

Test Kit Instruction

September 14, 2016

VICAM AFLATEST QUANTITATIVE AFLATOXIN TEST

FORWARD

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the Mycotoxin Handbook for information on use of this test kit in official FGIS inspections including sampling, general sample preparation, reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

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1. GENERAL INFORMATION

The VICAM AflaTest method uses immunoaffinity column chromatography to isolate aflatoxins B1, B2, G1, and G2 for fluorometric detection and quantification at the parts-per-billion (ppb) level.

Approved Test Kit Information	
Test Kit Vendor:	<i>VICAM, A Waters Business (508) 482-4935</i>
Test Kit Name:	VICAM AflaTest
Product Number:	12022
Effective Date of Instructions:	09/14/2016
Instructions Revision Number:	0
Type of Service:	Quantitative
Conformance Range:	5.0 – 300 ppb
Number of Analyses to Cover Conformance Range:	1
Approved Commodities:	Corn (including Dent or Field Corn, Corn Meal, Corn Flour, Cracked Corn, Corn Grits or Polenta, and Corn Screenings), Corn Gluten Meal, Distillers Dried Grain with Solubles, Popcorn, Milled Rice (including Brewer's Rice and Glutinous Rice) and Sorghum
Extraction method:	Blend 50 gram sample with 5 grams of sodium chloride and 100 milliliters (mL) 80% methanol/20% distilled or deionized water (v/v) for 1 minute (for DDGS use 200 mL 80% methanol/20% distilled or deionized water). For Corn Gluten meal blend 50 gram sample with 10 grams of sodium chloride and 200 milliliters (mL) 60% methanol/40% distilled or deionized water (v/v) for 3 minutes.
Test Format:	Immunoaffinity column with fluorometric detection
Detection Method:	VICAM Series 4 EX fluorometer

2. PREPARATION OF TESTING MATERIALS AND EQUIPMENT

Before beginning the procedures, please read the “Precautions Section” on page 10.

a. VICAM 4EX Fluorometer Calibration.

(1) General.

- (a) To ensure accurate results, calibrate the fluorometer before use each day and verify calibration using the yellow vial.
- (b) Turn the fluorometer on with the On/Off switch located on the rear panel. Once the fluorometer is turned on, it may be left on until close of business for the day.
- (c) After turning the fluorometer on, it will identify itself and perform a set of self-tests. If any error message appears, consult the operator’s manual or call Vicam.

(2) Calibration Procedures.

- (a) Set the date and time by pressing the OPTIONS key until “Settings” appears on the display; then press ENTER. “Set Date and Time” will appear on the display. Press ENTER, and follow the prompts.
- (b) Press the STOP key to return the fluorometer to the “VICAM ready” mode.
- (c) Press the OPTIONS key until “Calibrate Test” appears on the display. Press ENTER. Press the SELECT TEST key until “AflaTest FGIS” is on the display. Press ENTER, and follow the prompts.
- (d) When prompted to insert a calibration vial, clean the vial with a Kimwipe and insert it into the bottom of the well. Ensure the vial is fully inserted and touches the bottom of the well.
- (e) Enter the correct calibration value (see table below) for the high calibrator (red vial) and low calibrator (green vial).
- (f) Check the calibration by testing the yellow vial.

Calibration Vials	Series 4 EX
Red	160
Green	-2.0
Yellow	79 ± 5

- (g) Press the SELECT TEST key. The display should read "AflaTest FGIS." Press ENTER, and then insert the yellow vial. Record the results for the yellow vial on the work record.
- (h) If the value of the yellow calibration vial is not within FGIS specifications, repeat the calibration process (steps b through f); then check the yellow vial again. If the reading for the yellow vial remains above or below FGIS specifications, contact Vicam and let TSD know about the incident.
- (i) Calibrate the fluorometer at least once a week or before analyzing any test samples if more than 1 day has elapsed since the last test using the yellow vial.

b. Reagent Testing.

After calibrating the fluorometer, check the following reagents for background fluorescence by performing the procedures listed below.

- (1) Methanol: Dispense 2.0 mL of HPLC grade methanol into a clean cuvette, and place the cuvette in the fluorometer. The digital display should read zero (0).
- (2) Water: Dispense 2.0 mL of deionized or distilled water into a clean cuvette, and place the cuvette in the fluorometer. The digital display should read 0.
- (3) If any of the readings is greater than zero (0), replace reagent from a pure source, recheck fluorometer calibration, or replace cuvette.

c. Developer Solution.

- (1) Add 5 milliliters (mL) of AflaTest concentrated developer solution to 45 mL of distilled or deionized water. Mix well. The concentrated developer solution should have a slight yellowish brown color. If the solution is colorless, it has lost potency and should not be used. When preparing smaller quantities of the solution be sure to maintain the 1 part concentrated developer to 9 parts distilled or deionized water ratio.
- (2) Combine 1.0 mL of HPLC grade methanol and 1.0 mL of dilute developer solution in a clean VICAM borosilicate glass cuvette. Place the cuvette in the fluorometer to check for background fluorescence. The digital display reading should read 0. If the reading is greater than 0, check each reagent separately to determine which reagent is causing the problem and replace it, recheck calibration, or replace cuvette.
- (3) Label the dilute developer solution bottle with the date and time of preparation and the technician's initials. Fresh developer solution must be prepared daily and used within 8 hours.
- (4) Label each stock bottle of concentrated developer with the date it was first opened. Do not use concentrated developer after 30 days have elapsed.

d. Extraction Solvent.

- (1) Extraction Solvent: 80% MeOH/20% deionized or distilled water (v/v). Premixed 80/20 (MeOH/water) solution may be purchased from VICAM (part # 100000211- 20L or part # 100000212-4L). One may prepare extraction solvent in a well-ventilated area as follows:
 - (a) Using a 1000 mL graduated cylinder, measure 800 mL of methanol (ACS grade or better), and pour it into a clean carboy with spigot.
 - (b) Using a 250 mL graduated cylinder, measure 200 mL deionized or distilled water, and add to the methanol. Shake until completely mixed. Allow to reach room temperature before use.
 - (c) Label the container stating the mixture (80% methanol and 20% water), date of preparation, and technician's initials.
 - (d) Store this solution at room temperature in a tightly closed container until needed.
- (2) Extraction Solvent: 60% MeOH/40% deionized or distilled water (v/v) for corn gluten meal only. Premixed 60/40 (MeOH/water) may be purchased from VICAM (part # 100000213-20L or part # 100000214-4L). One may prepare extraction solvent in a well-ventilated area as follows:
 - (a) Using a 1000 mL graduated cylinder, measure 600 mL of methanol (ACS grade or better), and pour it into a clean carboy with spigot.
 - (b) Using a 1000 mL graduated cylinder, measure 400 mL deionized or distilled water and add to the methanol. Shake until completely mixed. Allow to reach room temperature before use.
 - (c) Label the container stating the mixture (60% methanol and 40% water), date of preparation, and the technician's initials.
 - (d) Store this solution at room temperature in a tightly closed container until needed.

e. Column chromatography setup.

- (1) The Test Procedures will require a VICAM pump stand with a glass syringe barrel reservoir and a manually operated syringe pump or an adjustable automated air pump to push the sample extract and reagents through the AflaTest column.
- (2) Before beginning Test Procedures, remove the top and bottom caps from the AflaTest affinity column, and gently shake the buffer solution from the top of the column.

- (3) When attaching the column to the glass syringe barrel on the pump stand, use the appropriate column coupling. Create a coupling by snipping off the bottom of the top cap after removing it from the column. Coupling can be reused.

3. EXTRACTION PROCEDURES

Place 50 g (\pm 0.2) of ground sample into blender jar, and add the appropriate reagents before blending, filtering and diluting (step d).

- a. Reagents for corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), milled rice (including brewer's rice and glutinous rice), popcorn and sorghum.

- (1) Add 5 grams of analytical, USP grade sodium chloride (NaCl) or food grade non-iodized salt.
- (2) Add 100 mL of the 80/20 methanol/water (v/v) extraction solution.

- b. Reagents for distillers dried grain with solubles.

- (1) Add 10 grams of analytical, USP grade sodium chloride (NaCl) or food grade non-iodized salt.
- (2) Add 200 mL of the 80/20 methanol/water (v/v) extraction solution.

- c. Reagents for corn gluten meal.

- (1) Add 10 grams of analytical, USP grade sodium chloride (NaCl) or food grade non-iodized salt.
- (2) Add 200 mL of 60% methanol/40% /water (v/v) extraction solution.

- d. Blending, Filtering and Dilution.

- (1) Cover jar and blend at high speed for 1 minute. (For corn gluten meal blend for 3 minutes)
- (2) Pour the extract through a clean funnel lined with fluted filter paper into a clean VICAM disposable beaker labeled with sample identification.
- (3) After collecting approximately 20 ml of filtered extract, carefully dispose of filter and its contents. Extract must be used within 30 minutes.
- (4) Pipette 10 mL of distilled or deionized water using 5 mL pipettor (VICAM # G4055) into a hard plastic beaker (VICAM # 36010). Add 5 mL of filtered extract (using a new pipette tip) and mix it by swirling the beaker or pipetting up and down 5 times.

- (5) Line a small, clean funnel with a VICAM microfiber filter. Fold filter gently without making a sharp crease to avoid breaking it. Pour diluted extract through the lined funnel into a 12 x 75 mm disposable borosilicate glass cuvette (VICAM # 34000) or hard plastic beaker (VICAM # 36010). This is the diluted filtered extract and ready for the analysis.
- (6) Immediately carry out the test procedure.

4. TEST PROCEDURES

Perform the appropriate sample cleanup procedures before proceeding to step d (Separation and Measurement) on page 9.

a. Sample cleanup for corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), milled rice (including brewer's rice and glutinous rice), popcorn and sorghum.

- (1) Pipette 1.0 mL of the diluted filtered extract into the headspace of the AflaTest column.
- (2) Use the column coupling to attach the AflaTest column to the bottom of the glass syringe barrel on the pump stand. Place a waste collection vessel under the column outlet. Insert the coupling on the end of pump tubing into the glass syringe barrel, and activate the pump. Maintain a steady flow rate of approximately 1 drop of extract per second until all the extract has passed through the column. Maintain pressure on the column until air passes through the column.
- (3) Detach the test column from the syringe barrel and fill the column headspace with deionized or distilled water (about 1mL). Reattach column to the syringe barrel, and activate the pump. Maintain a steady flow rate of 1 to 2 drops of water per second until all the water has passed through the column.
- (4) Repeat step 3. Maintain pressure on the column until air passes through the column.

b. Sample cleanup for distillers dried grain with solubles.

- (1) Pipette 1.0 mL of the filtered dilute extract into the headspace of the AflaTest column.
- (2) Use the column coupling to attach column to the bottom of the glass syringe barrel on the pump stand. Place a waste collection vessel under the column outlet. Insert the coupling on the end of the pump tubing into the top of the glass syringe barrel, and activate the pump. Maintain a steady flow rate of approximately 1 drop of extract per second until all the extract has passed through the column.

- (3) Detach the column from the glass syringe barrel. Repeat steps 1 and 2. Maintain pressure on the column until air passes through the column.
- (4) Detach the column from the syringe barrel and fill the column headspace with deionized or distilled water (about 1mL). Reattach the column to the syringe barrel, add 5mL water to the syringe barrel and activate the pump. Maintain a steady flow rate of 1 to 2 drops of water per second until all the water has passed through the column (6ml total). Maintain pressure on the column until air passes through the column.

c. Sample Cleanup for Corn Gluten Meal.

- (1) Use the column coupling to attach the column to the glass syringe barrel on the pump stand. Place a waste collection vessel under the column outlet. Add 4 mL of the filtered extract to the syringe barrel. Insert the coupling at the end of the pump tubing into the top of the glass syringe barrel, and activate the pump. Maintain a steady flow rate of approximately 1 drop of extract per second until all the extract has passed through the column.
- (2) Add 10 mL of deionized or distilled water to the syringe barrel, and activate the pump. Maintain a steady flow rate of 1 to 2 drops of water per second until all the water has passed through the column.
- (3) Repeat step 2.

d. Separation and Measurement.

- (1) Place a clean VICAM borosilicate glass cuvette under the outlet of the IAC column.
- (2) Detach the column and pipette 1 mL of HPLC grade methanol directly into the column headspace. Reattach the column, and activate the pump. Apply just enough air pressure to pass the methanol through the column at a rate of approximately 1 drop every 2 seconds. Collect all the methanol (1mL) in the cuvette.
- (3) Add 1.0 mL of freshly made developer solution directly to the sample eluate in the cuvette. Vortex for 3 to 5 seconds. Clean the outside of the cuvette quickly with a Kimwipe.
- (4) Immediately place the cuvette in a previously calibrated fluorometer. Reader reads sample concentration after 60 seconds.
- (5) Sample results above 300 ppb are reported as "Aflatoxin exceeds 300 ppb."

5. REPORTING AND CERTIFYING TEST RESULTS

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

7. STORAGE CONDITIONS AND PRECAUTIONS

a. Storage conditions.

- (1) Store AflaTest columns at room temperature (64° to 86° F).
- (2) The standard solutions in the three standard vials (red, green, and yellow) degrade slowly in the presence of light. Keep vials in closed case. About 2 months before the expiration date, obtain a new set of AflaTest FGIS Calibration Standards (VICAM product # 33030).
- (3) Store the developer concentrate in a tightly closed bottle in a cool, dry area and away from sunlight.

b. Precautions.

- (1) The sample extract passed over the AflaTest column must be clear. If the diluted filtrate turns cloudy, filter again using a new glass microfiber filter before proceeding with testing.
- (2) Use the recommended equipment for procedures:
 - (a) Collection of extracts and filtrates: VICAM disposable hard plastic beakers (VICAM # 36010) or clean glass containers. Soft plastic may leach fluorescent materials.
 - (b) Fluorometer cuvettes: VICAM 12 x 75 mm borosilicate glass cuvettes (VICAM # 34000). Note: Cuvettes from other sources may emit significant fluorescence, resulting in inaccurate readings. Keep cuvette's optical surface free of lint, fingerprints, etc.
- (3) Add diluted developer solution directly to the cuvette containing the eluate from the AflaTest column. Use freshly made (within 8 hours) solution only. Mix well by vortexing or flicking bottom of the tube with fingers. DO NOT shake or invert cuvette using a thumb or finger as a cap. Doing this may elute fluorescent materials.
- (4) Rinse glass syringe barrels with approximately 10 ml of distilled/deionized water between assays.
- (5) Carry out extraction of sample with 80% methanol or 60% methanol (corn gluten meal only) in a well-ventilated area.

8. EQUIPMENT AND SUPPLIES

Materials required.

- a. VICAM Series 4EX Fluorometer (VICAM # FLSEREX)
- b. AflaTest FGIS Calibration Standards (VICAM # 33030)
- c. Pump stand with air pump (VICAM # 21020, 21030, 21040, 21045, G4061 or G1104)
- d. AflaTest developer solution (VICAM # 32010)
- e. AflaTest immunoaffinity columns (VICAM # 12022)
- f. HPLC grade methanol or 80% methanol premixed solution (VICAM # 100000211- 20L or #100000212-4L)
- g. HPLC grade methanol or 60% methanol premixed solution (VICAM # 100000213- 20L or #100000214-4L) for corn gluten meal only
- h. USP grade sodium chloride (NaCl) or food grade non-iodized salt (VICAM # G1124)
- i. Automatic pipettor
 - (1) 1 mL capacity for methanol (VICAM # 20501)
 - (2) 1 mL capacity for developer (VICAM # 20600)
- j. Blender base (VICAM # 20201)
- k. Eberbach Glass Blender Jar, 500mL (VICAM #20300)
- l. Cuvettes, disposable 12 x 75 mm borosilicate glass tube (VICAM # 34000)
- m. Cuvette rack (VICAM # 21010)
- n. Disposable beakers (VICAM # 36010)
- o. Fluted filter paper (VICAM # 31240)
- p. Funnel for fluted filter paper (VICAM # 36022)
- q. Funnel for glass microfiber filter paper (VICAM # 36020)
- r. Glass microfiber filter paper (VICAM # 31955)
- s. Graduated cylinder, 50 mL glass (VICAM # 20050)
- t. Syringe, glass 10 mL (VICAM # 34010)
- u. Water wash bottle (VICAM# 20700)
- v. Micro-pipettor, 1mL (VICAM# G4033)
- w. Micro-pipette Tips for 1mL Micro-pipettor, 100 (VICAM# 20656)
- x. Adjustable pipettor, 5mL (VICAM # G4055)
- y. Finntips for 5mL adjustable pipettor (VICAM #G4060)
- z. A package of all the basic equipment can be purchased from VICAM (# G8001).

9. REVISION HISTORY

Revision 0 (09/14/2016)