

CHAPTER 9

ROMER - FLUOROQUANT (FQ Afla) TEST METHOD

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## 9.1 GENERAL INFORMATION

The Romer Fluoroquant aflatoxin test method product number COKFA 1010 uses fluorescence technology to quantitatively measure total aflatoxins (B1, B2, G1, and G2) in parts per billion (ppb).

## 9.2 PREPARATION OF SOLUTIONS

### a. Developer Solution.

- (1) Prepare, store, and dispense developer in a 50 ml amber bottle with a 1 ml repipettor.
- (2) Transfer the contents of one developer concentrate vial into 25 ml of deionized/distilled water using the disposable transfer pipette. If the developer concentrate is not yellow/brown when pulled into the disposable pipette, discard and use another vial.
- (3) Rinse the empty vial three times with the prepared developer, returning the rinse to the 50 ml bottle each time.

**NOTE: The developer working reagent must be made fresh every 24 hours.**

### b. 80 Percent Methanol Solution (for corn, corn meal, rice, popcorn, sorghum, and wheat).

- (1) Using a graduated cylinder, measure 800 ml of methanol (ACS grade or better) and place it into a clean carboy with spigot.
- (2) Add 200 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- (3) Label the container stating the mixture (80 percent methanol and 20 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 8 parts methanol to 2 parts of deionized or distilled water.**

- c. 86 Percent Acetonitrile Solution HPLC grade only (for soybeans and corn/soy blend).
- (1) Using a graduated cylinder, measure 860 ml of acetonitrile and place it into a clean carboy with spigot.
  - (2) Add 140 ml deionized or distilled water.
  - (3) Label the container stating the mixture (86 percent acetonitrile and 14 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 86 parts acetonitrile to 14 parts of deionized or distilled water.**

### 9.3 FLUOROMETER CALIBRATION

- a. Calibration Procedures using the Series III Fluorometer.
- (1) Turn the power on and respond as indicated (no warm-up period is required). When first turned on, the fluorometer will go through a series of self-tests. The fluorometer should be calibrated daily before running the first sample and periodically as needed. A calibration card comes with each set of calibrators and values on the card are valid only for that set of calibrators.
  - (2) Press “continue”.
  - (3) Press “select”.
  - (4) Use card included with calibrators to select the commodity tested - “11” for corn.
  - (5) Press “enter”.
  - (6) When prompted, insert High Calibrator, press “enter”.
  - (7) When prompted, insert Low Calibrator, press “enter”.
  - (8) Press “enter” to continue.

- (9) Run the Yellow Calibrator and check reading against range listed on the card.
- (10) If the Yellow calibrator is within tolerance range listed on the calibration card, proceed to analyze test samples.
- (11) If not, try recalibrating instrument from start-up (turn-off/turn-on). If the control value is still out of range, contact TSD or Romer Labs Inc.

b. Calibration Procedures using the FQ Reader.

Please read the Romer Labs FQ Reader operating manual for a brief introduction of the instrument, unpacking, inspection, and precaution guidelines before installing instrument.

(1) Instrument Set-up.

- (a) Place the FQ Reader on a table or bench. This should be a flat level surface, which is reasonably clean, dry, and free from excessive wind drafts.
- (b) Allow at least 6 inches (16 cm) of clearance above the instrument to open and close the lid.
- (c) Position the instrument so that the touch screen faces forward.
- (d) Follow all Romer Labs operating instructions for Fluorometer operation for connection of power supply, software installation, and any optional equipment (e.g. computer, or printer).

(2) Calibration Method.

- (a) Turn the power on, the **Test Kit Selection Screen** will appear (no warm-up period is required). The test kit selection screen shows all test kits that are available for reading on the instrument.
- (b) Ensure that the correct Optical Kit is installed in the FQ-Reader for the test to be run. The FQ Module A should be installed for use with Fluoroquant Afla test kits.

- (c) Select the desired test by touching the corresponding button on the screen.
- (d) The **Commodity Selection Screen** appears following selection of the test kit. This screen will show all the commodities that are currently available for analysis using the test kit.
- (e) Select the commodity to be analyzed by touching its corresponding button on the screen. To return to the test kit selection screen, touch the green bar at the top of the screen containing the test kit name.
- (f) The **Calibration Screen** will appear automatically following the selection of the commodity to be analyzed.
- (g) When prompted, insert High Calibrator, close lid, and touch “OK”.
- (h) When prompted, insert Low Calibrator, close lid, and touch “OK”.
- (i) **The Home Screen** appears following a successful calibration. The FQ Reader will display the message: Calibration Complete! “Ready to Begin Testing”.

(3) Measuring Samples.

- (a) The Home screen will display the test kit in use (center gray box at top), the commodity for which the instrument has been calibrated (green box at top right), and the results of any measurements that have been performed.
- (b) Touch “Sample ID” to name the next sample to be analyzed (optional). Using the keypad, enter the sample name into the name field. Touch “Save” to save the sample ID.
- (c) After preparing the sample according to official test procedures, open lid of the FQ Reader and insert the test cuvette. Close the lid.

**Note: Only use cuvettes that have been supplied with the test kits.**

- (d) Measurement will commence automatically when the method delay countdown has reached zero. The FQ Reader will display the message “MEASUREMENT IN PROGRESS, DO NOT OPEN LID, DO NOT TOUCH SCREEN DURING MEASUREMENT”.
- (e) Record test sample results after measurement process complete.
- (f) If the test sample results do not meet instrument criteria, the instrument will display “Unstable Data, Please Retest”.
- (g) Insert the **Control Calibrator** after initial calibration and to periodically check the instrument calibration stability. The result of the control calibrator must fall within the range specified on the calibrator box for the commodity tested. If the reading is not in this range, try recalibrating the machine. If the control value is still out of range, contact TSD or Romer Labs Inc.

#### 9.4 EXTRACTION PROCEDURES

- a. Procedures for Corn, Corn Meal, Rice, Popcorn, Sorghum, and Wheat.
  - (1) Transfer 50-grams of ground sample into an extraction mixing jar.
  - (2) Add 100 ml of the methanol/water extraction solvent.
  - (3) Cover the extraction jar and blend on high speed for 1 minute.
  - (4) Remove the cover and funnel the extract through a Whatman No.1 filter or a coffee filter into a sample jar labeled with the sample identification.
  - (5) After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container (e.g., disposable plastic beaker).
- b. Procedures for soybeans and corn soy blend.
  - (1) Transfer 50-grams of ground sample into an extraction mixing jar.
  - (2) Add 200 ml of the acetonitrile/water extraction solvent.

- (3) Cover the extraction jar and blend on high speed for 30 seconds. Remove the cover and funnel the extract through a Whatman No.1 filter or a coffee filter into a sample jar labeled with the sample identification.
- (4) After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container (e.g., disposable plastic beaker).

## 9.5 TEST PROCEDURES

### a. Purification of Corn, Corn Meal, Rice, Popcorn, Sorghum, and Wheat.

- (1) Pipette 1 ml of sample extract into the top of the SolSep 2001 column.
- (2) Add 1 ml of the diluent and mix well by repeatedly filling and emptying the pipette tip three times into the column.

**Note: The solution maybe difficult to push through the column if liquids are not mixed well.**

- (3) Place column in a 12 x 75 mm cuvette.
- (4) Insert the syringe barrel and stopper into the top of the column.
- (5) Push the extract through the column until air comes out of the bottom. Alternately, a vacuum column stand may be used to push extract through column.
- (6) Transfer 0.5 ml of each purified sample extract to a clean 12 x 75 mm cuvette and cap.

### b. Purification of Soybeans and Corn/Soy Blend.

- (1) Pipet 2 ml of sample extract into the top of the SolSep 2001 column.
- (2) Place column in a 12 x 75 mm cuvette.
- (3) Insert the syringe barrel and stopper into the top of the column.
- (4) Push the extract through the column until air comes out of the bottom. Alternately, a vacuum column stand may be used to push extract through column.

- (5) Transfer 1 ml of each purified sample extract to a clean 12 x 75 mm cuvette and cap.

c. Derivatization and Fluorometric Reading.

- (1) Immediately add 1 ml of the developer working reagent to each purified sample extract.
- (2) Recap the tube and vortex for 5 seconds.
- (3) Wipe the cuvette with lint-free paper and place in the fluorometer for a reading.
- (4) After a 15-second delay, the result will appear on the fluorometer screen and a record will be printed out.

**NOTE: Once the developer reagent is added, the sample must be mixed, and the sample cuvette must be placed in the fluorometer quickly. Samples must be derivatized one sample at a time and then read before proceeding to the next sample.**

## 9.6 REPORTING AND CERTIFYING TEST RESULTS

- a. Record the digital readout as ppb total aflatoxins in the sample.
- b. Report all results on the pan ticket and the inspection log to the nearest whole ppb.
- c. Sample results over 100 ppb are reported as >100 ppb unless a supplemental analysis is performed.
- d. Refer to the Certification section of the handbook for more detailed certification procedures.

## 9.7 SUPPLEMENTAL ANALYSIS

### a. Diluting the Sample Extract.

If quantitative results are above the test method conformance limit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 100 ppb, the sample extract must be diluted so that a value between 5 and 100 ppb is obtained. The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

### b. Example.

If the original analysis reported the aflatoxin value at 300 ppb, the sample extract should be diluted using the following procedures in order to obtain a true value.

- (1) Dilute 5 ml of the original extract with 15 ml of the extraction solvent mixture (**methanol/water for corn, corn meal, rice, popcorn, sorghum, and wheat, or acetonitrile/water for soybeans and corn/soy blend**). The total volume is 20 ml. This is a 1 to 4 dilution (compares volume in the beginning with the total volume in the end).
- (2) Multiply the analytical results obtained by 4 to obtain the actual aflatoxin concentration. For example, if 80 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 320 ppb.

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

$$\begin{aligned} \text{True Aflatoxin Value} &= (20 \div 5) \times 80 \text{ ppb} \\ &= 4 \times 80 \text{ ppb} = 320 \text{ ppb} \end{aligned}$$

## 9.8 CLEANING LABWARE

### a. Negative Tests ( $\leq 20$ ppb).

#### (1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour the liquid down the drain and place the solid material in a garbage bag and discard.

## 9.9 WASTE DISPOSAL

a. Negative Results ( $\leq 20$  ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

## 9.10 EQUIPMENT AND SUPPLIES

- a. Blender with ½ pint jars, or equivalent.
- b. Syringe with rubber stopper.
- c. Cuvette rack.
- d. Pipettor and tips - 200 to 1000 µl (0.2 to 1 ml) adjustable.
- e. Vortex Mixer.
- f. Series III Fluorometer.
- g. FQ-Reader part number EQFFM3000 (optional printer).
- h. 100 ml graduated cylinder.
- i. Funnels.
- j. Timer.
- k. Whatman No.1 Filter Paper or Coffee Filters.
- l. Glass cuvettes (12 x 75 mm).
- m. Repipette Dispenser (1ml), Lab Industries Model LS830X3 or equivalent.
- n. Sample grinder.
- o. Balance.
- p. Methanol ACS grade or better (**for extraction solvent for corn, corn meal, rice, popcorn, sorghum, and wheat**).
- q. Acetonitrile HPLC grade only (**for extraction solvent for soybeans and corn/soy blend**).
- r. Deionized or Distilled Water.
- s. SolSep 2001 Fluoroquant “A” columns.
- t. Developer Concentrate.

- u. Diluent (**for corn, corn meal, rice, popcorn, sorghum, and wheat**).
- v. High, Low, and Control calibrator ampules.
- w. Positive Control Standard.

#### **9.11 STORAGE CONDITIONS**

- a. Shelf life of test kit is 9 months from the date of manufacture. Do not mix components from different lots or use kits after expiration date.
- b. Developer Concentrate - shipped in an amber bottle. Store in a tightly closed container in a cool, dry, well ventilated area away from direct sunlight, combustible materials, and incompatible substances.
- c. Store the columns at room temperature (64 - 86° F) in a sealed container.
- d. Calibrators - Room temperature.
- e. Diluent - In a cool place away from heat source.