

Program Notice

FGIS-PN-14-06

5/1/2014

CHARM SCIENCES ROSA WET FUMONISIN QUANTITATIVE TEST METHOD

1. PURPOSE

The purpose of this program notice is to add the Charm Rosa Wet Fumonisin test kit (Product Number LF-FUMQ-WET) to the list of approved mycotoxin test kits. The Charm ROSA WET Test kit uses lateral flow technology and Water Extraction Technology (WET) that eliminates the use of organic solvents (methanol, ethanol, etc.) to report fumonisin 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) provide instructions for the use of approved mycotoxin test kits. In an effort to offer official use of the Charm ROSA WET Fumonisin Quantitative Test in a timely 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 ROSA WET Fumonisin 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 fumonisins into the aqueous solvent. Fumonisin interacts with colored beads in the lateral flow test strip, and the color intensity in the test and control zones is measured by the ROSA-M Reader and displayed in parts per billion (ppb) for approved grains and commodities.

To report results in parts per million (ppm) divide the displayed results by 1000, (e.g. 5000 ppb = 5 ppm).

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. 1-978-687-9200				
Test Kit Name	Charm ROSA WET Fumonisin Quantitative Test				
Product Number	Conformance Limit		Type of Service	Temp Range	Supplemental Analysis
LF-FUMQ-WET	Min	Max	Quantitative	64 – 86 ° F	Yes
FOL Code	0.5 ppm	5.0 ppm			
FUMJ					
Grain/Commodities Approved for					
Corn, barley, millet, oats, popcorn, rough rice, sorghum, and wheat.					
Extraction Method: Shake only- 50 gram sample with contents of one (1) ROSA WET Extraction Powder and 150 milliliters (mL) distilled or deionized water for 1.5 minutes.					
Test Format: Lateral flow strip.					
Detection Method: ROSA-M Reader, Model # LF-ROSAREADER-M-NB.					

b. Preparation of Testing Materials.

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

(1) Negative Control.

The FUMQ-W Dilution Buffer is used as a Negative Control. To run the Negative Control, use 300 microliters (µL) following Test Procedures Section 1.

(2) Positive Control.

The Fumonisin Positive Control is supplied dry. Store refrigerated.

- (a) To reconstitute positive control add 3.0 milliliters (mL) FUMQ -W Dilution Buffer. Shake well. Allow to stand for 10 minutes at room temperature.
- (b) Mix again before use.
- (c) Store reconstituted Positive Control refrigerated for up to 1 week, or aliquot (at least 0.5 mL) to micro-centrifuge tubes, label, and freeze within 6 hours of reconstitution at - 15° C or below for up to 2 months. Thaw frozen Positive Control slowly (overnight in refrigerator or with cool water) and shake well before use.

- (d) Store thawed Positive Control refrigerated and use within 24 hours of thawing. DO NOT REFREEZE.
 - (e) To run Positive Control, use 300 μ L as your sample and test following Test Procedures section k.
- c. Equipment Preparation.
- (1) Incubator must be at $45\pm 1^{\circ}$ C (temperature indicator should match ROSA incubator temperature). Incubator must be clean and level.
 - (2) ROSA Incubator may take 10 minutes to reach proper temperature, depending on ambient temperature.
 - (3) Test Calibration Strips daily to verify performance of ROSA-M-Reader and test strips.
- d. FUMQ-W Dilution Buffer.
- (1) Use FUMQ-W Dilution Buffer supplied with each test kit only.
 - (2) Dispense FUMQ-W Dilution Buffer into a clean micro-centrifuge tube for each sample to be tested (see Sample Preparation Section j).
 - (3) Use pre-dispensed tubes and buffer solution at room temperature (18 to 30° C) during daily use for up to 12 hours.
 - (4) Store FUMQ-W Dilution Buffer bottle and pre-dispensed tubes refrigerated.
- e. WET Extraction Powder.
- (1) Store at room temperature in supplied packet.
 - (2) Do not open until ready to use.
 - (3) WET Extraction Powder is non-hazardous and may be disposed as normal (non-hazardous) waste.
 - (4) Use WET Extraction Powder pre-dispensed in the designated packet for a 50 gram test sample extraction.

Note: Use the designated WET Extraction Powder packet(s) for the 50 gram ground test sample only.

f. Test Strips.

- (1) Store refrigerated, tightly closed in supplied container.
- (2) 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.
- (3) 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).
- (4) Inspect/verify desiccant indicator in test strip container. Beads inside desiccant packets should be blue. Do not use test strips if blue beads have turned purple or pink.
- (5) 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.**

g. Extraction Solution.

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

h. Performance Testing Procedures.

- (1) Enter performance mode in ROSA-M Reader by selecting FUM channel in 3-line mode and sequentially pressing ESC, 5, ENTER. Follow ROSA-M-Reader prompts to test Calibration Strips and Controls.
- (2) Test calibration strips daily to verify ROSA-M-Reader performance. Calibration strips must test in specified ranges printed on the calibration strips.
- (3) Test Negative Control and Positive Control weekly to verify performance of equipment and test strips.
- (4) Valid Control Ranges are:
 - Negative Control must read less than 100 ppb.
 - Positive Control must read between 500 to 1100 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, DIOO, or TSD with any documented information for quality control purposes.

i. **Extraction Procedures for: Corn, barley, millet, oats, popcorn, rough rice, sorghum, and wheat.**

- (1) Obtain a representative sample according to official procedures for the requested commodity.
- (2) Grind/mill sample so that 60 to 75 percent passes through a 20 mesh sieve.
- (3) Mix thoroughly and transfer **50 grams** (+/- 0.2) portion of ground sample into a clean extraction container.
- (4) Add contents of **one (1)** packet WET Extraction Powder for a 50 gram ground sample portion.

For Barley: Add the contents of two (2) packets WET Extraction powder to the 50 gram ground sample portion.

- (5) Add 150 mL distilled/deionized water.

For Barley: Add 250 mL distilled/deionized water.

- (6) Shake vigorously for 1.5 minutes by hand or mechanical shaker (with similar motion). Allow sample mixture to settle for at least 3 minutes.
- (7) Add 1 to 1.5 mL sample extract using a transfer pipet into a clean micro-centrifuge tube (about $\frac{3}{4}$ full) and label.
- (8) Centrifuge in mini-centrifuge for 20 seconds. This is the **clarified extract**, proceed to Sample Preparation Procedures, Section j.

Note: Use the extract within 30 minutes of extraction or within 2 hours if centrifuged.

- (9) Additional filtering step for **popcorn, and sorghum.**
 - (a) Filter each extract by drawing into 1 mL syringe and passing through a (GF/CA) filter.

- (b) Collect filtered extract in a clean micro-centrifuge tube and label. This is the **filtered extract**, proceed to Sample Preparation Procedures, Section j.

j. Sample Preparation Procedures.

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

- (1) Preparation of the **Diluted Extract** for 500 to 1500 ppb (0.5 to 1.5 ppm) fumonisin quantitation.
 - (a) Pipet 900 μL FUMQ-W Dilution Buffer into a clean micro-centrifuge tube for each test sample, and label.
For Barley: Pipet 1000 μL FUMQ-W Dilution Buffer into a clean micro-centrifuge tube for each test sample, and label.
 - (b) Pipet 100 μL of **clarified or filtered extract** to pre-dispensed tube with 900 μL FUMQ-W Dilution Buffer. Cap, and mix well (shake vigorously or vortex), and label.
For Barley: Pipet 200 μL of **clarified extract** to a pre-dispensed tube with 1000 μL FUMQ-W Dilution Buffer. Cap and mix well (shake vigorously or vortex), and label.
 - (c) This tube contains the **Diluted Extract**.
 - (d) Repeat for additional samples.
 - (e) Proceed to Test Procedures Section k.
- (2) Preparation of the **Second Diluted Extract** from the Diluted Extract for 1000-5000 ppb (1.0 to 5.0 ppm) fumonisin quantitation.
 - (a) Pipet 900 μL FUMQ-W Dilution buffer into a clean micro-centrifuge tube for each sample to be tested. Label tubes as needed.
 - (b) Pipet 100 μL of the **Diluted Extract** to a pre-dispensed tube with 900 μL FUMQ-W Dilution Buffer. Cap, mix (shake vigorously or vortex), and label.
 - (c) This is the **Second Diluted Extract**.

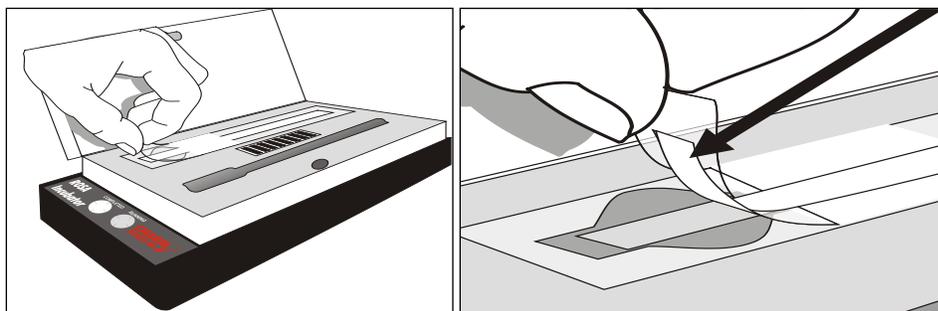
- (d) Repeat for additional samples.
- (e) Proceed to Test Procedures Section k.

Note: Laboratories may initially test the Second Diluted Extract if applicant request or levels typically reported in their market exceeds the 1.0 ppm. Use diluted extracts within 6 hours of preparation. Do not report quantitative results below 1.0 ppm using Second Diluted extract.

k. Test Procedures.

- (1) Check that the ROSA Incubator temperature is $45 \pm 1^\circ \text{C}$.
- (2) Label test strip(s) to identify sample. Avoid crushing sample compartment.
- (3) Open the incubator lid and place test strip in the ROSA-M Incubator with the flat side facing upward.

While holding the test strip flat on the incubator, use TAB to expose the sample compartment by peeling tape back to “Peel to Here” line. Avoid lifting the test strip and sponge under tape.

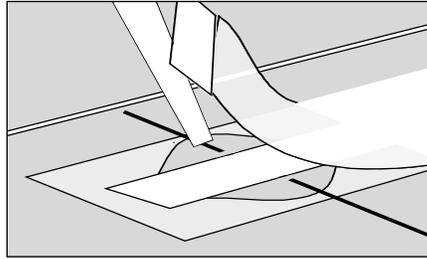


LF-INC4-5-45D: Quad incubator, 5 minute timer with display, set for 45°C .

- (4) Holding the pipet vertically slowly pipet $300 \mu\text{L}$ (Diluted, Second, Third (**Supplemental**) or Control) test sample extract into the sample compartment at the ROSA Incubator line (as shown).
 - (a) $300 \mu\text{L}$ of **Diluted extract**, for 500-1500 ppb (0.5 to 1.5 ppm) quantitation.
 - (b) $300 \mu\text{L}$ of **Second diluted extract**, for 1000-1500 (1.0 to 5.0 ppm) quantitation.

- (c) 300 μ L of **Third diluted extract**, for 1000-5000 ppb (1.0 to 5.0 ppm) quantitation. See Supplemental Analysis section for more information.
- (d) 300 μ L of **negative or positive control**.

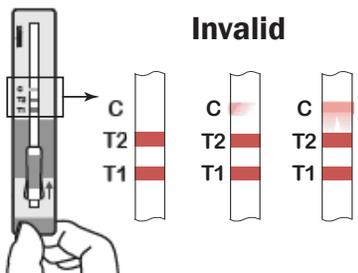
NOTE: Pipet very slowly.



- (5) Reseal the tape over the sample pad compartment. When performing multiple test(s) using a ROSA Incubator.
 - Peel, pipet, and reseal before starting next strip.
 - Complete all test strips within 1 minute.
- (6) Close lid on the ROSA incubator and tighten the latch. Timer starts and a red light will automatic illuminate.
- (7) **Incubate for 5 minutes.** After 5 minutes a beeper and alternating yellow and red blinking lights will start to flash.
- (8) Remove strip(s) from the ROSA Incubator. Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted.
 - Read within **2 minutes** of incubation completion.
 - **Lower ROSA Incubator** lid. Do not re-latch.

k. Visual Inspection.

While holding test strip vertically with sample compartment in the down position (do not squeeze sample compartment). Wipe foreign matter (dust etc.) off test strip.

 <p>Invalid</p>	<p>The test is INVALID if any of the following are observed:</p> <ul style="list-style-type: none">• 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. <p>If test is INVALID re-test the diluted extract or Control.</p> <p>DO NOT PUT INVALID TEST STRIPS IN ROSA-M READER</p>
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1. Interpreting the Lateral Flow Test Strip using the ROSA-M-NB 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-ROSAREADER-M-NB: ROSA-M Reader supplied with calibrators.

- (2) Read result on **FUM** channel (3-Line Mode, see Note¹) using the appropriate **MATRIX** (see Note 2). If desired, enter Sample and/or Operator. Press **ENTER** to read.

ROSA-M Reader results are stored in memory and can be recalled to display and download to a printer or computer.

- (3) **READING:** The number displayed is the concentration of fumonisin (ppb) in the diluted and second diluted sample extracts. A reading in ppb can be converted to ppm by dividing by 1000 (e.g. 500 ppb = 0.5 ppm).

A + sign reading on the 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 1000 - 5000 ppb (1.0 to 5.0 ppm) quantitation.

For example:

- **Diluted Extract:** READING of “**+1500 ppb**” on Matrix 00 indicates a value greater than 1500 ppb (1.5 ppm). For quantitation of 1000-5000 ppb (1.0 to 5.0 ppm), prepare the Second Diluted extract and use with another test strip.
- Read the prepared **Second Diluted Extract** on MATRIX 01 for quantitation from 1000-5000 ppb (**1.0 to 5.0 ppm**).
- **Second Diluted Extract READING** of less than **1000 ppb (1.0 ppm)** is not within the test sensitivity range and must be retested using the Diluted Extract to report quantitative results, or report test results (qualitative) as equal to or less than 1 ppm on the work record (≤ 1 ppm), certify as “Fumonisin equal to or less than 1 ppm”.
- **Second Diluted Extract READING** greater than **5000 ppb (5.0 ppm)** indicates a value greater than the GIPSA approved maximum conformance limit, and reported as exceeding 5.0 ppm for all grains and commodities, or use Supplemental Analysis procedures (Third Diluted extract) to report applicant requested test results over 5.0 ppm.

Note¹: See ROSA-M Reader manual to toggle between 2-line and 3-line modes.

Note²: Use the following table to determine the appropriate Matrix number to be used.

Matrix 00	Assay for analysis of <u>Diluted Extract</u> for 500-1500 ppb (0.5 – 1.5 ppm) quantitation.
Matrix 01	Assay for analysis of <u>Second Diluted Extract</u> for 1000-5000 ppb (1.0 to 5.0 ppm) quantitation.
Matrix 02	Assay for analysis of <u>Third (Supplemental Analysis) Diluted Extract</u> for 1000-5000 ppb (1.0 to 5.0 ppm) quantitation. <u>Uncorrected Fumonisin result.</u>

m. Supplemental Analysis Procedures

Supplemental analysis 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.

For example: The range for performance evaluation of quantitative Fumonisin test kits is 0.5 – 5.0 ppm. Therefore, supplemental analysis would be performed for a result above 5.0 ppm. 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., 0.5 – 5.0 ppm for fumonisin), and the result is then multiplied by the dilution factor to derive the final result.

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

The following describes the procedure for diluting and testing samples when the applicant requests certification above 5.0 ppm Fumonisin using the ROSA WET Fumonisin Quantitative Test kit.

A Supplemental Third Diluted Extract is prepared by diluting the Second Diluted Extract with FUMQ-W Dilution Buffer and tested on a new test strip to determine the **Uncorrected** sample concentration.

- (1) Prepare the Second Diluted Extract according to “Sample Preparation Procedures” section.
- (2) Prepare the Supplemental **Third Diluted Extract** from the Second Diluted Extract to dilute the sample concentration.
 - (a) Determine and record the Dilution Factor (DF) used to prepare the Supplemental Third Diluted Extract required to calculate the corrected final sample concentration.
 - (b) The DF is equal to the sum of the volume of the FUMQ-W 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	FUMQ-W Dilution Buffer	Second Diluted Extract Volume
4 =	300 µL	(plus) 100 µL
10 =	900 µL	(plus) 100 µL

- (c) Using the selected DF, pipette the correct volume of the FUMQ-W Dilution Buffer into a micro-centrifuge tube.
 - (d) Using the same DF, pipette the correct volume of the Second Diluted Extract into the micro-centrifuge tube containing the FUMQ-W Dilution Buffer. Cap, mix, and label the sample. This is the **Supplemental Third Diluted Extract**.
- (3) Follow instructions in “Test Procedures” for analysis of the **Supplemental Third Diluted Extract**.
 - (4) Read results on FUM (3-line mode) with **MATRIX 02**.

Valid Supplemental Third Diluted Extract **READING** must be within 1000 to 5000 ppb (1.0 to 5.0 ppm).

- A **READING** less than 1000 ppb (1.0 ppm) is below the detection range. Prepare another Supplemental Third Diluted Extract (step 2) with a lower Dilution Factor, and run another test strip to quantitate.
 - A **READING** greater than 5000 ppb (5.0 ppm) indicates that the concentration of the sample is greater than the test range. Prepare another Supplemental Third Diluted Extract with a higher Dilution Factor, and run another test strip to quantitate.
- (5) The number/result displayed on the ROSA-M Reader is the **Uncorrected Fumonisin Concentration** in the sample. Document on the work record.
 - (6) To convert the uncorrected Fumonisin result to the final **Corrected Fumonisin Concentration** in the sample multiply the result displayed on the ROSA-M Reader by the DF used to prepare the Supplemental Third Diluted Extract.

For Example:

If the Uncorrected Fumonisin result is 2000 ppb (as displayed on the ROSA-M Reader) and the DF is 4 the Corrected Fumonisin result concentration is 8,000 ppb ($2000 \text{ ppb} \times 4 = 8,000 \text{ ppb}$) or 8.0 ppm ($2.0 \text{ ppm} \times 4 = \underline{8.0 \text{ ppm}}$). Document corrected result on the work record.

n. Reporting and Certifying Test Results.

- (1) Report all results on the pan ticket and the inspection log to the nearest tenth ppm.

- (2) Sample results over the upper limit of the Diluted or Second Diluted Extracts are reported as greater than the stated sensitivity range.
- (3) Sample results **less than 0.5 ppm** are reported as such (i.e., < 0.5 ppm). Sample results **greater than 5.0 ppm** are reported as such (i.e., > 5.0 ppm).

Note: Applicants may request qualitative certification in lieu of retesting a sample with a result outside of the diluted or second diluted extract test sample sensitivity ranges/concentrations only.

o. 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.

p. Waste Disposal.

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

Fumonisin results that report over the Food and Drug Administration actionable limit (refer to Directive 9180.71) must be properly labeled (Not For Human Consumption) before landfill/routine disposal.

State and local regulations may be more stringent. Please review to ensure compliance.

q. Equipment and Supplies.

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

- (1) LF-FUMQ-WET 20K/-20ESK:
 - (a) 1 package containing 20 FUMQ-WET test strips packed in a moisture-resistant container.
 - (b) 1 – Fumonisin Positive Control (2 Controls in -20ESK)
 - (c) 1 FUMQ-W Dilution Buffer.

- (2) LF-FUMQ-WET-100K/-100ESK:
 - (a) 1 package containing 100 FUMQ-WET test strips packed in a moisture-resistant container.
 - (b) 1 – Fumonisin Positive Control (5 Controls in -100ESK).
 - (c) 1 FUMQ-W Dilution Buffer.
- (3) LF-FUMQ-WET-500K/-500ESK:
 - (a) 5 packages containing 100 FUMQ-WET- 100/-100ESK test strips packed in a moisture-resistant container.
 - (b) 5 – Fumonisin Positive Controls (25 Controls in -500ESK).
 - (c) 5 FUMQ-W Dilution Buffers.
- (4) Materials required but not provided:
 - (a) Sample grinder.
 - (b) Balance.
 - (c) Mechanical Shaker-minimum setting 300 rpm.
 - (d) Deionized or Distilled water.
 - (e) Sample extraction containers.
 - (f) 1.0 mL pipet and pipet tips.
 - (g) 500 μ L pipet and pipet tips.
 - (h) 300 μ L pipet and pipet tips.
 - (i) 100 μ L pipet and pipet tips.
 - (j) 250 mL Graduated cylinder
 - (k) 1.5 mL micro-centrifuge tubes.
 - (l) Transfer pipets.

- (5) Extraction Powder required for testing, purchased separately:
 - (a) LF-WET-EXT-50G-20 WET Extraction powder for 50 gram sample (20/pack).
 - (b) LF-WET-EXT-50G-100 WET Extraction powder for 50 gram sample (100/pack).
- (6) Materials required but not provided for testing popcorn, and sorghum.
 - (a) FIL-0-2UM-GFCA: 0.2 µm GF/CA filter to fit on a 1 mL syringe.
 - (b) SYRINGE-1ML-PK-NS: 1 mL plastic non-sterile syringe.
- (7) Equipment:
 - (a) MINICEN-110/220V: Mini-centrifuge.
 - (b) LF-ROSAREADER-M-NB: ROSA-M Reader supplied with calibration strips.
 - (c) LF-CALIB-RRM: ROSA-M Reader Calibration Strips.
 - (d) LF-INC4-5-45D: Quad ROSA Incubator (45°C and 5 minute timer with display).
 - (e) LF-INC2-5-45: Dual ROSA Incubator (45°C and 5 minute timer without display).

t. Storage Conditions and Precautions.

(1) Storage Conditions.

Store FUMQ-WET test strips, FUMQ-W Dilution Buffer and Fumonisin Positive Control refrigerated at 0 to 7° C.

(2) Precautions.

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.

- (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.

/s/Robert Lijewski

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