

CHAPTER 5

ROMER - FLUOROQUANT AFLA (FQ Afla IAC) TEST METHOD

Product Number COKFA4010/4020

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## 5.1 GENERAL INFORMATION

The Romer Fluoroquant Afla IAC aflatoxin test method uses fluorescence technology to quantitatively measure total aflatoxins (B1, B2, G1, and G2) using an immunoaffinity column for sample purification and reported in parts per billion (ppb).

## 5.2 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 screenings, and distillers dried grains w/solubles).

- (1) Using a graduated cylinder, measure 800 ml of methanol (HPLC grade) 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. 16 Percent Methanol in Water Rinse Solution.
- (1) Using a graduated cylinder, measure 40 ml of 80/20 methanol extraction solution and place it into a clean wash bottle.
  - (2) Add 160 ml deionized or distilled water to the wash bottle, and mix well.
  - (3) Label the container stating the mixture (16 percent rinse solution), date of preparation, time, and initials of technician who prepared the solution.
  - (4) Rinse solution must be made fresh every 24 hours.

### **5.3 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 enter the method number - “23”.
  - (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 the 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 and touch “FluoroQuant Afla IAC”.
- (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 Touch “IAC Method 1”.
- (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) Run the Yellow Calibrator and check reading against range listed on the card.

**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.

#### 5.4 EXTRACTION PROCEDURES

- a. Procedures for Corn, Corn Screenings, and Distillers Dried grains w/solubles.
  - (1) Transfer 50 grams of ground sample into an extraction mixing jar.
  - (2) Add 10 grams of Sodium Chloride (Salt).
  - (3) Add 200 ml of the methanol/water extraction solvent.
  - (4) Cover the extraction jar and blend on high speed for 1 minute.
  - (5) Remove the cover and funnel the extract through a Whatman No.1 filter or a coffee filter into a glass or polypropylene container labeled with the sample identification.
  - (6) After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container (e.g., disposable plastic beaker).

## 5.5 TEST PROCEDURES

- a. Purification of Corn, Corn Screenings, and Distillers Dried grains w/solubles.
- (1) Pipette 500 µl of sample extract into a clean cuvette, discard tip.
  - (2) Using a pipettor add 2000 µl of distilled or deionized water.
  - (3) Pierce the top seal of an AflaStar® IAC column with a pointed filter column and remove the bottom cap from the IAC column.
  - (4) Place the entire 2500 µl diluted sample into the syringe barrel of the pointed filter column.
  - (5) Insert the syringe plunger and stopper assembly into the top of the column.
  - (6) Push the extract completely through the column, at a rate of about 1 – 2 drops per second. (**Alternate method:** Use manual or powered vacuum column stand to push extract through column.)
  - (7) Place 2 ml of distilled or deionized water into the cuvette that was used for diluting the sample. Pour this water onto the pointed filter column and push completely through the IAC column.
  - (8) Remove the pointed filter column from the IAC column and discard.
  - (9) Rinse the IAC column with 2 ml of **16 percent methanol rinse solution.**
  - (10) Repeat this procedure once, so that a total of 4 ml of the 16 percent methanol rinse solution have been passed over the column.
  - (11) Place a clean cuvette under the IAC column.
  - (12) Elute the aflatoxin by adding 2 ml **methanol (HPLC grade)** to the IAC column.
  - (13) Push the methanol through the IAC column at a rate of about 1 – 2 drops per second.
- b. Derivatization and Fluorometric Reading.
- (1) Immediately add 1 ml of the developer working reagent to the 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 30-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.**

## 5.6 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. Refer to the Certification section of the handbook for more detailed certification procedures.

**Note: There is no supplemental analysis procedure for this test method. All test results over the test kit conformance limit must be reported as exceeding 100 ppb.**

## 5.7 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.

## 5.8 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.

## 5.9 EQUIPMENT AND SUPPLIES

- a. Blender with ½ pint jars, or equivalent.
- b. Syringe Plunger and stopper assembly or optional plunger stand.
- c. Cuvette rack.
- d. Vortex Mixer.
- e. Pipettes fixed 500 µl and 1000 µl with tips.
- f. Romer FQ-Reader Model 9200-000 or Series III Fluorometer Model RL-100.
- g. 100 ml graduated cylinder.
- h. Funnels.
- i. Timer.
- j. Pointed filter columns.
- k. Glass cuvettes (12 x 75 mm).
- l. Repipettor (1ml).
- m. Pipettor (2ml) for 16 percent methanol rinse solution.
- n. Sample grinder.
- o. Balance.
- p. Methanol HPLC grade only.
- q. Sodium Chloride (Salt).
- r. Deionized or Distilled Water.
- s. IAC Aflatoxin columns.

- t. Developer Concentrate.
- u. High, Low, and Control calibrator ampules.
- v. Positive Control Standard.

#### **5.10 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 is 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. Refrigerate the AflaStar® IAC columns in a sealed container at 2 - 8° C.
- d. Calibrators should be stored at room temperature (64 - 86° F).