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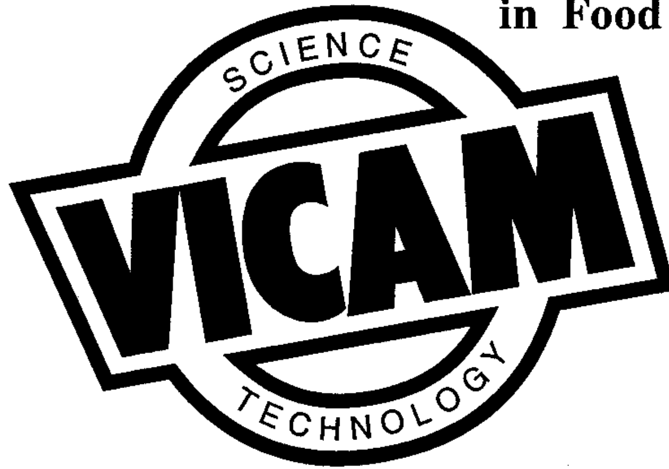

AflaTest®

Instruction Manual

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1.1 INTENDED USER

AflaTest® is a quantitative method for the detection of aflatoxin in many commodities. Vicam's advanced biotechnology permits the measurement of all the major aflatoxins (including AFB1, AFB2, AFG1, AFG2 and AFM1) without the use of toxic solvents like chloroform or methylene chloride. AflaTest® aflatoxin testing is used in a wide variety of locations from the local farm elevator to food processing quality control laboratories to government testing laboratories - anyplace where quick, easy to perform and highly accurate aflatoxin analysis can prevent contamination and improve the quality of the food supply.

1.2 PRINCIPLE

Aflatoxin, a toxin from a naturally occurring mold, is a Group 1 carcinogen proven to cause cancer in humans. Aflatoxin can also cause economic losses in livestock due to disease or reduced efficiency of production. AflaTest® is a fast, simple, safe and highly accurate method for quantitatively measuring aflatoxin in many commodities.

Samples are prepared by mixing with an extraction solution, blending and filtering. The extract is then applied to the AflaTest® column bound with specific antibodies to aflatoxin. At this stage, the aflatoxin binds to the antibody on the column. The column is then washed with water to rid the immunoaffinity column of impurities. By passing methanol through the column, the aflatoxin is removed from the antibody. This methanol solution can then be injected into an HPLC system or measured in a fluorometer. These steps are outlined in section 1.7, AflaTest® Overview.

1.3 APPLICABILITY AND APPROVALS

AflaTest® has been optimized for quantitative measurement of aflatoxins in many commodities. The Table of Contents lists the testing protocols developed for specific commodities as of the publication date of this manual. Assistance in measuring aflatoxin in commodities not listed in this manual can be obtained by contacting our Technical Assistance Department.

AflaTest® methods vary in the amount of sample passed through the affinity column. Greater amounts of sample passed through the column results in lower limits of detection. However, when lesser amounts of sample are passed over the column, the range of the assay is wider and the test can be completed quicker. In general, 0.2g methods have a wider testing range and are faster. 1.0g methods have a lower limit of detection. Both methods are accurate.

AflaTest® has been validated by the AOAC Research Institute under the *Performance Tested*[sm] *Program* to detect aflatoxin residues in grain and grain products. AflaTest® was granted *Performance Tested*[sm] status and is licensed under certification mark number 940801. This quantitative test kit also underwent evaluation by the United States Department of Agriculture, Federal Grain Inspection Services (FGIS) for the detection of total aflatoxin (B_1, B_2, G_1 and G_2) for corn, corn germ meal, corn gluten feed, corn gluten meal, corn meal, corn/soy blend, milled rice, popcorn, sorghum, soybeans and wheat. Under the authority of the United States Grain Standards Act, this test kit was found to meet or exceed all design and test performance criteria as defined in "Design Criteria and Test Performance Specifications for Quantitative Aflatoxin Test kits". This test kit is cited in the AOAC® Official Methods Program, as official method 991.31 applicable for the determination of aflatoxin B_1, B_2, G_1 and G_2 both by fluorometry and HPLC analysis. AflaTest® has final action AOAC approval.

1.4 LIMITATIONS

This test has been designed for use with the procedure and reagents described on the following pages. Do not use materials beyond the expiration date. Deviation from these instructions may not yield optimum results.

1.5 SAMPLING

Mycotoxins do not occur in every kernel in a lot and may only occur in a small percentage of the kernels in a lot. Because of the wide range in mycotoxin concentrations among individual kernels in a contaminated lot, variation from sample to sample can be large. It is important to obtain a representative sample from a lot. Product should be collected from different locations in a static lot based on a probing pattern. The probe should draw from the top to the bottom of the lot. The samples obtained from the probes should be ground and mixed well and a subsample taken for testing. For further information on grain sampling, refer to the following FGIS publications:

FGIS Aflatoxin Handbook
FGIS Grain Inspection Handbook, Book 1, Grain Sampling
FGIS Mechanical Sampling Systems Handbook

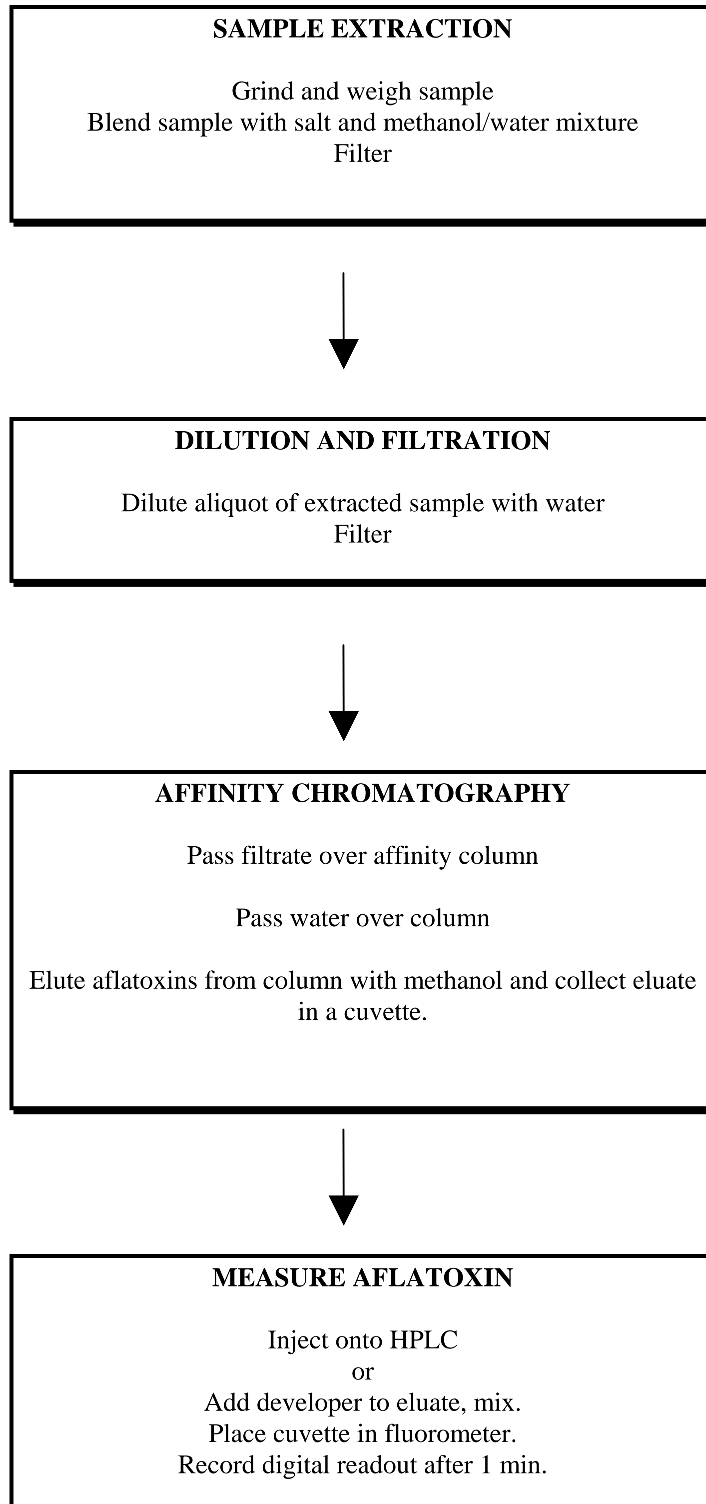
Limited quantities of these publications are available free, upon request. Send requests to:

USDA-APHIS, MSD-HSB PDMS
Room 1A28
4700 River Road
Riverdale, MD 20737

1.6 SHELF LIFE AND STORAGE CONDITIONS

Store at room temperature. Storage at temperatures above 30°C for prolonged periods of time may reduce shelf life. If storage temperatures above 30°C are anticipated, all components may be stored at 4°C. It is recommended that reagents should be at room temperature (18 - 22°C) for usage.

1.7 AFLATEST® OVERVIEW



2.1 FLUOROMETER CALIBRATION FOR SEQUOIA-TURNER MODEL 450 (SERIES 1 AND 2)

Important: Fluorometer calibration is dependent upon the model of fluorometer and the procedure used. The series 1 fluorometer uses a white lamp and the series 2 fluorometer uses a blue lamp. The lamp color can be determined by turning on the instrument and opening the lid to the cuvette holder. Pull up on the trapezoidal part to the left of the cuvette holder labeled NB360 and look into the instrument to check the color of the bulb. Consult the specific procedure to determine the correct gain levels and calibration standard values.

1. Turn on the power with switch at left rear of the Sequoia-Turner Model 450 Fluorometer.

2. Allow fluorometer to warm up for 20-30 minutes.

3. Use only Calibration standards indicated in the specific procedure.

4. Set the gain selector knob to the appropriate level.

5. Turn the span knob all the way clockwise.

6. Place the GREEN standard in the sample chamber and close the lid.

7. Adjust the zero knob until the digital display reads the desired value.

8. Remove the GREEN standard from the chamber. Place the RED standard in the sample chamber and close the lid.

9. Adjust the span knob until the digital display reads the desired value.

   Note: If display cannot be adjusted with the span knob to read the desired values, it may be necessary to readjust the gain knob. If the digital display value is too low, increase the gain level to the next selection. If the display level is too high, decrease the gain level. After changing the gain level, start calibration process again at step #5.

10. Remove the RED standard from the chamber.

11. Recheck the calibration of the GREEN standard to make sure that it reads the correct value. Adjust with the zero knob if necessary.

12. Insert yellow vial. The result of this measurement should be within the range indicated in the procedure. If the result is not within the range specified, recalibrate the fluorometer.

13. Place all standards back in the case. Do not leave standards exposed to light.

   The calibration of the fluorometer is accurate within the linear binding capacity of the affinity column.

2.2 FLUOROMETER CALIBRATION - TORBEX MODEL FX-100 SERIES 3

The following section details the calibration procedures for the TorBex Model FX-100 Series 3 fluorometer. For more detailed information on the operation of this fluorometer consult the TorBex Fluorometer Model FX-100 Operator's Manual which was supplied with the fluorometer.

A. Starting the Fluorometer

1. Turn power ON using the on/off switch on the rear panel.
2. The model FX-100 will identify itself on the LCD then proceed through a series of self tests. If any error messages appear consult the operator's manual.
3. The fluorometer will then proceed through a printer check. If any error messages appear consult the operator's manual.
4. Set Date and Time

When date and time are first shown, the display reads:

DATE XX/XX/XX	**Time XX:XX:XX**
Change	**Continue**

If the displayed date and time are correct, depress the arrow key under **CONTINUE**.

If not correct, depress the arrow key under **CHANGE**. The display then asks if date or time is to be changed.

One is selected and changed using the numerical keypad.

When the correct time or date are entered, depress the **ENTER** key. The system returns to display the time and date message given above with the new data entered.

To change date if time has already been set, select arrow key under **CHANGE**, select **DATE**, enter the new date and continue as above.

When the time and date are correct, depress the arrow key under **CONTINUE**.

5. Set Test Delay Time

The following message will be displayed:

TEST DELAY TIME
60 SEC PRESS ENTER

This allows the user to set the delay time, which is the time from when the sample is placed into the fluorometer to the time it is actually measured. For AflaTest® applications the delay time is 60 seconds.

The keypad will read **60**, press **ENTER**.

6. Set Answer Format

After test delay time has been set, the display reads the following:

SELECT ANSWER FORMAT
INTEGERS DECIMALS

Selecting integers will give whole numbers only, selecting decimals will give numbers less than ten with one decimal place. Select **DECIMALS.**

7. Select measurement units

The following message will be displayed:

SELECT MEASUREMENT UNITS
PPM PPB

This allows you to select the readout in ppm or ppb. Select **PPB**.

B. Calibration Procedure

After selecting the answer format, the following message is displayed briefly:

START CALIBRATION
CHECKING GAINS. WAIT

Then the message changes to read:

INSERT RED VIAL

Select high calibration vial (RED) and place it in the sample chamber. Be sure the vial is fully inserted and touches the bottom of the sample chamber. The display now shows:

CALIBRATION VALUE
24.0 PPB PRESS ENTER

While 24 ppb is the default value, any calibration value which is greater than the blank value, up to 1000 ppb, may be used. Consult the specific AflaTest® procedure for the appropriate value.

Enter the desired value and push **ENTER**. Pressing **ENTER** will start the calibration sample measurement. The display will read:

MEASURING CALIBRATOR

When calibration is completes the following message will appear:

REMOVE RED VIAL

Remove the calibration sample vial from the sample chamber. The next message to appear will read:

INSERT GREEN VIAL
CONTINUE CALIBRATION

At this time the blank sample vial (GREEN) should be selected and placed in the sample chamber. Be sure that the vial is fully inserted and touches the bottom of the sample chamber. The display will now read:

BLANK VALUE
-1.0 PPB PRESS ENTER

While -1.0 ppb is the default value, any blank value which is less than the calibration value may be used, including negative numbers. Consult the specific AflaTest® procedure for the appropriate value.

Enter the desired value using the number keys. Pressing **ENTER** will start the blank calibration measurement. At this time the display will read:

MEASURING BLANK

When the blank measurement is completed, the message will read as follows:

MEASURING BLANK
REMOVE GREEN VIAL

Remove blank vial at this time. The display will now read:

PUSH ENTER TO CONTINUE
RECAL <SOFT KEYS> RETEST

This display defines the two active arrow keys. The left one (**RECA**L) is pressed at the end of a measurement if the system is to be recalibrated. The right arrow key (**RETEST**) is pressed at the end of a measurement if the sample is to be remeasured (without delay time). To read a vial with a 5 second delay, press the middle arrow key before inserting the vial.

The calibration process is now completed. Press **ENTER** key to prepare instrument for measuring unknown samples. The display will read:

READY TO START TESTING
INSERT SAMPLE TO MEASURE

At this point the calibration may be checked by inserting the YELLOW calibration vial for measurement. The result of this measurement should correspond to the appropriate value for the calibration parameters found in the specific AflaTest® procedure. If it does not, recalibrate the fluorometer with a different set of standards. To restart the calibration process press the left arrow key (**RECAL**).

C. Sample Measurement

1. To measure aflatoxin concentration in sample:

Place sample cuvette in sample chamber. Be sure that the sample is fully inserted and touches the bottom of the chamber. The display will read:

TEST DELAY IN PROGRESS
_ _ _ SECS TILL MEASUREMENT

The number of seconds remaining before the measurement starts will decrement until it reaches zero(0) and then the message will read:

PERFORMING MEASUREMENT

When the measurement is finished, the results will be displayed as follows:

TEST RESULTS _ _ _ PPB
REMOVE SAMPLE VIAL

2. Re-Measurement Options

At this time there are three actions which can be selected by the operator:

a. Remove sample cuvette and proceed to next sample for measurement. The LCD will display:

PREV RESULTS _ _ _ PPB
INSERT NEXT SAMPLE

when the next sample is inserted in well the display will change to read:

PREV RESULTS _ _ _ PPB
_ _ _ SECS TILL MEASUREMENT

The sample will be measured and the results displayed as before.

b. Repeat Measurement

The sample can be re-measured without delay time by pressing the right arrow key (**RETEST**). The sample will be immediately measured and the results displayed as before.

c. If the sample has been removed, a result can be obtained in 5 seconds by pressing the middle arrow key before inserting the cuvette into the fluorometer.

d. Re-calibrate

If it is desired, the fluorometer can be re-calibrated. To initiate the calibration process press the left arrow key (**RECAL**). The instrument will then proceed to the calibration process as described in Section B above.

2.3 FLUOROMETER CALIBRATION FOR VICAM V1 SERIES 4

1. Turn power on using the ON/OFF switch on the back of the instrument.

2. Press the **SELECT TEST** key until AflaTest® appears on the display. Then press **ENTER**.

3. The fluorometer will read:

   START CALIBRATION... OPEN THE LID
   INSERT RED VIAL

   Open the lid and insert the red mycotoxin calibration vial**. Make sure that the vial is fully inserted and touches the bottom of the well.**

4. The display will read:

   HIGH CAL 22 PPB

   If this is the red vial setting desired for the procedure you are using press **ENTER**. Otherwise, enter the desired calibration setting on the keypad, from the specific procedure. Confirm that the desired value appears on the display and press **ENTER**.

5. The display will read:

   READING HIGH CAL... SAVING HIGH INTENSITY

   and then read:

   OPEN THE LID
   INSERT GREEN VIAL

   Open the lid, remove the red vial and insert the green mycotoxin calibration vial, again making sure that the vial is fully inserted and touches the bottom of the well.

6. The display will read:

   LOW CAL - 1.0 PPB

   If this is the green vial setting desired for the method you are using press **ENTER**. Otherwise enter the desired calibration setting on the keypad from the specific procedure. Confirm that the desired value appears on the display and press **ENTER**.

7. The display will read:

   READING LOW CAL... SAVING LOW INTENSITY

   and then read:
   OPEN THE LID

   Open the lid and remove the green vial.

8. The display will read:

VICAM V1.1 READY

The display may show other numbers to indicate what software version you have installed.

Press **SELECT TEST**. The display will read:

AFLATEST

Press **ENTER**.

9. The display will read:

START RUN TEST OPEN THE LID

10. Insert the yellow mycotoxin calibration standard. The yellow vial reading should be in the range listed in the Procedures Section.

11. The fluorometer is now ready for samples to be inserted. The series 4 fluorometer needs to be calibrated only once a week.

To recalibrate the fluorometer**:** Press the **STOP** key. Press the **OPTIONS** key until the display reads:

CALIBRATE TEST

Then press **ENTER**. The screen should display:

AFLATEST

If it doesn't, press **SELECT TEST** until "**AFLATEST**" appears on the display and then press **ENTER**. Insert red and green mycotoxin calibration vials and calibrate as described above.

To run a test: press **SELECT TEST** until the display reads:

AFLATEST (or the desired test)

Then press **ENTER**.

To leave any procedure: press **STOP**.

For more details on the use of the fluorometer, please consult the fluorometer Operator's Manual.

2.4 CALIBRATION FOR MF-2000™ MINI FLUOROMETER VICAM V1.0

Calibration will be required only if the unit has been turned off or after 24 hours has elapsed since the previous calibration.

1. Turn power on using the ON/OFF switch on the back of the instrument.

2. The green **POWER** and **READY** light will illuminate indicating that the instrument is operational and ready to proceed with testing. Allow instrument to warm up for 15 minutes.

3. Press the **RUN/STEP** button. The red **INSERT RED VIAL** light will illuminate. Perform the next step within 15 seconds or the **READY** light will illuminate.

4. Open the lid and insert the red calibration vial. **Make sure that the vial is fully inserted and touches the bottom of the well**.

5. Press the **RUN/STEP** button. The calibration will be completed when the red **INSERT GREEN VIAL** light is illuminated. Perform the next step within 15 seconds or the **READY** light will illuminate.

6. Open the lid and insert the green calibration vial. **Make sure that the vial is fully inserted and touches the bottom of the well.**

7. Press the **RUN/STEP** button. The calibration will be completed when the green **INSERT SAMPLE** light is illuminated.

8. Open the lid and remove the green vial. **INSERT SAMPLE** light will illuminate.

9. Insert yellow vial to check the calibration. Press **RUN/STEP** button. The **BAR GRAPH** lights will illuminate. Verify result with the acceptable range listed in the appropriate procedure.

10. **READY** light will illuminate.

11. Press **RUN/STEP** button. The **INSERT SAMPLE** light will illuminate.

12. Insert cuvette containing sample eluate and press **RUN/STEP** button. The **BAR GRAPH** lights will illuminate. Read toxin concentration using the appropriate overlay.

13. Repeat steps 11 and 12 to read more samples.

14. The red **OVER HIGH CAL** light will turn on when the toxin concentration is higher than the red vial concentration.

15. The red **ERROR** light will illuminate when there is an initialization error, when the sample concentration is out of range (too low or too high), when the high calibrator has more fluorescence than the instrument can measure or when the low calibrator has less fluorescence than the instrument can detect.

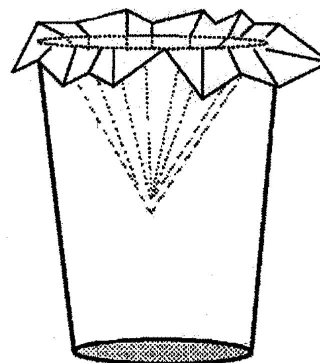
2.5 COMPARISON OF FLUOROMETER CALIBRATION SETTINGS

The fluorometer calibration settings vary depending on the method and the model of the fluorometer. The following table contains a general description of the appropriate fluorometer settings. Please see the specific method in the procedures section for the calibration setting for each method.

	Sequoia-Turner Model 450		Torbex FX-100	Vicam V1	MF-2000™
	series 1	series 2	series 3	series 4	overlay
Gram equivalent of method	Red/green (yellow)	Red/green (yellow)	Red/green (yellow)	Red/green (yellow)	
0.2	100 / 0 (50 ± 5)	130 / 0 (65 ± 5)	120 / -2 (59 ± 5)	110 / -2 (54 ± 5)	AflaTest® 0.2 g
0.5	40 / 0 (20 ± 2)	52 / 0 (26 ± 2)	48 / -1 (24 ± 2)	44 / -1 (22 ± 2)	AflaTest® 0.5 g
1.0	20 / 0 (10 ± 2)	26 / 0 (13 ± 2)	24 / -1 (12 ± 2)	22 / -1 (11 ± 2)	AflaTest® 1.0 g
FGIS			150 / -3 (74 ± 5)	140 / -3 (69 ± 5)	AflaTest® USDA-FGIS

2.6 PREPARATION OF FILTRATION STEPS

Fluted Filter

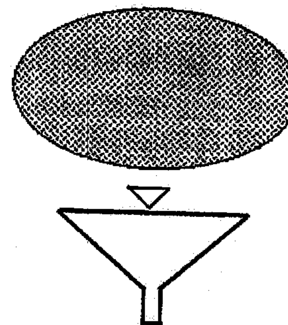

Fluted Filter Assembly

The first filtration step is a simple gravity filtration through fluted filter paper to separate the sample extract solution from the coarse particulate sample solids. The filtrate is collected in a clean container or graduated cylinder.

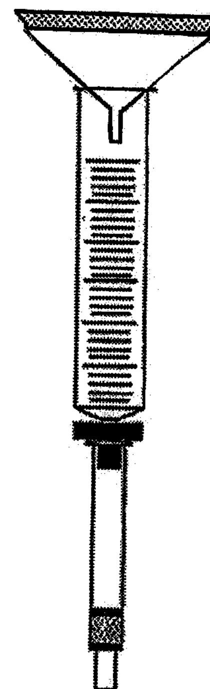
1. Open one fluted filter carefully and insert into clean container. (Optional: a funnel may be used to hold the filter).

2. Fold edges of filter over rim of cup to hold in place. Maintain the fluted folds of the filter paper to maximize surface area. This will increase speed of filtration.

3. It is not necessary to wait for all the extract to pass through the filter before continuing.



Microfibre Filter



The second filtration step is the gravity filtration of the extract through a microfibre filter. This removes any precipitates in the extract and assures that the extract will easily pass through the affinity column. Microfibre filtration is performed just prior to affinity chromatography.

1. Place a small funnel in top outlet of syringe barrel or clean collecting cup.

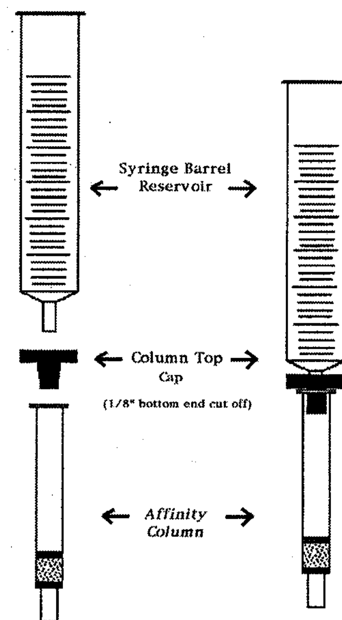
2. Place one microfibre filter gently into small funnel by pressing filter into funnel with index finger. Be careful not to rip or puncture the filter.

2.7 PUMP STAND SETUP

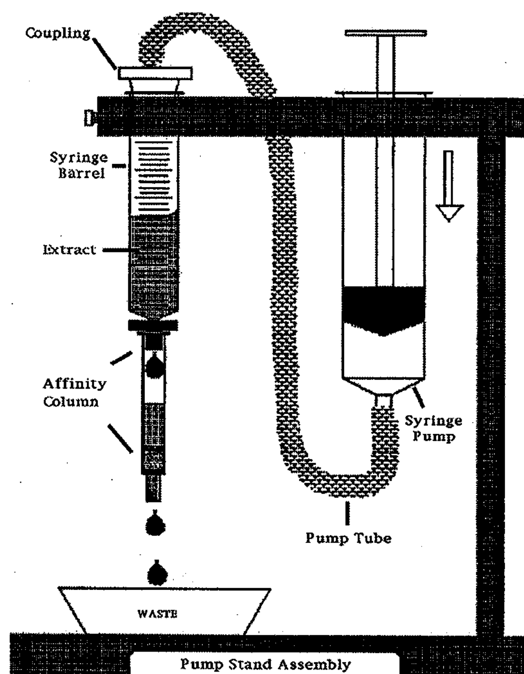
AflaTest® affinity chromatography is easily performed with the AflaTest® affinity column attached to a pump stand. The stand has a 10 mL glass syringe barrel that serves as a reservoir for the column. A large plastic syringe with tubing and coupling provides air pressure to manually push liquids through the column. An adjustable air pump (Vicam part #20650) can be attached to the pump tube instead of the large pump syringe barrel to operate without using hand pressure. Double position pump stands (part # 21030), four-position pump stands with aquarium pumps (Vicam part #21045), and twelve-position pump stands with aquarium pumps (Vicam part # G1104) are available for running multiple samples at one time.

1. Remove large top cap from column.
2. Cut bottom 1/8 inch off the end of the top cap with scissors or sharp blade. This provides a reusable coupling for attaching the column.
3. Attach column to coupling and place waste collection cup under column outlet. Keep bottom cap on column.
4. Pour extract into microfibre filter (see previous section) and collect desired amount of extract in glass syringe barrel using markings on the syringe barrel to measure extract.
5. Pull up on the plastic syringe piston.
6. Inset coupling on end of tube into syringe barrel. Remove column bottom cap.
7. Apply pressure to piston of plastic syringe to push liquid through the column. Maintain a flow rate of 1-2 drops per second. Push all liquid through the column. Repeat for wash and elution steps (see procedures).

Note: Avoid pulling up on plastic syringe piston while coupling is attached to glass syringe barrel. This may displace the antibody coated support beads and affect test results.



Affinity Column Syringe Barrel Connection



Pump Stand Assembly

2.8 CLEANING EQUIPMENT

Before Starting AflaTest® Testing

To eliminate background fluorescence make sure the equipment is clean and not contaminated with materials that might cause background fluorescence. This is particularly important when using brand new equipment or equipment that has not been used for a long period of time.

Before using the equipment, it should be washed with a mild detergent solution and then rinsed thoroughly with purified water. This includes the glass syringe barrels used for sample reservoirs. The syringe barrels are treated with a lubricant for use with a piston plunger. Wash new syringe barrel for pump stands using a brush with soap and water. Then rinse with purified water and methanol before using to remove lubricant. Other pieces of equipment that need to be cleaned with detergent before using are graduated cylinders, funnels and blender jars. Repipetters need only to be rinsed with methanol before use.

Between Assays:

After each assay, the blender jar assembly needs to be washed with a mild detergent solution and rinsed thoroughly with purified water. The same cleaning procedure must be performed for any equipment that will be reused to hold, collect or transfer sample extracts.*

Do not wash repipetter with soap. Methanol repipetter needs only to be refilled with methanol.

In between each assay, the syringe barrel reservoir can be rinsed with methanol followed by a rinse with purified water. This will be sufficient to prevent cross-contamination of samples. After a number of samples have been tested, the glass syringe barrel should be washed with a brush and detergent and rinsed well with water.

It is not recommended to wash and reuse the cuvettes. These cuvettes are designed for one-time use and should be discarded.

Other Important Precautions

Use only equipment specified by Vicam. Avoid contact of any test reagents or solutions (such as methanol, water, extract, column eluate or developer) with rubber or soft flexible plastic. These materials may leach contaminating fluorescent materials into the sample and thereby affect results.

Note: Some blender jar lids are lined with waxed cardboard. These liners are not resistant to methanol and water solutions and will breakdown when used for sample extraction. The extract will then become contaminated with materials which may cause background fluorescence. Lids with cardboard liner should not be used.

* More details on decontamination can be found in JAOAC **48**, 681 (1965); Am. Hyg. Assoc. J. **42**, 398 (1981); and IARC Sci. Publ. No. 37, IARC, Lyon, France, 1980.

3.1 PREPARATION OF EXTRACTION SOLUTIONS

The AflaTest® procedure uses a methanol or a methanol/water solution to extract aflatoxin out of the sample.

To prepare extraction solution:

Use reagent grade (or better - i.e. HPLC grade) methanol when preparing extraction solutions.

Solution desired (methanol:water)	Methanol (mL)	Purified Water (mL)	Total Volume (mL)
80:20	800	200	1000 (1 liter)
70:30	700	300	1000 (1 liter)
60:40	600	400	1000 (1 liter)

CAUTION: Extraction solvent is flammable. Keep container tightly capped when not in use. Prepare extraction solution every week or as needed. The formulas above will prepare 1 liter of solution. Solution volume may be increased or decreased as needed provided the proportion of reagents is kept consistent.

3.2 PREPARATION OF AFLATEST® DEVELOPER SOLUTION

To Prepare Dilute AflaTest® Developer solution:

1. Measure 5.0 mL AflaTest® Developer concentrate solution and place in the 2 oz. amber glass bottle of a 50 mL bottle dispenser for developer. (Vicam part # 20600).

2. Add 45.0 mL purified water and mix well.

3. Secure the bottle dispenser top tightly. Keep the dilute Developer solution tightly capped when not in use. Do not use dilute Developer more than 8 hours after preparation.

To use Developer:

Pipet exactly 1.0 mL of dilute Developer solution directly into the cuvette containing the affinity column eluate and mix well before reading this solution in the fluorometer. When using the bottle dispenser make sure there are no bubbles in the tubing before dispensing the Developer solution.

To assure maximum performance of AflaTest® Developer solution follow these recommendations:

1. Make the dilute Developer solution every 8 hours. If potency of dilute Developer is in question, it is better to make up a new dilute solution from the Developer concentrate.

2. Avoid contamination of the bottle of concentrated Developer, glassware and pipetters with dirt, dust and other liquids. Keep the bottle tightly capped when not in use. The stock solution of concentrated Developer solution should have a definite yellow color. This color is a good indication of its potency. Do not use if the concentrated solution is colorless.

3. Label each new bottle of concentrated Developer with the date on which it was first opened. Do not use more than 30 days after opening.

4. Test the dilute Developer solution for background fluorescence. Put 2.0 mL dilute Developer into a cuvette. Place the cuvette in a calibrated fluorometer. The fluorometer digital display should be 0. If readout does not equal 0, see Section 3.5, Reagent Check.

3.3 PREPARATION OF DILUTION/WASH SOLUTIONS

The formulas below will prepare 1 liter of solution. Solution volume may be increased or decreased as needed provided the proportion of reagents is kept consistent.

1. Methanol:Water solutions; prepare every week or as needed

Solution desired (methanol:water)	HPLC Grade Methanol (mL)	Purified Water (mL)	Total Volume (mL)
10:90	100	900	1000 (1 liter)
15:85	150	850	1000 (1 liter)
20:80	200	800	1000 (1 liter)

2. Tween-20 solutions; prepare every month or as needed

Solution desired	Tween-20 (mL)	Purified Water (mL)	Total Volume (mL)
10% Tween-20	100	900	1000 (1 liter)
15% Tween-20	150	850	1000 (1 liter)
20% Tween-20	200	800	1000 (1 liter)

3. Zinc Acetate/Aluminum Chloride ($Zn(OAc)_2/AlCl$); prepare every three months or as needed

   200g $Zn(C_2H_3)_2)_2$ (Zinc Acetate)
   5 g $AlCl_3$ (Aluminum Chloride)
   bring to 1000 mL (1 liter) with purified water

3.4 PREPARATION OF HPLC SOLUTIONS

1. HPLC Mobile Phase

Solution desired (methanol:water)	HPLC Grade Methanol (mL)	Purified Water (mL)	Total Volume (mL)
45:55	450	550	1000 (1 liter)

Solution should be filtered and degassed before use.

2. Iodine solution (0.05%)

0.5 g Iodine
100 mL Methanol
900 mL purified water

Dissolve iodine in methanol, stirring until completely dissolved. While stirring add purified water. Mix solution for at least 30 minutes. Filter solution through 0.45 micron nylon filter. This solution can be used for 2 weeks from preparation.

3.5 REAGENT CHECK

In AflaTest®procedures, aflatoxin levels are detected and quantified by fluorometry. For accurate determination of aflatoxin concentration it is critical that only aflatoxin in the cuvette is emitting fluorescence. Background fluorescence and/or chemiluminescence caused by reagents or the cuvettes will be erroneously measured as aflatoxin by the fluorometer.

It is good practice and strongly recommended to check the reagents and the cuvettes to make sure that they are not fluorescent and will not contribute to the fluorescence measured by the fluorometer. This is an easy process and should be performed daily or whenever a new batch of reagents or cuvettes is used.

To Check Reagents and Cuvettes:

1. Calibrate fluorometer.
2. Pipet 2 mL methanol used for column elution into a cuvette.
3. Measure background fluorescence in fluorometer. The readout should be 0.
4. Pipet 2 mL purified water used for column washes into a cuvette.
5. Measure background fluorescence in fluorometer. The readout should be 0.
6. Pipet 2 mL dilute Developer into a cuvette.
7. Measure background fluorescence in fluorometer. The readout should be 0.
8. Pipet 1 mL methanol into a cuvette and add 1 mL dilute Developer. Mix well.
9. Measure background fluorescence in fluorometer. The readout should be 0.

***** IMPORTANT *****

Solutions that do not give a 0 readout must be retested with a new cuvette. If the solution does not read 0, it should be discarded and a new solution prepared and tested.

If all three solutions tested give readouts above 0, recheck fluorometer calibration. If calibration is satisfactory, then there is a good possibility that the cuvettes are defective and a new batch of cuvettes should be obtained. Be sure to use cuvettes purchased from Vicam. Other cuvettes may contain fluorescent material.

Helpful suggestion: Before starting sample testing, a good check of procedures, reagents and equipment is to run a complete assay without any sample. The fluorometer reading of a blank assay should be 0.

4.1 MATERIALS AND EQUIPMENT REQUIRED FOR FLUOROMETER PROCEDURES

Materials Required

Description	Part #
AflaTest®-P Columns, for Fluorometer & HPLC (50/box)	12022
*Disposable Pipets (50 per pack)	20652
*VICAM Fluted Filter Paper, 24 cm (100)	31240
*Microfibre Filters, 1.5µm, 11 cm (100)	31955
*Kim Wipes Tissues (1 box)	31967
*AflaTest® Developer (50 mL)	32010
Calibration Standards as specified in procedure	
*Mycotoxin Calibration Standards	33020
AflaTest® FGIS Calibration Standards	33030
AflaTest®-M Calibration Standards	33040
Tween-20 (50 mL) (for nutmeg, oregano & alfalfa)	33501
*Disposable Cuvettes (250 per pack)	34000
Methanol, HPLC Grade (4 x 4 L)	35016
*Disposable Plastic Beakers (25 per pack)	36010
Distilled, reverse osmosis or deionized water	
Noniodized sodium chloride (salt, NaCl)	
Zinc Acetate (for cocoa)	
Aluminum Chloride (for cocoa)	
0.22 micron nylon syringe disk filters (for peanut hulls & corn gluten)	

Equipment Required

Description	Part #
*Graduated cylinder, 50 mL	20050
*Digital Scale with AC Adapter	20100
*Commercial Blender with Stainless Steel Container	20200
*Graduated cylinder, 250 mL	20250
*500 mL Bottle Dispenser for Methanol (0-3 mL range)	20501
*50 mL Bottle Dispenser for developer (0-3 mL range)	20600
*Wash Bottle, 500 mL	20700
*Cuvette Rack	21010
*Single Position Pump Stand	21020
*Filter Funnel, 65 mm (10 per pack)	36020
*Series 4 fluorometer or	G8000
MF-2000™ Mini Fluorometer	G8200
centrifuge, 2000g (for milk test only)	

* Included in AflaTest® Fluorometer Series 4 Basic Equipment Package - 110V, U.S.A. (Vicam part # G8001) and 220V, International (Vicam part # G8002).

Suggested but not required

Description	Part #
Vortex Mixer	23040
Filter Funnel, 105 mm (4 funnels)	36222

4.2 AFLATEST® FLUOROMETER PROCEDURE FOR ALFALFA (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
2.4 Prepare 10% Tween 20 solution.
2.5 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.6 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 100 mL methanol:water (80:20).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 5.0 mL filtered extract into a clean vessel.
4.2 Dilute extract with 20 mL of 10% Tween 20 solution. Mix well.
4.3 Filter dilute extract through glass microfibre filter into glass syringe barrel using markings on barrel to measure 4 mL.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb.

4.3 AFLATEST® FLUOROMETER PROCEDURE FOR COCOA (0.5 GRAM SAMPLE EQUIVALENT, 0 - 100 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-1	48	24 ± 2
Vicam V1 series 4	-1	44	22 ± 2

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare $Zn(OAc)_2/AlCl_3$ solution (see section 3.3).
2.4 Prepare methanol:water (20:80 by volume) every week or as needed.
2.5 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.6 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 100 mL 100% methanol.
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 10 mL filtered extract into a clean vessel.
4.2 Dilute extract with 40 mL of $Zn(OAc)_2/AlCl_3$ solution. Mix well.
4.3 Filter dilute extract through glass microfibre filter into glass syringe barrel using markings on barrel to measure 10 mL.

5.0 Column Chromatography

5.1 Pass 10 mL filtered diluted extract (10 mL = 0.5 g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of methanol:water (20:80) through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 3 ppb.

7.0 Recovery: Average of 40%.

4.4 AFLATEST® FLUOROMETER PROCEDURE FOR CORN, MILO, GRAINS & FEEDS (0 – 50 PPB, 1.0 GRAM SAMPLE EQUIVALENT)

1.0 Assay range:

Instrument	Assay Range
Series 1	0 - 50 ppb
Series 2	0 - 50 ppb
Series 3	0 - 50 ppb
Series 4	0 - 50 ppb
MF-2000™	0 - 24 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 1	1.0 ppb
Series 2	1.0 ppb
Series 3	1.0 ppb
Series 4	1.0 ppb
MF-2000™	1.5 ppb

3.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 1	0	20	10 ± 2	50
Sequoia-Turner series 2	0	26	13 ± 2	10
TorBex Model FX-100 series 3	-1	24	12 ± 2	
Vicam V1 series 4	-1	22	11 ± 2	
MF-2000™	Overlay AflaTest® 1.0g		10 - 12	

*Initial settings. Setting may need to be increased to accommodate values.

4.0 Set up:

4.1 Calibrate fluorometer.
4.2 Prepare AflaTest® developer solution daily.
4.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
4.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
4.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

5.1 Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar.
5.2 Add to jar 100 mL methanol: water (80:20).
5.3 Cover blender jar and blend at high speed for 1 minute.
5.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.0 Extract Dilution

6.1 Pipet or pour 10 mL filtered extract into a clean vessel.

6.2 Dilute extract with 40 mL purified water. Mix well.

6.3 Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.

7.0 Column Chromatography

7.1 Pass 10 mL filtered diluted extract (10 mL = 1.0 g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.

7.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.

7.3 Repeat step 7.2 once more until air comes through column.

7.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.

7.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.5 AFLATEST® PROCEDURE FOR CORN, GRAINS AND FEEDS (0 – 100 PPB, 0.5 GRAM SAMPLE EQUIVALENT)

1.0 Assay range:

Instrument	Assay Range
Series 1	0 - 100 ppb
Series 2	0 - 100 ppb
Series 3	0 - 100 ppb
Series 4	0 - 100 ppb
MF-2000™	0 - 48 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 1	1.0 ppb
Series 2	1.0 ppb
Series 3	1.0 ppb
Series 4	1.0 ppb
MF-2000™	1.5 ppb

3.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 1	0	40	20 ± 3	50
Sequoia-Turner series 2	0	52	26 ± 2	50
TorBex Model FX-100 series 3	-1	48	24 ± 2	
Vicam V1 series 4	-1	44	22 ± 2	
MF-2000™	Overlay AflaTest® 0.5g		21 - 24	

*Initial settings. Setting may need to be increased to accommodate values.

4.0 Set up:

4.1 Calibrate fluorometer.
4.2 Prepare AflaTest® developer solution daily.
4.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
4.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
4.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

- **5.1** Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar.
- **5.2** Add to jar 100 mL methanol:water (80:20).
- **5.3** Cover blender jar and blend at high speed for 1 minute.
- **5.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.0 Extract Dilution

- **6.1** Pipet or pour 10 mL filtered extract into a clean vessel.
- **6.2** Dilute extract with 40 mL of purified water. Mix well.
- **6.3** Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 5 mL.

7.0 Column Chromatography

- **7.1** Pass 5 mL filtered diluted extract (5 mL = 0.5g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
- **7.2** Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
- **7.3** Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
- **7.4** Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.6 AFLATEST® FLUOROMETER PROCEDURE FOR CORN, MILO, GRAINS & FEEDS (0 – 300 PPB, 0.2 GRAM SAMPLE EQUIVALENT)

1.0 Assay range:

Instrument	Assay Range
Series 1	0 - 300 ppb
Series 2	0 - 300 ppb
Series 3	0 - 300 ppb
Series 4	0 - 300 ppb
MF-2000™	0 - 120 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 1	2.0 ppb
Series 2	2.0 ppb
Series 3	2.0 ppb
Series 4	2.0 ppb
MF-2000™	4.1 ppb

3.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 1	0	100	50 ± 5	200
Sequoia-Turner series 2	0	130	65 ± 5	50
TorBex Model FX-100 series 3	-2	120	59 ± 5	
Vicam V1 series 4	-2	110	54 ± 5	
MF-2000™	Overlay AflaTest® 0.2g		53 – 65	

*Initial settings. Setting may need to be increased to accommodate values.

4.0 Set up:

4.1 Calibrate fluorometer.
4.2 Prepare AflaTest® developer solution daily.
4.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
4.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
4.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

- **5.1** Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar.
- **5.2** Add to jar 100 mL methanol: water (80:20).
- **5.3** Cover blender jar and blend at high speed for 1 minute.
- **5.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.0 Extract Dilution

- **6.1** Pipet or pour 10 mL filtered extract into a clean vessel.
- **6.2** Dilute extract with 40 mL purified water. Mix well.
- **6.3** Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 2 mL.

7.0 Column Chromatography

- **7.1** Pass 2 mL filtered diluted extract (2mL = 0.2 g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
- **7.2** Pass 5 mL of purified water through the column at a rate of about 2 drops/second.
- **7.3** Repeat step 7.2 once more until air comes through column.
- **7.4** Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
- **7.5** Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.7 AFLATEST® FLUOROMETER USDA-FGIS PROCEDURE FOR CORN, CORN MEAL, CORN/SOY BLEND, MILLED RICE, POPCORN, SORGHUM & SOYBEANS

1.0 Assay range:

Instrument	Assay Range
Series 1	0 - 320 ppb
Series 2	0 - 320 ppb
Series 3	0 - 320 ppb
Series 4	0 - 320 ppb
MF-2000™	0 - 320 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 1	< 5.0 ppb
Series 2	< 5.0 ppb
Series 3	< 5.0 ppb
Series 4	< 5.0 ppb
MF-2000™	4.7 ppb

3.0 Calibration Settings: use FGIS AflaTest® calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-3	150	74 ± 5
Vicam V1 series 4	-3	140	69 ± 5
MF-2000™	Overlay AflaTest® FGIS		66 - 74

4.0 Set up:

4.1 Calibrate fluorometer.
4.2 Prepare AflaTest® developer solution daily.
4.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
4.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
4.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

5.1 Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar.
5.2 Add to jar 100 mL methanol: water (80:20).
5.3 Cover blender jar and blend at high speed for 1 minute.
5.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.0 Extract Dilution

6.1 Pipet or pour 10 mL filtered extract into a clean vessel.
6.2 Dilute extract with 20 mL purified water. Mix well.
6.3 Filter dilute extract through glass microfibre filter into clean collecting vessel.

7.0 Column Chromatography

7.1 Pass 1 mL filtered diluted extract (1 mL = 0.167 g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.

7.2 Pass 1 mL of purified water through the column at a rate of 1 -2 drops/second. If AflaTest® column is attached to glass syringe barrel, remove AflaTest® column from glass syringe and place first wash directly into AflaTest® column headspace.

7.3 Pass another 1 mL of purified water through the column until air comes through column.

7.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.

7.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

Note: For samples reading over 150ppb on the MF-2000™, rerun sample by passing 0.5mL filtered diluted extract over the AflaTest® column at step 7.1. Run the rest of the procedure as written, but multiply the readout on the fluorometer by 2.

4.8 AFLATEST® AOAC FLUOROMETER PROCEDURE FOR CORN, RAW PEANUTS AND PEANUT BUTTER (0 - 50 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow
Sequoia-Turner series 1	0	20	10 ± 2
Sequoia-Turner series 2	0	26	13 ± 2
TorBex Model FX-100 series 3	-1	24	12 ± 2
Vicam V1 series 4	-1	22	11 ± 2

Readout will be in parts per billion total aflatoxin.

2.0 Set up:

2.1 Calibrate fluorometer weekly.
2.2 Make Aflatest developer solution daily.
2.3 Make up methanol:water (70:30 by volume) solution every week or as needed.
2.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads ppb.
2.5 Make sure 2 mL wash water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample extraction:

3.1 Weigh 25 g ground sample with 5 g NaCl and place in blender jar.
3.2 Add to jar 125 mL 70% methanol:30% water.
3.3 Cover blender jar and blend at high speed for 2 minutes.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract dilution:

4.1 Pipet or pour 15.0 mL filtered extract into a clean vessel.
4.2 Dilute extract with 30 mL distilled water. Mix well.
4.3 Filter dilute extract through glass microfiber glass filter into glass syringe barrel using marking on barrel to measure 15 mL.

5.0 Column chromatography:

5.1 Pass 15 mL of filtered extract completely through the Aflatest column at a rate of 1-2 drops/seconds (15 mL = 1.0 g sample equivalent).
5.2 Wash the column with 10 mL of distilled water at a rate of 1-2 drops/seconds.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute Aflatest column with 1.0 mL HPLC grade methanol at a rate of 1-2 drops/second and collect all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of freshly made Aflatest Developer to eluate in the cuvette. Mix well and measure fluorescence in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of detection:

Note: The AOAC Collaborative study ran samples at a low level of 10 ppb. Other studies at Vicam showed detection possible at levels of 2 ppb.

4.9 AFLATEST® FLUOROMETER PROCEDURE FOR CORN GERM MEAL AND WHEAT (USDA-FGIS METHOD) (0 - 320 PPB)

1.0 Calibration Settings: use FGIS AflaTest® calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-3	150	74 ± 5
Vicam V1 series 4	-3	140	69 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) every week or as needed.
2.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 50g sample with 10g salt and place in blender jar.
3.2 Add to jar 200 mL methanol:water (80:20).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Filtration

4.1 Pipet or pour 10 mL filtered extract into a clean vessel.
4.2 Dilute extract with 20 mL of purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a clean vessel.

5.0 Column Chromatography

5.1 Pass 2.0 mL filtered diluted extract completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 1.0 mL of purified water through the column at a rate of 1-2 drops/second. If AflaTest® column is attached to a glass syringe barrel, remove AflaTest® column from glass syringe and place first wash directly into AflaTest® column headspace.
5.3 Pass another 1 mL of purified water through the column until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of freshly prepared AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of detection: Less than 5 ppb.

4.10 AFLATEST® FLUOROMETER PROCEDURE FOR CORN GLUTEN MEAL AND CORN GLUTEN FEED (0.26 GRAM SAMPLE EQUIVALENT, USDA-FGIS METHOD, 0 - 320 PPB)

1.0 Calibration Settings: use FGIS AflaTest® calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-3	110	54 ± 5
Vicam V1 series 4	-3	100	49 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (60:40 by volume) solution every week or as needed.
2.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 250 mL methanol:water (60:40).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 10 mL filtered extract into a clean vessel.
4.2 Dilute extract with 20 mL purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a clean vessel.
4.4 Pipet or pour 6 mL filtered extract into a clean 10 mL syringe barrel with 0.22 micron nylon syringe disk filter attached (Gelman #09-730-191, Corning #09-754-22 or Fisher CAMEO II #DDR 02T2550).
4.5 Insert piston into syringe barrel, push extract through filter and collect in a clean vessel.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.26 g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of purified water through the column at a rate of 1-2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: less than 5 ppb

4.11 AFLATEST® FLUOROMETER PROCEDURE FOR COTTONSEED MEAL & WHOLE COTTONSEED (0.5 GRAM SAMPLE EQUIVALENT, 0 – 100 PPB)

1.0 Assay range:

Instrument	Assay Range
Series 2	0 - 100 ppb
Series 3	0 - 100 ppb
Series 4	0 - 100 ppb
MF-2000™	0 - 48 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 2	Interpolated to be 1.0 ppb
Series 3	Interpolated to be 1.0 ppb
Series 4	Interpolated to be 1.0 ppb
MF-2000™	Interpolated to be 1.5 ppb

3.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 2	0	52	26 ± 2	50
TorBex Model FX-100 series 3	-1	48	24 ± 2	
Vicam V1 series 4	-1	44	22 ± 2	
MF-2000™	Overlay AflaTest® 0.5g		21 - 24	

*Initial settings. Setting may need to be increased to accommodate values.

4.0 Set up:

4.1 Calibrate fluorometer.
4.2 Prepare AflaTest® developer solution daily.
4.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
4.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
4.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

5.1 Weigh 50g sample with 10g salt (NaCl) and place in blender jar.
5.2 Add to jar 200 mL methanol:water (80:20).
5.3 Cover blender jar and blend at high speed for 5 minutes.
5.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.0 Extract Dilution

6.1 Pipet or pour 10 mL filtered extract into a clean vessel.
6.2 Dilute extract with 40 mL of purified water. Mix well.
6.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 10 mL.

7.0 Column Chromatography

7.1 Pass 10 mL filtered diluted extract (10 mL = 0.5 g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
7.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
7.3 Repeat step 7.2 once more until air comes through column.
7.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
7.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

8.0 Notes

8.1 25 g of sample with 5 g of salt and 100 mL methanol:water (80:20) can also be used at step 5.1 and 5.2. The rest of the procedure should be followed as written.
8.2 Whole cottonseed sometimes will not blend well with 200 mL methanol:water (80:20) at step 5.2. In this case, blend 50 g sample with 15 g salt and 300 mL methanol:water (80:20). Pass 15 mL of diluted and filtered extract over the AflaTest®-P column at step 7.1. The extract dilution, fluorometer calibration settings, column washes and methanol elution should be followed as written.

4.12 AFLATEST® FLUOROMETER PROCEDURE FOR COTTONSEED MEAL & WHOLE COTTONSEED (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Assay range:

Instrument	Assay Range
Series 2	0 - 300 ppb
Series 3	0 - 300 ppb
Series 4	0 - 300 ppb
MF-2000™	0 - 120 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 2	Interpolated to be 2.0 ppb
Series 3	Interpolated to be 2.0 ppb
Series 4	Interpolated to be 2.0 ppb
MF-2000™	Interpolated to be 4.1 ppb

3.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 2	0	130	65 ± 5	50
TorBex Model FX-100 series 3	-2	120	59 ± 5	
Vicam V1 series 4	-2	110	54 ± 5	
MF-2000™	Overlay AflaTest® 0.2g		53 – 59	

*Initial settings. Setting may need to be increased to accommodate values.

4.0 Set up:

4.1 Calibrate fluorometer.
4.2 Prepare AflaTest® developer solution daily.
4.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
4.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
4.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

5.1 Weigh 50g sample with 10g salt (NaCl) and place in blender jar.
5.2 Add to jar 200 mL methanol:water (80:20).
5.3 Cover blender jar and blend at high speed for 5 minutes.
5.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.0 Extract Dilution

6.1 Pipet or pour 10 mL filtered extract into a clean vessel.
6.2 Dilute extract with 40 mL of purified water. Mix well.
6.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

7.0 Column Chromatography

7.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
7.2 Pass 5 mL of purified water through the column at a rate of about 2 drops/second.
7.3 Repeat step 7.2 once more until air comes through column.
7.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
7.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

8.0 Notes

8.1 25 g of sample with 5 g of salt and 100 mL methanol:water (80:20) can also be used at step 5.1 and 5.2. The rest of the procedure should be followed as written.
8.2 Whole cottonseed sometimes will not blend well with 200 mL methanol:water (80:20) at step 5.2. In this case, blend 50 g sample with 15 g salt and 300 mL methanol:water (80:20). Pass 6 mL of diluted and filtered extract over the AflaTest®-P column at step 7.1. The extract dilution, fluorometer calibration settings, column washes and methanol elution should be followed as written.

4.13 AFLATEST® FLUOROMETER PROCEDURE FOR FLUID MILK

1.0 Assay range:

Instrument	Assay Range
Series 1	0 - 2.0 ppb
Series 2	0 - 2.0 ppb
Series 3	0 - 2.0 ppb
Series 4	0 - 2.0 ppb
MF-2000™	0 - 2.2 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 1	0.10 ppb
Series 2	0.10 ppb
Series 3	0.10 ppb
Series 4	0.10 ppb
MF-2000™	0.13 ppb

3.0 Calibration Settings: use AflaTest®-M calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 2**	-0.10	2.50	1.25 ± 0.2	50
TorBex Model FX-100 series 3	-0.10	2.20	1.10 ± 0.2	
Vicam V1 series 4	-0.10	2.0	1.0 ± 0.2	
MF-2000™	Overlay AflaTest® Milk		0.94 - 1.1	

*Initial settings. Setting may need to be increased to accommodate values.
**Set red at 25 and green at -1. Divide all results on display by 10.

4.0 Set up:

4.1 Calibrate fluorometer.
4.2 Prepare AflaTest® developer solution daily.
4.3 Prepare methanol:water (10:90 by volume) solution every week or as needed.
4.4 Prepare methanol:water (80:20 by volume) solution every week or as needed.
4.5 Make sure that reagent blank (1 mL methanol:water (80:20) + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
4.6 Make sure that 2 mL methanol:water (10:90) in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

5.1 Add 1g salt to 50 mL fluid milk sample and mix well.
5.2 Centrifuge milk at 2000g for 10 minutes.
5.3 Carefully remove the skim portion (bottom layer) of the milk for analysis without disturbing the top fat layer. You can use a syringe needle to poke a hole into the bottom of a plastic centrifuge tube to remove the bottom layer.
5.4 Immediately before affinity chromatography analysis, filter the skim sample through a microfibre filter.

6.0 Column Chromatography

6.1 Pass 10 mL filtered skim milk completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
6.2 Remove AflaTest® column from the loading syringe barrel.
6.3 Fill AflaTest® column headspace with methanol:water (10:90) solution.
6.4 Place AflaTest column on a clean glass syringe barrel. Fill glass syringe barrel with 10 mL methanol:water (10:90) solution.
6.5 Pass 10 mL of methanol:water (10:90) through the column at a rate of about 2 drops/second.
6.6 Repeat step 6.5 once more until air comes through column.
6.7 Elute affinity column by passing 1.0 mL methanol:water (80:20) through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
6.8 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.14 AFLATEST® FLUOROMETER PROCEDURE FOR NUTMEG (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use FGIS AflaTest® calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
2.4 Prepare 15% Tween 20 solution.
2.5 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.6 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 100 mL 80% methanol:20% water.
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 5.0 mL filtered extract into a clean vessel.
4.2 Dilute extract with 20 mL of 15% Tween 20 solution. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb

4.15 AFLATEST® FLUOROMETER PROCEDURE FOR ONIONS (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use FGIS AflaTest® calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
2.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 100 mL methanol:water (80:20).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 10 mL filtered extract into a clean vessel.
4.2 Dilute extract with 40 mL of purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb

4.16 AFLATEST® FLUOROMETER PROCEDURE FOR OREGANO (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use FGIS AflaTest® calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
2.4 Prepare 10% Tween 20 solution.
2.5 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.6 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 100 mL methanol:water (80:20).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 5.0 mL filtered extract into a clean vessel.
4.2 Dilute extract with 20 mL of 10% Tween 20 solution. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb

4.17 AFLATEST® FLUOROMETER PROCEDURE FOR PAPRIKA, CHILI PEPPER & RED PEPPER (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use FGIS AflaTest® calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
2.4 Prepare methanol:water (20:80 by volume) solution every week or as needed.
2.5 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.6 Make sure that 2 mL methanol:water (20:80) in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 100 mL methanol:water (80:20).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 10 mL filtered extract into a clean vessel.
4.2 Dilute extract with 40 mL of purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of methanol:water (20:80) through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb

4.18 AFLATEST® FLUOROMETER PROCEDURE FOR PARSLEY (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use FGIS AflaTest® calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
2.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 200 mL methanol:water (80:20).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel. Do not allow extract to sit more than 15 minutes before proceeding to step 4.1

4.0 Extract Dilution

4.1 Pipet or pour 10 mL filtered extract into a clean vessel.
4.2 Dilute extract with 40 mL of purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings to measure 8 mL.

5.0 Column Chromatography

5.1 Pass 8 mL filtered diluted extract (8 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb.

4.19 AFLATEST® FLUOROMETER PROCEDURE FOR PEANUTS, PEANUT MEAL, PEANUT BUTTER, ALMONDS, PISTACHIOS, APRICOT NUTS AND CASHEWS (0 – 50 PPB, 1.0 GRAM SAMPLE EQUIVALENT)

1.0 Assay range:

Instrument	Assay Range
Series 1	0 - 50 ppb
Series 2	0 - 50 ppb
Series 3	0 - 50 ppb
Series 4	0 - 50 ppb
MF-2000™	0 - 24 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 1	1.0 ppb
Series 2	1.0 ppb
Series 3	1.0 ppb
Series 4	1.0 ppb
MF-2000™	1.5 ppb

3.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 1	0	20	10 ± 2	50
Sequoia-Turner series 2	0	26	13 ± 2	10
TorBex Model FX-100 series 3	-1	24	12 ± 2	
Vicam V1 series 4	-1	22	11 ± 2	
MF-2000™	Overlay AflaTest® 1.0g		10 - 12	

*Initial settings. Setting may need to be increased to accommodate values.

4.0 Set up:

4.1 Calibrate fluorometer.
4.2 Prepare AflaTest® developer solution daily.
4.3 Prepare methanol:water (60:40 by volume) solution every week or as needed.
4.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
4.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

5.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
5.2 Add to jar 125 mL methanol:water (60:40).
5.3 Cover blender jar and blend at high speed for 1 minute.
5.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.0 Extract Dilution

6.1 Pipet or pour 20 mL filtered extract into a clean vessel.
6.2 Dilute extract with 20 mL of purified water. Mix well.
6.3 Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.

7.0 Column Chromatography

7.1 Pass 10 mL filtered diluted extract (10 mL = 1.0g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
7.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
7.3 Repeat step 7.2 once more until air comes through column.
7.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
7.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.20 AFLATEST® FLUOROMETER PROCEDURE FOR PEANUTS, PEANUT MEAL AND PEANUT BUTTER (0 – 100 PPB, 0.5 GRAM SAMPLE EQUIVALENT)

1.0 Assay range:

Instrument	Assay Range
Series 1	0 - 100 ppb
Series 2	0 - 100 ppb
Series 3	0 - 100 ppb
Series 4	0 - 100 ppb
MF-2000™	0 - 48 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 1	1.0 ppb
Series 2	1.0 ppb
Series 3	1.0 ppb
Series 4	1.0 ppb
MF-2000™	1.5 ppb

3.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 1	0	40	20 ± 3	50
Sequoia-Turner series 2	0	52	26 ± 2	50
TorBex Model FX-100 series 3	-1	48	24 ± 2	
Vicam V1 series 4	-1	44	22 ± 2	
MF-2000™	Overlay AflaTest® 0.5g		21 - 24	

*Initial settings. Setting may need to be increased to accommodate values.

4.0 Set up:

4.1 Calibrate fluorometer.
4.2 Prepare AflaTest® developer solution daily.
4.3 Prepare methanol:water (60:40 by volume) solution every week or as needed.
4.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
4.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

5.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
5.2 Add to jar 125 mL methanol:water (60:40).
5.3 Cover blender jar and blend at high speed for 1 minute.
5.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.0 Extract Dilution

6.1 Pipet or pour 20 mL filtered extract into a clean vessel.
6.2 Dilute extract with 20 mL of purified water. Mix well.
6.3 Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 5 mL.

7.0 Column Chromatography

7.1 Pass 5 mL filtered diluted extract (5 mL = 0.5g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
7.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
7.3 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
7.4 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.21 AFLATEST® FLUOROMETER PROCEDURE FOR RAW SHELLED PEANUTS, LOW LIMIT OF DETECTION (2.0 GRAM SAMPLE EQUIVALENT, 0 - 25 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow
Vicam V1 series 4	-0.3	11	5 ± 1

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (70:30 by volume) solution every week or as needed.
2.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 5g salt (NaCl) and place in Eberbach blender jar (500 mL) (Vicam cat # 20300). **Use of the 500 mL jar ensures proper blending, larger volume jars may give less precise results.**
3.2 Add to jar 125 mL methanol:water (70:30).
3.3 Cover blender jar and blend at high speed for 2 minutes.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 25 mL filtered extract into a clean vessel.
4.2 Dilute extract with 50 mL of purified water. Mix well.
4.3 Filter dilute extract through **1.0 µm glass microfiber filter (Vicam cat. # G2005)** into glass syringe barrel using marking on barrel to measure 30 mL. A 10 mL glass syringe barrel can be filled three times.

5.0 Column Chromatography

5.1 Pass 30 mL of filtered extract completely through the Aflatest column at a rate of 1-2 drops/second (30 mL = 2.0g sample equivalent).
5.2 Wash the column with 10 mL of distilled water at a rate of 1-2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute Aflatest column with 1.0 mL HPLC grade methanol at a rate of 1-2 drops/second and collect all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of freshly made Aflatest Developer to the eluate in the cuvette. Mix well and measure fluorescence in a calibrated fluorometer. Read total aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: 0.5 ppb

4.22 AFLATEST® FLUOROMETER PROCEDURE FOR PEANUT HULLS (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (60:40 by volume) solution every week or as needed.
2.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 10g salt (NaCl) and place in blender jar.
3.2 Add to jar 250 mL methanol:water (60:40).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 20 mL filtered extract into a clean vessel.
4.2 Dilute extract with 20 mL of purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a clean vessel.

5.0 Column Chromatography

5.1 Attach 0.22 micron nylon syringe disk filter (Gelman #09-730-191, Corning #09-754-22 or Fisher CAMEO II #DDR 02T2550) to the end of the glass syringe barrel. Attach AflaTest®-P column to the disk filter using coupling.
5.2 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through filter and AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.3 Remove filter and reattach AflaTest®-P affinity column directly to glass syringe barrel.
5.4 Pass 5 mL of purified water through the column at a rate of about 2 drops/second.
5.5 Repeat step 5.4 once more until air comes through column.
5.6 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.7 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb

4.23 AFLATEST® FLUOROMETER PROCEDURE FOR PECANS & WALNUTS (1.0 GRAM SAMPLE EQUIVALENT, 0 - 50 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-1	24	12 ± 1
Vicam V1 series 4	-1	22	11 ± 1

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (20:80 by volume) solution every week or as needed.
2.4 Prepare methanol:water (60:40 by volume) solution every week or as needed.
2.5 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.6 Make sure that 2 mL methanol:water (20:80) in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 125 mL methanol:water (60:40).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 20 mL filtered extract into a clean vessel.
4.2 Dilute extract with 20 mL of purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.

5.0 Column Chromatography

5.1 Pass 10 mL filtered diluted extract (10 mL = 1.0g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of methanol:water (20:80) through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of detection: Interpolated to be 2 ppb.

4.24 AFLATEST® FLUOROMETER PROCEDURE FOR BLACK PEPPER AND TUMERIC (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use FGIS AflaTest® calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
2.4 Prepare 10% Tween 20 solution.
2.5 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.6 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 100 mL methanol:water (80:20).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 5.0 mL filtered extract into a clean vessel.
4.2 Dilute extract with 20 mL of 10% Tween 20 solution. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb.

4.25 AFLATEST® FLUOROMETER PROCEDURE FOR WHITE PEPPER (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use FGIS AflaTest® calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
2.4 Prepare 10% Tween 20 solution.
2.5 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.6 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g ground sample and place in blender jar (no salt).
3.2 Add to jar 100 mL methanol:water (80:20).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 5.0 mL filtered extract into a clean vessel.
4.2 Dilute extract with 20 mL of 10% Tween-20 solution. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb.

4.26 AFLATEST® FLUOROMETER PROCEDURE FOR POPPED POPCORN (1 GRAM SAMPLE EQUIVALENT, 0 - 50 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 2	0	26	13 ± 2	50
TorBex Model FX-100 series 3	-1	24	12 ± 2	
Vicam V1 series 4	-1	22	11 ± 2	

*Initial settings. Setting may need to be increased to accommodate values.

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
2.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 200 mL methanol:water (80:20).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 10 mL filtered extract into a clean vessel.
4.2 Dilute extract with 40 mL of purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 20 mL.

5.0 Column Chromatography

5.1 Pass 20 mL filtered diluted extract (20 mL = 1g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 1 ppb.

4.27 AFLATEST® FLUOROMETER PROCEDURE FOR SOY SAUCE (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (20:80 by volume) solution every week or as needed.
2.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.5 Make sure that 2 mL methanol:water (20:80) in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 20g sample with 2g salt (NaCl) and place in blender jar.
3.2 Add to jar 80 mL 100% methanol.
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 10 mL filtered extract into a clean vessel.
4.2 Dilute extract with 40 mL of purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of methanol:water (20:80) through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb.

4.28 AFLATEST® FLUOROMETER PROCEDURE FOR WHOLE STILLAGE, THIN STILLAGE & SYRUP (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.

2.2 Prepare AflaTest® developer solution daily.

2.3 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.

2.4 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 20g liquid sample with 2g salt (NaCl) and place in blender jar.

3.2 Add to jar 80 mL 100% methanol.

3.3 Cover blender jar and blend at high speed for 1 minute.

3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 10 mL filtered extract into a clean vessel.

4.2 Dilute extract with 40 mL of purified water. Mix well.

4.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.

5.2 Pass 5 mL of purified water through the column at a rate of about 2 drops/second.

5.3 Repeat step 5.2 once more until air comes through column.

5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.

5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 2 ppb.

4.29 AFLATEST® FLUOROMETER PROCEDURE FOR TOBACCO (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-2	120	59 ± 5
Vicam V1 series 4	-2	110	54 ± 5

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
2.4 Prepare methanol:water (20:80 by volume) solution every week or as needed.
2.5 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.6 Make sure that 2 mL methanol:water (20:80) in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 100 mL methanol:water (80:20).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 10 mL filtered extract into a clean vessel.
4.2 Dilute extract with 40 mL of purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

5.0 Column Chromatography

5.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of methanol:water (20:80) through the column at a rate of about 2 drops/second.
5.3 Repeat step 5.2 once more until air comes through column.
5.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 3 ppb.

4.30 AFLATEST® FLUOROMETER PROCEDURE FOR VEGETABLE OIL (0.5 GRAM SAMPLE EQUIVALENT, 0 - 100 PPB)

1.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow
TorBex Model FX-100 series 3	-1	48	24 ± 2
Vicam V1 series 4	-1	44	22 ± 2

2.0 Set up:

2.1 Calibrate fluorometer.
2.2 Prepare AflaTest® developer solution daily.
2.3 Prepare methanol:water (60:40 by volume) solution every week or as needed.
2.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

3.0 Sample Extraction:

3.1 Weigh 25g sample with 5g salt (NaCl) and place in blender jar.
3.2 Add to jar 125 mL methanol:water (60:40).
3.3 Cover blender jar and blend at high speed for 1 minute.
3.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

4.0 Extract Dilution

4.1 Pipet or pour 20 mL filtered extract into a clean vessel.
4.2 Dilute extract with 20 mL of purified water. Mix well.
4.3 Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 5 mL.

5.0 Column Chromatography

5.1 Pass 5 mL filtered diluted extract (5 mL = 0.5g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
5.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
5.3 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
5.4 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

6.0 Limit of Detection: Interpolated to be 1 ppb.

4.31 AFLATEST® FLUOROMETER PROCEDURE FOR WHEAT MIDDS, OATS, CALF MIXING PELLETS, DRIED DISTILLERS GRAIN, CANOLA SEED, CANOLA MEAL, SAFFLOWER SEED, SAFFLOWER MEAL & HIGH FIBER SAMPLES (0.5 GRAM SAMPLE EQUIVALENT, 0 – 100 PPB)

1.0 Assay range:

Instrument	Assay Range
Series 2	0 - 100 ppb
Series 3	0 - 100 ppb
Series 4	0 - 100 ppb
MF-2000™	0 - 48 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 2	Interpolated to be 1.0 ppb
Series 3	Interpolated to be 1.0 ppb
Series 4	Interpolated to be 1.0 ppb
MF-2000™	Interpolated to be 1.5 ppb

3.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 2	0	52	26 ± 2	50
TorBex Model FX-100 series 3	-1	48	24 ± 2	
Vicam V1 series 4	-1	44	22 ± 2	
MF-2000™	Overlay AflaTest® 0.5g		21 - 24	

*Initial settings. Setting may need to be increased to accommodate values.

4.0 Set up:

4.1 Calibrate fluorometer.

4.2 Prepare AflaTest® developer solution daily.

4.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.

4.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.

4.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

5.1 Weigh 50g sample with 10g salt (NaCl) and place in blender jar.
5.2 Add to jar 200 mL methanol:water (80:20).
5.3 Cover blender jar and blend at high speed for 1 minute.
5.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.0 Extract Dilution

6.1 Pipet or pour 10 mL filtered extract into a clean vessel.
6.2 Dilute extract with 40 mL of purified water. Mix well.
6.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 10 mL.

7.0 Column Chromatography

7.1 Pass 10 mL filtered diluted extract (10 mL = 0.5 g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
7.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
7.3 Repeat step 7.2 once more until air comes through column.
7.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
7.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

8.0 Notes

25 g of sample with 5 g of salt and 100 mL methanol:water (80:20) can also be used at step 5.1 and 5.2. The rest of the procedure should be followed as written.

4.32 AFLATEST® FLUOROMETER PROCEDURE FOR WHEAT MIDDS, OATS, CALF MIXING PELLETS, DRIED DISTILLERS GRAIN, CANOLA SEED, CANOLA MEAL, SAFFLOWER SEED, SAFFLOWER MEAL & HIGH FIBER SAMPLES (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 Assay range:

Instrument	Assay Range
Series 2	0 - 300 ppb
Series 3	0 - 300 ppb
Series 4	0 - 300 ppb
MF-2000™	0 - 120 ppb

2.0 Limit of detection (LOD):

Instrument	LOD
Series 2	Interpolated to be 2.0 ppb
Series 3	Interpolated to be 2.0 ppb
Series 4	Interpolated to be 2.0 ppb
MF-2000™	Interpolated to be 4.1 ppb

3.0 Calibration Settings: use Mycotoxin calibration standards

Instrument	Green	Red	Yellow	Gain*
Sequoia-Turner series 2	0	130	65 ± 5	50
TorBex Model FX-100 series 3	-2	120	59 ± 5	
Vicam V1 series 4	-2	110	54 ± 5	
MF-2000™	Overlay AflaTest® 0.2g		53 - 59	

*Initial settings. Setting may need to be increased to accommodate values.

4.0 Set up:

4.1 Calibrate fluorometer.
4.2 Prepare AflaTest® developer solution daily.
4.3 Prepare methanol:water (80:20 by volume) solution every week or as needed.
4.4 Make sure that reagent blank (1 mL methanol + 1 mL developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
4.5 Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer.

5.0 Sample Extraction:

5.1 Weigh 50g sample with 10g salt (NaCl) and place in blender jar.

5.2 Add to jar 200 mL methanol:water (80:20).

5.3 Cover blender jar and blend at high speed for 1 minute.

5.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

6.0 Extract Dilution

6.1 Pipet or pour 10 mL filtered extract into a clean vessel.

6.2 Dilute extract with 40 mL of purified water. Mix well.

6.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

7.0 Column Chromatography

7.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.

7.2 Pass 5 mL of purified water through the column at a rate of about 2 drops/second.

7.3 Repeat step 7.2 once more until air comes through column.

7.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.

7.5 Add 1.0 mL of AflaTest® Developer to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

8.0 Notes

25 g of sample with 5 g of salt and 100 mL methanol:water (80:20) can also be used at step 5.1 and 5.2. The rest of the procedure should be followed as written.

4.33 AFLATEST® FLUOROMETER PROCEDURE WORKSHEET

Consult desired AflaTest® procedure and fill in with appropriate volumes and amounts.

Sample: ______________

Sample extraction:

1. Weigh ______________ g ground sample and place in blender jar.
2. Add ________________ g NaCl (salt) to sample in jar.
3. Add to jar ____________ mL of ___________% methanol:water extraction solvent.
4. Cover blender jar and blend at high speed for __________ minute(s).
5. Remove cover from jar and pour about 50 mL extract into fluted filter paper. Collect filtrate in a clean vessel.

Extract Dilution:

6. Pipet or pour ________ mL filtered extract into a clean cup or container.
7. Dilute extract with ___________ mL distilled water. Mix well.
8. Filter dilute extract through glass microfibre filter (Vicam part # 31955). Collect filtrate in a clean cup or container.

AflaTest® Affinity Chromatography:

9. Prepare AflaTest® column for affinity chromatography.
10. Pipet ________ mL filtered extract through the AflaTest® column (= __________g sample).
11. Push the extract through the column slowly.
12. Pipet __________ mL distilled water into the column and push it through the column slowly.
13. Repeat the column wash with another equal portion of distilled water ________ times.
14. Elute the aflatoxins from the AflaTest® column with **1.0** mL HPLC grade methanol and collect eluate in a glass cuvette.
15. Add **1.0** mL dilute AflaTest® Developer (made up fresh daily) directly to eluate in the cuvette. Mix well.
16. Place cuvette in a calibrated fluorometer. Record digital readout after 60 seconds. Readout will be in parts per billion (ppb) total aflatoxins for the sample extracted.

Fluorometer Calibration: Green = ____________ Red = __________ Yellow = _______

Fluorometer series _____________ Calibration standards: ______________

5.1 MATERIALS AND EQUIPMENT REQUIRED FOR HPLC PROCEDURES

Materials Required

Description	Part #
AflaTest®-P Columns, for Fluorometer & HPLC (50/box)	12022
Disposable Pipets(50 per pack)	20652
VICAM Fluted Filter Paper, 24 cm (100)	31240
Microfibre Filters, 1.5µm, 11 cm (100)	31955
Disposable Cuvettes (250 per pack)	34000
Methanol, HPLC Grade (4 x 4 L)	35016
Disposable Plastic Beakers (25 per pack)	36010
Distilled, reverse osmosis or deionized water	
Noniodized sodium chloride (salt, NaCl)	
Zinc Acetate (for cocoa)	
Aluminum Chloride (for cocoa)	

Equipment Required

Description	Part #
Graduated cylinder, 50 mL	20050
Digital Scale with AC Adapter	20100
Commercial Blender with Stainless Steel Container	20200
Graduated cylinder, 250 mL	20250
Wash Bottle, 500 mL	20700
Cuvette Rack	21010
Single Position Pump Stand	21020
500 mL Bottle Dispenser for Methanol (0-3 mL range)	20501
Filter Funnel, 65 mm (10 per pack)	36020
HPLC System as specified in procedure	

Suggested but not required

Description

Adjustable Pipetter, 1 mL handheld

5.2 AFLATEST® HPLC PROCEDURE FOR COCOA (0 - 100 PPB)

1.0 HPLC Set up:

1.1 Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm).
1.2 Mobile phase: methanol:water (45:55) isocratic degassed.
1.3 Flow rate: 0.8 mL/min.
1.4 Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm
1.5 Post column:
Post column iodine: 0.05% iodine solution, see section 3.4, Preparation of HPLC Solutions.
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

2.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
2.2 Add to jar 100 mL 100% methanol.
2.3 Cover blender jar and blend at high speed for 1 minute.
2.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

3.1 Pipet or pour 5.0 mL filtered extract into a clean vessel.
3.2 Dilute extract with 20 mL of $Zn(OAc)_2/AlCl_3$ solution. Mix well.
3.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 10 mL.

4.0 Column Chromatography

4.1 Pass 10 mL filtered diluted extract (10 mL = 0.5 g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
4.2 Pass 10 mL of methanol:water (20:80) through the column at a rate of about 2 drops/second.
4.3 Repeat step 4.2 once more until air comes through column.
4.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
4.5 Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

5.0 Recovery: Average recovery of 42% at 20 ppb.

5.3 AFLATEST® HPLC PROCEDURE FOR CORN, GRAINS & FEEDS (1.0 GRAM SAMPLE EQUIVALENT, 0 - 50 PPB)

1.0 HPLC Set up:

1.1 Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm).

1.2 Mobile phase: methanol:water (45:55) isocratic degassed.

1.3 Flow rate: 1.0 mL/min with photochemical reactor, 0.8 mL/min with post column iodine

1.4 Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm

1.5 Post column:
Photochemical reactor: Aura Industries, Staten Island, NY or use post column iodine.
Post column iodine: 0.05% iodine solution, see section 3.4, Preparation of HPLC Solutions.
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

2.1 Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar.

2.2 Add to jar 100 mL methanol:water (80:20).

2.3 Cover blender jar and blend at high speed for 1 minute.

2.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

3.1 Pipet or pour 10 mL filtered extract into a clean vessel.

3.2 Dilute extract with 40 mL of purified water. Mix well.

3.3 Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.

4.0 Column Chromatography

4.1 Pass 10 mL filtered diluted extract (10 mL = 1g sample equivalent) completely through AflaTest ®-P affinity column at a rate of about 1-2 drops/second until air comes through column.

4.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.

4.3 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.

4.4 Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

5.0 Limit of Detection: (using post column photochemical reaction)

Commodity	Aflatoxin	Limit of Detection (ppb)
Corn	$7B_1:1B_2:3G_1:1G_2$	0.1
Corn	B_1	0.05
Corn	B_2	0.02
Corn	G_1	0.25
Corn	G_2	0.08

Note: Better limit of detection may be obtained using post column iodine

6.0 Recovery: (average over 0 - 50 ppb range)

Commodity	Aflatoxin	% Recovery
Corn	$7B_1:1B_2:3G_1:1G_2$	*77
Corn	B_1	69
Corn	B_2	79
Corn	G_1	77
Corn	G_2	81

***Average of the percentage recoveries from Aflatoxin B_1, B_2, G_1 and G_2**

5.4 AFLATEST® HPLC PROCEDURE FOR CORN, RAW PEANUTS, PEANUT BUTTER (AOAC METHOD) (1.0 GRAM SAMPLE EQUIVALENT, 0 - 50 PPB)

1.0 HPLC Set up:

1.1 Column: 4.6 mm x 25 cm, 5µm , C18 (Rainin)
1.2 Mobile phase: water:acetonitrile:methanol (3:1:1) degassed
1.3 Flow rate: 1.0 mL/min.
1.4 Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission >420 nm cut off emission filter
1.5 Post column:
Post column iodine: 0.05% iodine solution, see section 3.4, Preparation of HPLC Solutions.
Flow rate: 0.3 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

2.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
2.2 Add to jar 125 mL methanol:water (70:30).
2.3 Cover blender jar and blend at high speed for 2 minutes.
2.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

3.1 Pipet or pour 15 mL filtered extract into a clean vessel.
3.2 Dilute extract with 30 mL of purified water. Mix well.
3.3 Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography

4.1 Pass 15 mL filtered diluted extract (15 mL = 1g sample equivalent) completely through AflaTest ®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
4.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
4.3 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
4.4 Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

6.0 Limit of Detection: Note: The AOAC Collaborative study ran samples at a low level of 10 ppb. Other studies at Vicam showed detection possible at levels of 2 ppb.

7.0 Recovery: Greater than 70% on aflatoxin B_1, B_2, G_1, G_2.

5.5 AFLATEST® HPLC PROCEDURE FOR COTTONSEED MEAL & WHOLE COTTONSEED (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:

1.1 Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm).
1.2 Mobile phase: methanol:water (45:55) isocratic degassed.
1.3 Flow rate: 0.8 mL/min.
1.4 Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm
1.5 Post column:
Post column iodine: 0.05% Iodine (see Solution Preparation section).
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

2.1 Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar.
2.2 Add to jar 200 mL methanol:water (80:20).
2.3 Cover blender jar and blend at high speed for 5 minutes.
2.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

3.1 Pipet or pour 10 mL filtered extract into a clean vessel.
3.2 Dilute extract with 40 mL of purified water. Mix well.
3.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

4.0 Column Chromatography

4.1 Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest ®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
4.2 Pass 5 mL of purified water through the column at a rate of about 2 drops/second.
4.3 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
4.4 Add 1.0 mL of purified water to eluate. Inject 50-200 µL onto HPLC.

Note: For greater sensitivity, more sample volume may be passed over column in step 4.1.

5.0 Limit of Detection: extrapolated to be 1 ppb

5.6 AFLATEST PROCEDURE FOR FLUID MILK SAMPLES (0 – 0.5 PPB)

1.0 HPLC Set up:

1.1 Column: Whatman Partisphere RTF C_{18} 4.6 X 150 mm or Merck 5 mm X 12.5 cm C_{18} column 5 µm, Waters Novapak® C_{18}, 5 mm x 100 mm 4 µM
Mobile phase: 45% methanol:55% water isocratic degassed
Flow rate: 0.8 mL/minute
Fluorescence Detector: Kratos 950 Fluorescence Detector, excitation = 360 nm, emission = 440 nm
Peak retention time: ~5 minutes

1.2 Column: Rainin 4.6 mm X 25 cm Microsorb - C_{18}
Mobile phase: 50% methanol:50% water isocratic degassed
Flow rate: 0.7 mL/minute
Absorbance Detector: Beckman 160 detector 365 nm
Peak retention time: ~8 minutes

2.0 Sample Extraction:

2.1 Add 1g NaCl to 40 mL fluid milk sample and mix well.

2.2 Centrifuge milk at 2000 g for 10 minutes.

2.3 Carefully remove the skim portion (bottom layer) of the milk for analysis without disturbing the top, fat layer (a syringe needle can be used to poke a hole into the bottom of a plastic centrifuge tube).

2.4 Immediately before affinity chromatography analysis, filter the skim sample through glass microfiber filter paper.

3.0 Column Chromatography:

3.1 Remove two end caps from Aflatest affinity column.

3.2 Cut off tip of column top cap to use as a coupling. Attach column to outlet of 10 mL reservoir on pump stand.

3.3 Pass 25 mL of filtered milk sample through the Aflatest column at a steady slow flow rate of about 1-2 drops per second.

3.4 After milk sample has completely passed through column, transfer Aflatest column to a clean syringe barrel and pass 10 mL 10% methanol:90% water solution through Aflatest column twice at about 2 drops per second flow rate. Make sure all the liquid has passed through the column.

3.5 Elute Aflatest column at flow rate of about 1-2 drops per second with 1.0 mL 80% methanol:20% water and collect in a clean glass cuvette.

3.6 Concentrate to 100 µL and inject entire eluate if using absorbance detector..

Note: Greater sensitivity is possible with fluorescence detection at excitation 360 nm, emission 440 nm. 1 mL eluate at step 3.5 can be diluted with 1 mL water and injected directly into HPLC without concentrating. Sample can also be eluted with 1 mL 100% methanol at step 3.5

4.0 Limit of detection: 0.05 ppb

5.7 AFLATEST® HPLC PROCEDURE FOR NUTMEG (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:

1.1 Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm.
1.2 Mobile phase: methanol:water (45:55) isocratic degassed.
1.3 Flow rate: 0.8 mL/min.
1.4 Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm
1.5 Post column:
Post column iodine: 0.05% iodine solution, see section 3.4, Preparation of HPLC Solutions.
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

2.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
2.2 Add to jar 100 mL methanol:water (80:20).
2.3 Cover blender jar and blend at high speed for 1 minute.
2.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

3.1 Pipet or pour 5.0 mL filtered extract into a clean vessel.
3.2 Dilute extract with 20 mL of 15% Tween-20 solution. Mix well.
3.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

4.0 Column Chromatography

4.1 Pass 4 mL of filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
4.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
4.3 Repeat step 4.2 once more until air comes through column.
4.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
4.5 Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0 Limit of Detection: Extrapolated to be 1 ppb total aflatoxins.

6.0 Recovery: 77% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

5.8 AFLATEST® HPLC PROCEDURE FOR DRIED ONIONS (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:

1.1 Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm.
1.2 Mobile phase: methanol:water (45:55) isocratic degassed.
1.3 Flow rate: 0.8 mL/min.
1.4 Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm
1.5 Post column:
Post column iodine: 0.05% iodine solution, see section 3.4, Preparation of HPLC Solutions.
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

2.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
2.2 Add to jar 100 mL methanol:water (80:20).
2.3 Cover blender jar and blend at high speed for 1 minute.
2.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

3.1 Pipet or pour 10 mL filtered extract into a clean vessel.
3.2 Dilute extract with 40 mL of purified water. Mix well.
3.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

4.0 Column Chromatography

4.1 Pass 4 mL of filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
4.2 Pass 10 mL of purified water through the column at a rate of 1 -2 drops/second.
4.3 Repeat step 4.2 once more until air comes through column.
4.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
4.5 Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

6.0 Limit of Detection: Extrapolated to be 1 ppb total aflatoxins.

7.0 Recovery: 87% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

5.9 AFLATEST® HPLC PROCEDURE FOR OREGANO (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:

1.1 Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm.

1.2 Mobile phase: methanol:water (45:55) isocratic degassed.

1.3 Flow rate: 0.8 mL/min.

1.4 Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm

1.5 Post column:
Post column iodine: 0.05% iodine solution, see section 3.4, Preparation of HPLC Solutions.
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

2.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.

2.2 Add to jar 100 mL methanol:water (80:20).

2.3 Cover blender jar and blend at high speed for 1 minute.

2.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

3.1 Pipet or pour 5.0 mL filtered extract into a clean vessel.

3.2 Dilute extract with 20 mL of 10% Tween-20 solution. Mix well.

3.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

4.0 Column Chromatography

4.1 Pass 4 mL of filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.

4.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.

4.3 Repeat step 4.2 once more until air comes through column.

4.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.

4.5 Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

6.0 Limit of Detection: Extrapolated to be 1 ppb.

7.0 Recovery: 57% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

5.10 AFLATEST® HPLC PROCEDURE FOR PAPRIKA, CHILI PEPPER & RED PEPPER (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:

1.1 Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm.)
1.2 Mobile phase: methanol:water (45:55) isocratic degassed.
1.3 Flow rate: 0.8 mL/min.
1.4 Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm
1.5 Post column:
Post column iodine: 0.05% iodine solution, see section 3.4, Preparation of HPLC Solutions.
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

2.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
2.2 Add to jar 100 mL methanol:water (80:20).
2.3 Cover blender jar and blend at high speed for 1 minute.
2.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

3.1 Pipet or pour 10 mL filtered extract into a clean vessel.
3.2 Dilute extract with 40 mL of purified water. Mix well.
3.3 Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.

4.0 Column Chromatography

4.1 Pass 4 mL of filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
4.2 Pass 10 mL of methanol:water (20:80) through the column at a rate of about 2 drops/second.
4.3 Repeat step 4.2 once more until air comes through column.
4.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
4.5 Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0 Limit of Detection:
Extrapolated to be 1 ppb.

6.0 Recovery:
76% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

5.11 AFLATEST® HPLC PROCEDURE FOR PARSLEY (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:

- **1.1** Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm.)
- **1.2** Mobile phase: methanol:water (45:55) isocratic degassed.
- **1.3** Flow rate: 0.8 mL/min.
- **1.4** Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm
- **1.5** Post column:
  Post column iodine: 0.05% iodine solution, see section 3.4, Preparation of HPLC Solutions.
  Flow rate: 0.2 mL/min.
  Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
  Reaction time: ~1 minute.

2.0 Sample Extraction:

- **2.1** Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 200 mL methanol:water (80:20).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

- **3.1** Pipet or pour 10 mL filtered extract into a clean vessel.
- **3.2** Dilute extract with 40 mL of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 8 mL.

4.0 Column Chromatography

- **4.1** Pass 8 mL of filtered diluted extract (8 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through column.
- **4.4** Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
- **4.5** Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0 Limit of Detection: Extrapolated to be 1 ppb.

6.0 Recovery: 87% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

5.12 AFLATEST® HPLC PROCEDURE FOR PEANUTS, CASHEWS, APRICOT NUTS, ALMONDS, PISTACHIOS, WALNUTS & PECANS (1.0 GRAM SAMPLE EQUIVALENT, 0 - 50 PPB)

1.0 HPLC Set up:

- **1.1** Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm.)
- **1.2** Mobile phase: methanol:water (45:55) isocratic degassed.
- **1.3** Flow rate: 1.0 mL/min with photochemical reactor, 0.8 mL/min with post column iodine
- **1.4** Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm
- **1.5** Post column:
  Photochemical reactor: Aura Industries, Staten Island, NY or use post column iodine.
  Post column iodine: 0.05% iodine solution, see section 3.4, Preparation of HPLC Solutions.
  Flow rate: 0.2 mL/min.
  Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
  Reaction time: ~1 minute.

2.0 Sample Extraction:

- **2.1** Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 125 mL methanol:water (60:40).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

- **3.1** Pipet or pour 20 mL filtered extract into a clean vessel.
- **3.2** Dilute extract with 20 mL of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 10 mL.

4.0 Column Chromatography

- **4.1** Pass 10 mL of filtered diluted extract (10 mL = 1g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
- **4.3** Repeat step 4.2 once more until air comes through column.
- **4.4** Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
- **4.5** Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

5.0 Limit of Detection: Extrapolated to be 0.5 ppb total aflatoxins.

6.0 **Recovery:** Average recovery of 70% total aflatoxins (7B1:1B2:3G1:1G2 ratio) over 2 - 20 ppb range

5.13 AFLATEST® HPLC PROCEDURE FOR BLACK PEPPER & TUMERIC (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:

1.1 Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm.)
1.2 Mobile phase: methanol:water (45:55) isocratic degassed.
1.3 Flow rate: 0.8 mL/min.
1.4 Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm
1.5 Post column:
Post column iodine: 0.05% iodine solution, see section 3.4, Preparation of HPLC Solutions.
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

2.1 Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar.
2.2 Add to jar 100 mL methanol:water (80:20).
2.3 Cover blender jar and blend at high speed for 1 minute.
2.4 Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

3.1 Pipet or pour 5.0 mL filtered extract into a clean vessel.
3.2 Dilute extract with 20 mL of 10% Tween 20 solution. Mix well.
3.3 Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

4.0 Column Chromatography

4.1 Pass 4 mL of filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
4.2 Pass 10 mL of purified water through the column at a rate of about 2 drops/second.
4.3 Repeat step 4.2 once more until air comes through column.
4.4 Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
4.5 Add 1.0 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0 Limit of Detection: Extrapolated to be 1 ppb total aflatoxins.

6.0 Recovery: 97% from black pepper at 20 ppb, 83% from tumeric at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix)

5.14 AFLATEST® HPLC PROCEDURE FOR WHEAT MIDDS, OATS, CALF MIXING PELLETS, SAFFLOWER SEED, SAFFLOWER MEAL, CANOLA SEED, CANOLA MEAL, DRIED DISTILLERS GRAIN & HIGH FIBER SAMPLES (0.2 GRAM SAMPLE EQUIVALENT, 0 - 300 PPB)

1.0 HPLC Set up:

- **1.1** Column: reverse phase C18 (Waters Nova pak C18, 5mm X 100mm, 4µm cartridge, Whatman Partisphere RTF C18, 4.6 X 150mm or Merck C18 column, 5 mm X 12.5 cm, 5µm).
- **1.2** Mobile phase: methanol:water (45:55) isocratic degassed.
- **1.3** Flow rate: 0.8 mL/min.
- **1.4** Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission 440 nm
- **1.5** Post column:
  Post column iodine: 0.05% Iodine (see Solution Preparation section).
  Flow rate: 0.2 mL/min.
  Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
  Reaction time: ~1 minute.

2.0 Sample Extraction:

- **2.1** Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar.
- **2.2** Add to jar 200 mL methanol:water (80:20).
- **2.3** Cover blender jar and blend at high speed for 1 minute.
- **2.4** Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

- **3.1** Pipet or pour 10 mL filtered extract into a clean vessel.
- **3.2** Dilute extract with 40 mL of purified water. Mix well.
- **3.3** Filter dilute extract through glass microfibre filter into a glass syringe barrel using markings on barrel to measure 4 mL.

4.0 Column Chromatography

- **4.1** Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest ®-P affinity column at a rate of about 1-2 drops/second until air comes through column.
- **4.2** Pass 5 mL of purified water through the column at a rate of about 2 drops/second.
- **4.3** Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1 mL) in a glass cuvette.
- **4.5** Add 1.0 mL of purified water to eluate. Inject 50-200 µL onto HPLC.

Note: For greater sensitivity, more sample volume may be passed over column in step 4.1.

5.0 Limit of Detection: extrapolated to be 1 ppb

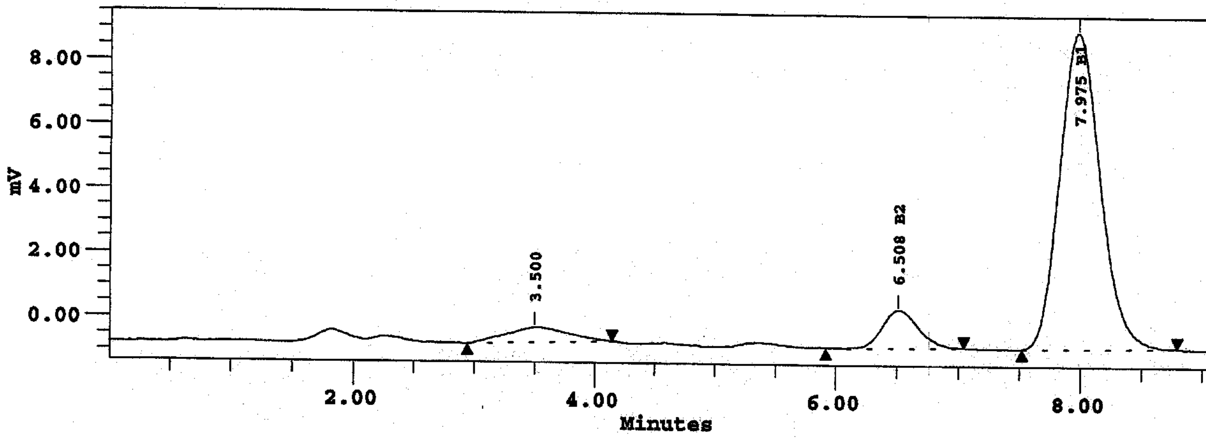
6.0 Recovery:

Commodity	**Spike level** $7B_1{:}1B_2{:}3G_1{:}1G_2$ aflatoxin mix	**% recovery**
Wheat midds	30 ppb	70
Oats	30 ppb	73
Calf mixing pellets	30 ppb	65
Safflower seed	20 ppb	71
Safflower meal	20 ppb	51
Canola seed	20 ppb	75
Canola meal	20 ppb	66

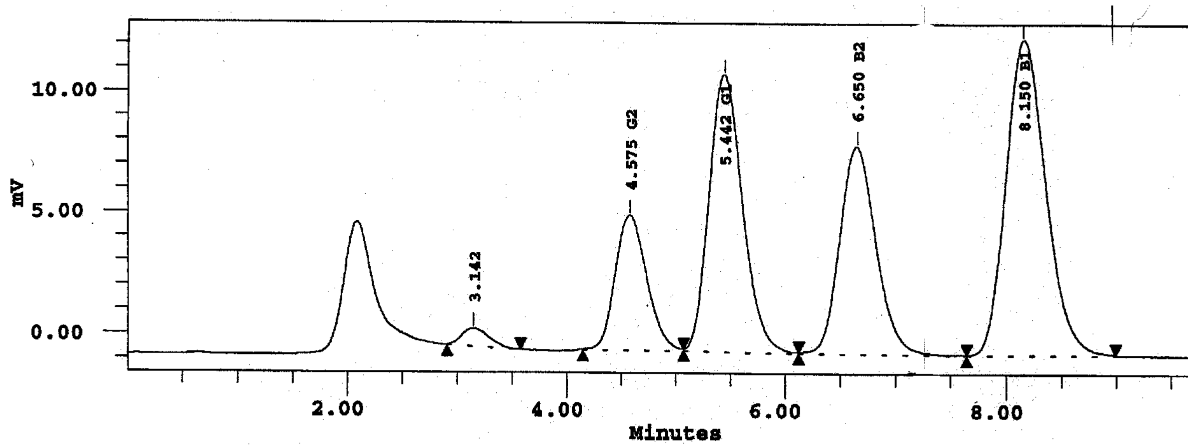
5.15 REPRESENTATIVE CHROMATOGRAMS

AflaTest® Representative Chromatogram for Corn, by Fluorescence Detection with Post-Column Iodine

3.7 ppb aflatoxin contaminated corn (3.5 B1, 0.2 B2)



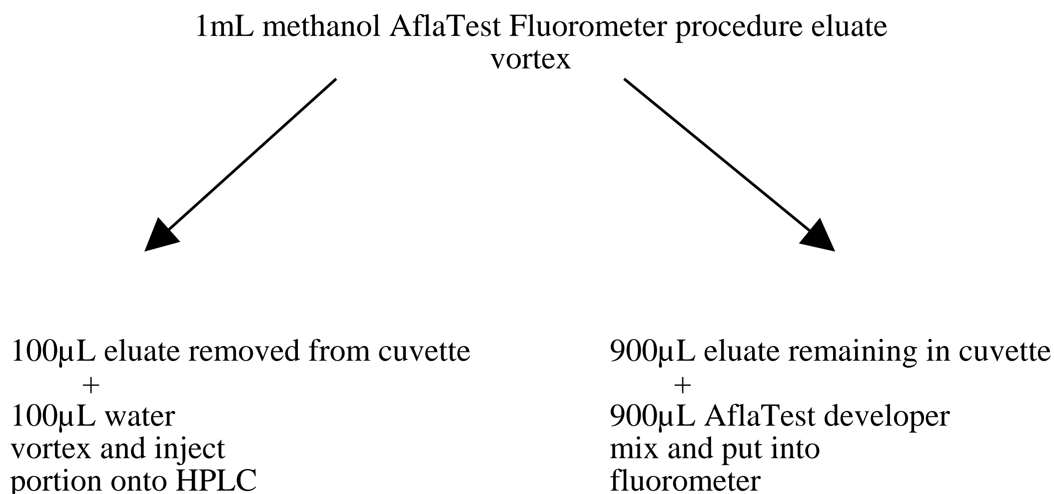
13 ppb total (5.0 B1, 1.5 B2, 5.0 G1, 1.5 G2) aflatoxin standard



6.0 AFLATEST® PROCEDURE FOR SIMULTANEOUS FLUOROMETER AND HPLC CLEANUP

The 1 mL methanol eluate from an AflaTest Fluorometer procedure can be split. A small portion of this eluate can be injected onto the HPLC. AflaTest developer can be added to the larger portion of this eluate in a one to one ratio and the sample read in the fluorometer.

A 1mL methanol AflaTest Fluorometer procedure eluate before adding AflaTest developer can be split in the following manner:



Note: It is normal for HPLC results to be lower than fluorometer results as the fluorometer is calibrated to account for loss of aflatoxin in the extract clean up and the HPLC is not.

7.1 GENERAL PRECAUTIONS FOR FLUOROMETER PROCEDURES

Depending on the specific procedure, make sure to add salt to sample before extraction. Make sure salt has no additives.

Make sure methanol dispensing tube is primed and free of air bubbles before dispensing.

Always use good, clean equipment and reagents (HPLC grade methanol for sample elution and purified, reverse osmosis or deionized water). Check reagents for background fluorescence as described in section 3.5, Reagent Check. Cuvettes not purchased from Vicam may give background fluorescence and should never be used with Vicam's tests.

Do not exceed the flow rates recommended in the procedure.

Use clean cuvettes and avoid contamination of eluate solution in cuvette. Check for contaminants inside the cuvette (lint, particle or air bubble) or dirt or fingerprints on the outside. Wipe outside of cuvette with Kim-wipe and make sure there are no particles inside cuvette before taking fluorometric readings.

Protect calibration standards from light and replace every year.

Load sample on column immediately after microfibre filtration.

Perform test from beginning to end without interruptions.

Use only equipment specified by Vicam. Avoid contact of any test reagents or solutions (such as methanol, extract, column eluate or developer) with rubber or soft flexible plastic. These materials may leach fluorescence into the sample causing false high readings.

7.2 TROUBLESHOOTING FOR FLUOROMETER PROCEDURES

1. **Problem:** False high readings

   Solution:

   Check reagents. Make sure purified water, wash solution and eluting solution read 0.

   Do not mix cuvette by putting thumb on top of cuvette and shaking.

   Make developer fresh every 8 hours.

   Make sure sample is clear after microfiber filtration.

   Avoid contact of reagents with soft flexible plastic or rubber.

   Make sure there is no dust or particles in cuvettes.

   Wash new syringe barrel for pump stands using a brush with soap and water. Then rinse with purified water and methanol before using to remove lubricant.

   Do not put AflaTest® developer solution through the AflaTest® column.

2. **Problem:** False low readings

   Solution:

   Check to make sure method is followed correctly.

   Make sure extraction solution is made correctly and is less than one week old.

   Maintain the recommended flow rates through the affinity column during sample passing, washing and elution.

3. **Problem:** Inconsistent readings

   Solution:

   Be sure to compare readings from the same columns run with the same sample filtrate at the same time. Make sure samples have been mixed very well. Different samples can give variations in readings due to variations in aflatoxin content. Even different portions of a sample can vary in aflatoxin content.

   Protect calibration standards from light and replace every year.

   Calibrate fluorometer correctly for the procedure you are using.

   Follow instructions carefully. Run a sample from start to finish without stopping.

   Run a daily sample of a known value to serve as a day-to-day precision control.

   Mix filtrate well after diluting.

   Make sure methanol dispensing tube is primed and free of air bubbles before dispensing.

   Collect all of the sample eluate in the cuvette.

   Use a 60 second time delay.

TorBex Model FX-100 Series 3 Troubleshooting

1. **Problem:** "Lamp data ready error" reading on samples but not calibration standard vials.

   Solution: Make sure final eluate in cuvette is not cloudy or strongly colored.

2. **Problem:** Words don't appear on screen after turning on instrument.

   Solution: Make sure brightness knob on right side is turned up. Check fuse.

3. **Problem:** Printer prints nonsense.

   Solution: Check dip switch settings.

   The dip switches for the Seiko Epson Model P-40 S is correctly set if the dip switches on the rear face of the printer are set as follows:

	1	2	3	4	5	6	7	8
UP	*			*	*		*	
DOWN		*	*			*		*

   The dip switches for the Seiko DPU-411-11BU are correctly set if the dip switches on the underside of the printer are set as follows:

	1	2	3	4	5	6	7	8		1	2	3	4	5	6
UP (REAR)			*	*			*	*		*					*
DOWN (FRONT)	*	*			*	*					*	*	*	*	

   The dip switches for the Seiko DPU-411 printer are correctly set if the dip switches on the underside of the printer are set as follows:

	1	2	3	4	5	6	7	8
UP (REAR)	*					*		
DOWN (FRONT)		*	*	*	*		*	*

   The dip switches for the Seiko DPU-414 printer are internally stored. These settings were set by Vicam before shipment. If your DPU-414 is printing nonsense please call Technical Services.

4. **Problem:** Fluorometer is not holding calibration

   Solution:

   The testing room should be maintained at a consistent temperature.

   Check that calibration vials are clear.

   Gently blow out sample chamber with canned air.

   Read yellow vial immediately after calibration and then periodically during the day. Vial can be re-read by pressing middle arrow key before putting vial into fluorometer. If value shifts significantly over time, every day, when room temperature is consistent, then the fluorometer needs to be returned for repair. Please call Vicam before returning any fluorometer for repair.

5. **Problem:** Error message appears after turning on instrument (i.e. “range check failure” or “lamp check failure”).

   Solution: Turn instrument off, then back on again. If problem is consistent, the instrument needs to be returned for repair. Please call Vicam before returning any fluorometer for repair.

6. **Problem:** Can the fluorometer be left on overnight?

   Solution: Fluorometer can be left on permanently but should be recalibrated daily. Fluorometer can be recalibrated by pressing left arrow key under readout.

7. **Problem:** Yellow vial consistently does not read in range indicated**.**

   Solution: Try using a different set of standards. Standards should be replaced every year.

8. **Problem:** "Range check failure" reading.

   Solution: Check fuse.

Vicam Series 4 Fluorometer Troubleshooting

1. **Problem:** Display problem

   Solution: Clear memory and set the display using the following procedure:

   At the "VICAM READY" type this number sequence: 8,3,1,1,5. The display will show "CLEAR MEMORY?", press ENTER. The display will show "CONFIRM CLEAR?", press 1. The memory is now cleared. Next, at the "VICAM READY" display press these numbers once each time: 7,5,7,6,1,2. You won't see any change in the display "VICAM READY". For instruments with serial numbers greater than 177, press the number 2. For instruments with serial numbers of 177 or less, press the number 1. This will set the display to the latest revision. After that, test the instrument for normal operation.

2. **Problem:** Results vary from 0 to 270 ppb on a calibration vial.

   Solution: Be sure to push the standard vials and cuvettes fully into the instrument so that the bottom of the vial touches the bottom of the sample well.

AflaTest® Milk Procedure Troubleshooting

1. **Problem:** Unable to push milk through column.

   Solution:

   Whole milk needs to be centrifuged, the bottom layer must be taken without disturbing the top layer of fat. Try removing bottom layer by piercing the bottom of a plastic centrifuge tube with an 18 gauge syringe needle.

   Centrifuge at 2000 g for 10 to 15 minutes. The rpm value that corresponds to 2000g will vary depending on the centrifuge rotor. For a JA18 rotor, 4500 rpm equals 2000g. Use a nomogram to identify the rpm corresponding to 2000g for your centrifuge rotor.

   Remember to add salt and filter sample through microfibre filter.

Milk is best run at room temperature.

2. Problem: False positives.

Solution:

Make sure to switch AflaTest® column to a clean syringe after passing milk over column and before 10% methanol wash. Place part of first wash directly into column headspace.

Elute with 80% methanol.

3. Problem: False negatives.

Solution: Calibrate fluorometer with AflaTest® Milk standards.

7.3 GENERAL PRECAUTIONS FOR HPLC PROCEDURES

Absorbance detection is possible at 365 nm without post column iodine. This detection is less sensitive than fluorescence detection with post column iodine. For greater sensitivity, add 100µl purified water to elute and concentrate the volume of the eluate to about 100 - 200 µL on a steam plate, under nitrogen or on an evaporator. Inject entire sample quantitatively. If drying is performed, use siliconized vials to avoid irreversible binding of aflatoxins to the tube walls.

Fluorescence detection is also possible with pre column trifluoroacetic acid or post column derivitization with electrochemically generated bromide (Kobra cell).

8.0 TECHNICAL ASSISTANCE

For assistance please contact your local distributor or Vicam Technical Services:

Phone:	800-338-4381	Canada, Mexico and the United States
	617-926-7045	all International and United States customers
Fax:	617-923-8055	
e-mail:	techservice@vicam.com	

9.0 LIABILITY

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10.0 REFERENCES

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3. Joshua, H., *Journal of Chromatography*, Determination of aflatoxins by reversed-phase high-performance liquid chromatography with post-column in-line photochemical derivitization and fluorescence detection, **654** (1993) 247-254.

4. Truckess, M. W., Stack. M. E., Nesheim, S., Page, S. W., Albert, R. H., Hansen, T. J. and Donahue, K. F., *Journal of the Association of the Official Analytical Chemistry*, Immunoaffinity column coupled with solution fluorometry or liquid chromatography post column derivatization for determination of aflatoxins in corn, peanuts and peanut butter: collaborative study, **74** (1) (1991) 81-88.

11.0 ORDERING INFORMATION

To place an order contact your local Vicam distributor or Vicam at:

In the United States:

Phone:	877-228-4244	Canada and the United States
	800-338-4381	Mexico
	617-926-7045	all International and United States customers
Fax:	617-923-8055	
e-mail:	vicam@vicam.com	

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