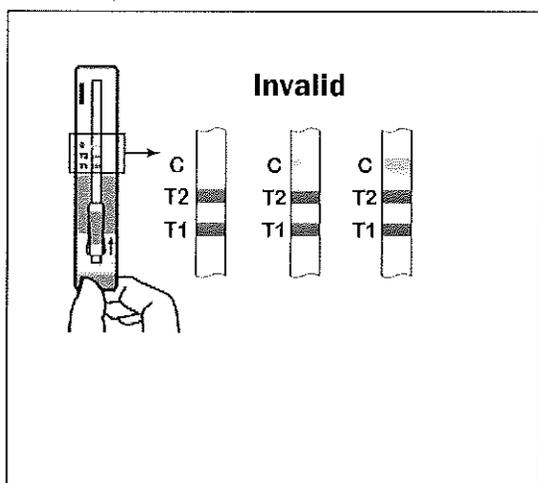
Holding test strip flat in ROSA Incubator, use TAB to **expose sample compartment by peeling tape back to “Peel to Here” line**. Avoid lifting the test strip and sponge under tape.

Step 3 • Holding pipet vertically, slowly pipet 300 μL (± 15 μL) of (Diluted extract or Control) into sample compartment at ROSA Incubator indicator line (as shown).



- Step 4 • Reseal tape** over sample compartment.
 Note: When performing multiple tests in a ROSA Incubator:
- Peel, pipet and reseal before starting next test strip.
 - Complete all test strips within 1 minute.
- Step 5 • Close lid** on ROSA Incubator and latch. Timer starts and red light illuminates.
- Step 6 • Incubate** for 5 minutes, but not more than 7 minutes. At 5 minutes, a beeper and alternating yellow and red blinking lights start.
- Step 7 • Remove test strip** from ROSA Incubator. Do not squeeze sample compartment. Hold test strip with sample compartment in the down position until interpreted.
- Wipe any foreign matter (dust, etc.) off test strip.
 - Inspect and read all test strips within 1 minute (60 seconds) of incubation completion.
 - **Lower ROSA Incubator lid.** Do not re-latch.

h. Visual Inspection.



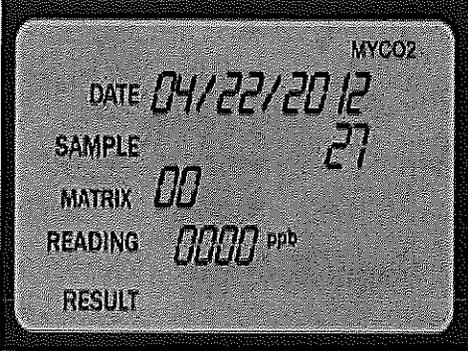
The test strip is **INVALID** if any of the following are observed:

- **C** (Control) line is missing.
- **T1, T2** (Test) or **C** line is smeared or uneven.
- **T1, T2** or **C** line is obscured by Diluted extract or Control.
- Beads do not flow past **T1, T2** or **C** lines.

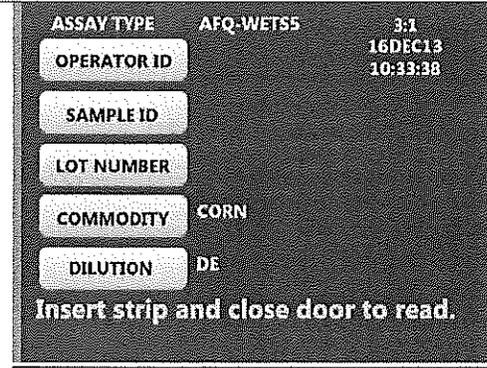
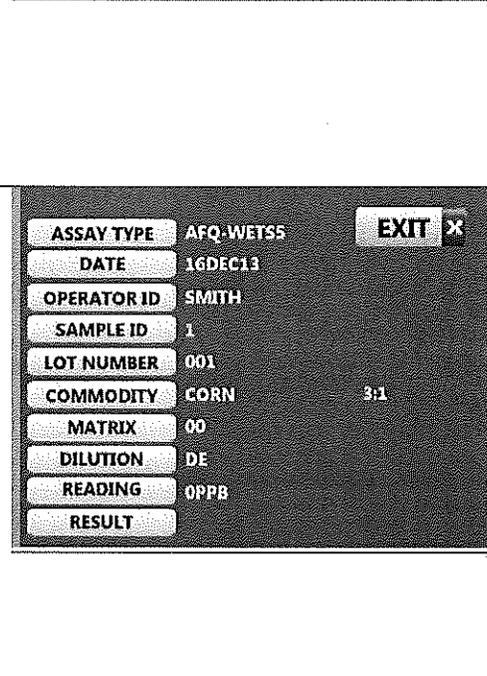
If test is **INVALID** re-test the Diluted extract or Control.

DO NOT PUT INVALID TEST STRIPS IN ROSA-M READER OR CHARM EZ-M.

i. Interpretation with ROSA-M-Reader.

| | |
|---|---|
|  | <p>Insert clean and valid test strip into ROSA-M Reader. Slide test strip completely into slot until it stops.</p> <p>Read results on MYCO2 channel (3-line mode), (MYCO2 flasing) using the appropriate MATRIX. If dersired, enter Sample and /or Operator. Press ENTER to read.</p> <p>Results are stored in memory and can be recalled to display and downloaded to printer or computer.</p> <p>Note: See ROSA-M Reader Manual to toggle between 2-line and 3-line modes, and to select the appropriate MATRIX</p> <p>The appropriate MATRIX numbers are as follows:</p> <p>MATRIX 00: Assay of Diluted Extract for Barley, Corn, Corn grits, Corn meal, Hominy, Soybeans.</p> <p>MATRIX 01: Assay of Diluted Extract for Corn Flour, Corn Screenings, Corn/Soy Blend, Distillers Dried Grain with Solubles, Oats, Rough Rice, Sorghum, Wheat.</p> <p>MATRIX 02 Supplemental Diluted Extract for 100 to 1000 ppb quantitation (corn only)</p> |
|  | <p>READING: The number displayed is the concentration of aflatoxin (ppb) in the sample.</p> <p>A Diluted Extract READING greater than 100 ppb indicates that the concentration of the sample is greater than the Sensitivity range of the sample dilution.</p> <p>An applicant can request a supplemental analysis (corn only) option to report test results above the Diluted Extract sensitivity range of 100 ppb..</p> <p><u>Note Do not certify any sample above 100 ppb unless Supplemental Analysis Procedures are performed.</u></p> |

j. Interpretation with Charm EZ-M (Read only mode).

| | |
|--|--|
|  | <p>Insert clean and valid test strip into Charm EZ-M. Slide test strip into the slot with the sample compartment in the down position until it stops. Select AFQ-WETS5 from the TESTS list (if required) followed by COMMODITY and DE (Diluted Extract for DILUTION). If desired, enter OPERATOR ID, SAMPLE ID, and/or LOT NUMBER. Close door to read.</p> <ul style="list-style-type: none"> • DE: Diluted Extract for 5 to 100 ppb quantitation (all commodities). • SUPP DE: Supplemental Diluted Extract for 100 to 1000 ppb quantitation (<u>corn only</u>). <p>Results are stored in memory and can be recalled to display and downloaded to printer or computer.</p> |
|  | <p>READING: The number displayed is the concentration of aflatoxin (ppb) in the sample.</p> <p>A Diluted Extract READING greater than 100 ppb indicates that the concentration of the sample is greater than the Sensitivity range of the sample dilution.</p> <p>An applicant can request a supplemental analysis (corn only) option to report test results above the Diluted Extract sensitivity range of 100 ppb.</p> <p><u>Note Do not certify any sample above 100 ppb unless Supplemental Analysis Procedures are performed.</u></p> |

k. Supplemental Analysis Procedures.

Supplemental analysis (corn only) is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation.

The range for performance evaluation of quantitative aflatoxin test kits is 5 – 100 ppb. Therefore, supplemental analysis would be performed for a result above 100 ppb. In supplemental analysis, the extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range (i.e., 5 – 100 ppb for aflatoxins), and a correction for dilution is applied to derive at the final result. For this test kit, the appropriate calibration setting is selected for automatic correction for the supplemental dilution performed.

Supplemental analysis is performed only at the request of the applicant.

For supplemental analysis, perform the following procedure.

Preparation and Assay of Supplemental Diluted Extract for 100 to 1000 ppb aflatoxin.

- (1) Prepare Diluted Extract according to Sample Preparation and Extraction Procedures.
- (2) Prepare Supplemental Diluted Extract from the Diluted Extract.
- (3) Pipet 1.0 mL AFQ Dilution Buffer into a clean micro-centrifuge tube.
- (4) Pipet 100 μ L Diluted Extract to micro-centrifuge tube containing 1.0 mL AFQ Dilution Buffer, cap, mix, and label. This sample is the Supplemental Diluted Extract.
- (5) Repeat for additional samples.
- (6) Use Supplemental Diluted Extract as test sample in Sample Analysis found in Test Procedures section.
- (7) Inspect and interpret the test strip as directed in Test Procedures section. Valid Supplemental Diluted Extract final result must be within 53 to 1000 ppb detection range of the sample dilution.

A final result less than 53 ppb is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 100 ppb.

A Supplemental Diluted Extract READING of “+1000 ppb” indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. Report test results as greater than 1000 ppb on the work record and certify “Aflatoxin exceeds 1000 ppb”.

1. 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 below the test kit conformance limit of 5 ppb are reported as less than 5 ppb. Sample results above the test kit conformance limit of 100 ppb are reported as greater than 100 ppb, unless supplemental analysis procedures are performed.

Refer to the Aflatoxin handbook for reporting and certification of test results.

m. Cleaning Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge labware, wash thoroughly, and then rinse with a copious amount of clean water before reusing.

n. Waste Disposal (Water Based Extraction Kits):

(1) Liquid and Solid Wastes:

After use, the liquid portion or extract of the water-based extraction kits can be disposed by down the drain. Any solid material such as the grain residue, filter paper and plastic bags can be disposed of in the regular trash.

(2) Aflatoxin results that report over the Food and Drug Administration actionable limit (20 ppb) must be properly labeled (Not For Human Consumption) before landfill/routine disposal.

o. Equipment and Supplies.

Materials supplied in test kits can be purchased that contain 20, 100, or 500 strips and include Control and AFQ-Dilution Buffer.

(1) LF-AFQ-WETS5 20K or 20ESK:

- (a) 1 package containing 20 AFQ-WETS5 test strips packed in a moisture-resistant container.
- (b) 1 – Aflatoxin B1 Positive Control (2 Controls in -20ESK).
- (c) 1 AFQ Dilution Buffer.

(2) LF-AFQ-WETS5-100K/-100ESK:

- (a) 1 package containing 100 AFQ-WETS5 test strips packed in a moisture-resistant container.
 - (b) 1 – Aflatoxin B1 Positive Control (5 Controls in -100ESK).
 - (c) 1 AFQ Dilution Buffer.
- (3) LF-AFQ-WETS5-500K/-500ESK:
- (a) 5 packages containing 100 AFQ-WETS5- 100/-100ESK test strips packed in a moisture-resistant container.
 - (b) 5 – Aflatoxin B1 Positive Controls (25 Controls in -500ESK).
 - (c) 5 AFQ Dilution Buffers.
- (4) Materials required but not provided:
- (a) Sample grinder.
 - (b) Balance.
 - (c) Deionized or Distilled water.
 - (d) Sample extraction containers.
 - (e) 1.0 mL pipet and pipet tips.
 - (f) 100-1000 μ L variable volume pipet and pipet tips.
 - (g) 300 μ L pipet and pipet tips.
 - (h) 100 μ L pipet and pipet tips.
 - (i) 250 mL Graduated cylinder.
 - (j) 1.5 mL micro-centrifuge tubes.
 - (k) Micro-centrifuge tube rack.
 - (l) Storage bottle.
 - (m) Transfer pipets.

- (5) Extraction Powder required for testing, purchased separately:
 - (a) LF-WET-EXTS-50G-20 WET Extraction powder for 50 gram sample (20/pack).
 - (b) LF-WET-EXTS-50G-100 WET Extraction powder for 50 gram sample (100/pack).
- (6) Equipment:
 - (a) MINICEN-110/220V: Mini-centrifuge.
 - (b) LF-ROSAREADER-M-NB: ROSA-M Reader supplied with Calibration strips.
 - (c) LF-ROSA-EZ-M Reader supplied with Calibration strips.
 - (d) LF-CALIB-RRM: ROSA-M Reader Calibration Strips.
 - (e) LF-INC4-5-45D: Quad ROSA Incubator (45° C and 5 minute timer with display).
 - (f) LF-INC2-5-45: Dual ROSA Incubator (45° C and 5 minute timer without display).

Note: Optional Printers are available for both ROSA-M Reader and Charm EZ-M upon request.

p. Storage Conditions and Precautions.

- (1) Storage Conditions:
 - (a) Store LF-AFQ-WETS5 test strips refrigerated tightly in the supplied container.
 - (b) Store AFQ Dilution Buffer, and predispensed micro-centrifuge tubes refrigerated.
 - (c) Store WET-S Extraction Powder at room temperature in supplied packet.
 - (d) Store reconstituted EP Control refrigerated (0 to 7° C) for up to one week or aliquot (at least 1.5 ml) to clean micro-centrifuge tubes, label, and freeze within six hours of reconstitution (-15° C or below) for up to two months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed EP Control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.

- (e) Reconstituted positive Control can be refrigerated for up to 1 week and freeze within 6 hours of reconstitution at -15°C or below for up to 2 months.

(2) Precautions.

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

- (a) 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.
- (b) Keep ROSA Incubator lid lowered, but not latched unless performing a test procedure.
- (c) 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 new Mycotoxin Handbook is released to include the test method stated herein.

5. QUESTIONS

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


Robert Lijewski, Director
Field Management Division