AflaTest® Fluorometer
Instruction Manual

VICAM, A Waters Business
34 Maple Street
Milford, MA 01757 U.S.A.
Tel: 800-338-4381, 508-482-4935
Fax: 508-482-4972
e-mail: vicam@vicam.com
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Introduction

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). 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 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 underwent evaluation by the United States Department of Agriculture, Grain Inspection, Packers and Stockyards Administration (USDA-GIPSA) for the detection of total aflatoxin (B_1, B_2, G_1 and G_2) for barley, corn, corn bran, corn flour, corn gluten feed, corn gluten meal, corn meal, corn/soy blend, corn screenings, flaking corn grits, condensed distillers solubles, dried distillers grain, dried distillers grain with solubles, milled rice, rough rice, rice bran, 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, September 2010 version". 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 Official Method status.

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. Do not freeze columns or reagents.

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.

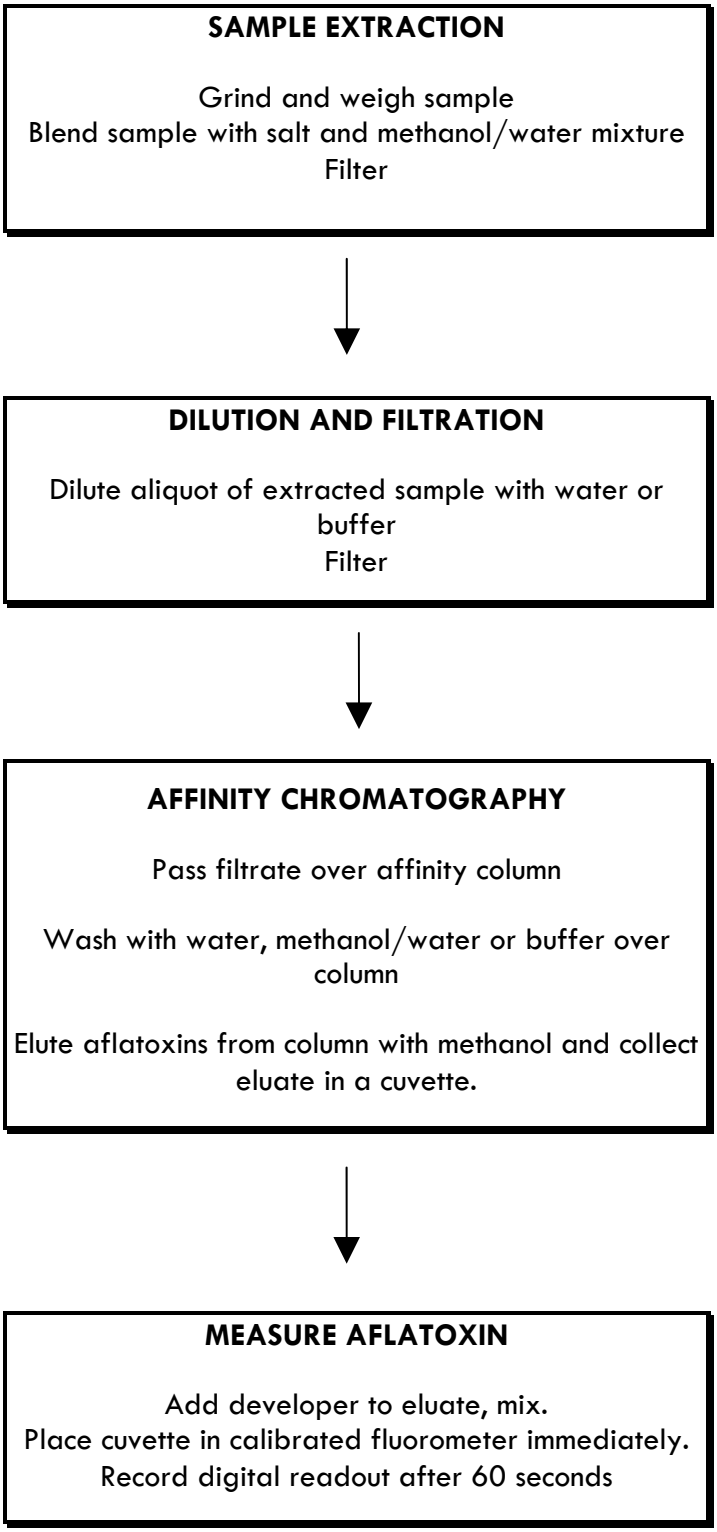
These can be viewed online at:

- www.gipsa.usda.gov Click on "Federal Grain Inspection" then "Publications" on the left.
- European community sampling procedures can be found in Commission Regulation EC No 401/2006 of 23 February 2006.

1.6 Shelf life and Storage Conditions

Store AflaTest columns 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 in the refrigerator (2 - 8°C). Do not freeze columns or reagents. It is recommended that reagents should be at room temperature (18 - 22°C) for usage.

1.7 AflaTest Fluorometer Method Overview



Equipment Preparations

2.1 Fluorometer Calibration for Vicam Series - 4 and 4EX

1. Press the **OPTIONS** key until Calibrate Test appears on the display. Then press **ENTER**. 2. Press the **SELECT TEST** key until AflaTest appears on the display. Then press **ENTER**.	The fluorometer will read: • **Vicam Ready** • **Calibrate Test** • **AflaTest**
3. Open the lid and insert the red calibration vial. Make sure that the vial is fully inserted and touches the bottom of the well.	The fluorometer will read: • **OPEN THE LID** • **INSERT RED VIAL**
4. Close the lid.	The fluorometer will read: • **HIGH CAL 22PPB**
5. If the red vial setting desired for the procedure you are using is displayed press **ENTER**. *Otherwise, enter the desired calibration setting on the specific procedure using the keypad. Confirm that the desired value appears on the display and press **ENTER**.*	The fluorometer will read: • **Reading HI Cal...** • **Saving HI Intensity**
6. Open the lid, remove the red vial and insert the green calibration vial, again making sure that the vial is fully inserted and touches the bottom of the well.	The fluorometer will read: • **OPEN THE LID** • **INSERT GREEN VIAL**
7. Close the lid.	The fluorometer will read: • **LOW CAL -1.0PPB**
8. If the green vial setting desired for the procedure you are using is displayed press **ENTER**. *Otherwise, enter the desired calibration setting on the specific procedure using the keypad. Confirm that the desired value appears on the display and press **ENTER**.*	The fluorometer will read: • **Reading Low Cal...** • **Saving Low Intensity**
9. Open the lid and remove green vial. The calibration settings for AflaTest will now print and go back to the main screen.	The fluorometer will read: • **OPEN THE LID** • **VICAM READY**
10. Press the **SELECT TEST** key, AflaTest should appear, if it does not press the **SELECT TEST** key till it appears on the display. Then press **ENTER**.	The fluorometer will read: • **AflaTest** • **Start Run Test**
11. Open the lid. Insert the yellow calibration standard. The yellow vial reading should be in the range listed in the Procedures Section.	The fluorometer will read: • **OPEN THE LID** • **Insert Sample**

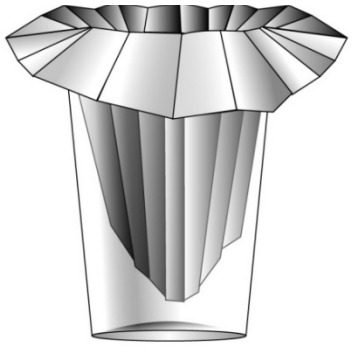
The fluorometer is now ready for samples to be inserted. The series-4 and series-4EX fluorometers need to be calibrated only once a week.

To recalibrate the fluorometer:

1. Press the **STOP** key to return to the main screen.	The fluorometer will read: • **Vicam Ready**
2. Follow the above instructions to calibrate the fluorometer.	

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

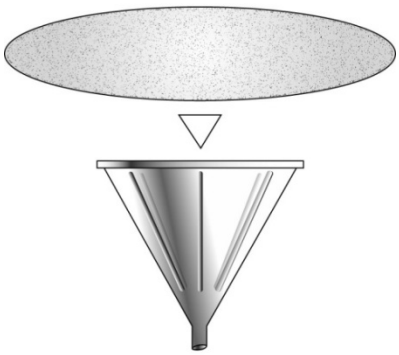
2.2 Preparation of Filtration Steps



Fluted Filter

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.



Microfiber 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.

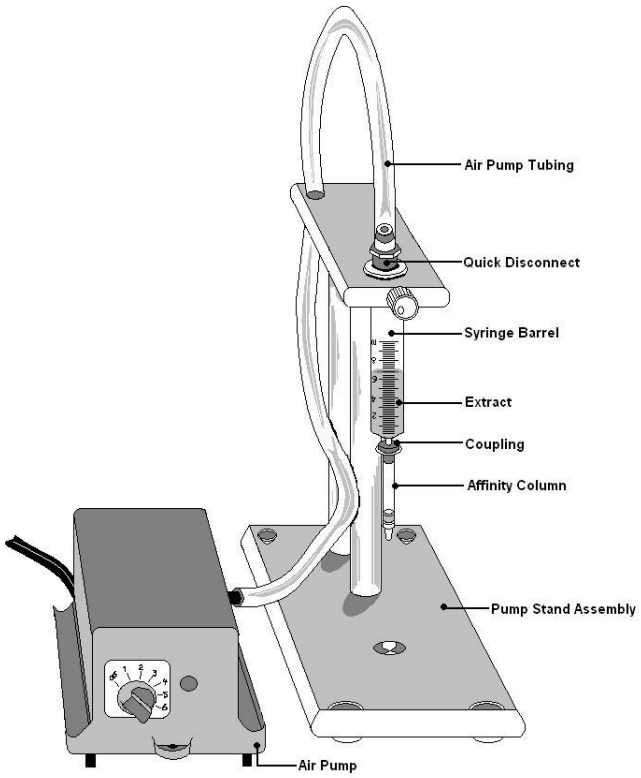
2.3 Pump Stand Setup

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. An adjustable electric aquarium air pump (VICAM part #20650) with tubing and coupling provides air pressure to push liquids through the column. Double position pump stands (part # 21040), 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.

When using a pump stand:

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. Use markings on the syringe barrel to measure 4 to 10 mL extracts.
5. Insert coupling on end of tube into syringe barrel. Remove column bottom cap.
6. Apply pressure by means of adjusting the air pump pressure dial 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). The methanol elution requires less pressure to maintain the 1 drop/second flow rate. The quick disconnect can be loosened or pulsed to provide less pressure for the methanol elution.

Affinity Column Syringe Barrel Connection



2.4 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. Wash new syringe barrel for pump stands using a brush with soap and water. Then rinse with purified water and methanol. Other pieces of equipment that need to be cleaned with detergent before using are graduated cylinders, funnels and blender jars. Bottle dispensers need only to be rinsed with methanol before use.

Between Assays:

- **Blender Jars:** After each assay, the blender jar assembly needs to be washed with a mild detergent solution and rinsed thoroughly with water. The same cleaning procedure must be performed for any equipment that will be reused to hold, collect or transfer sample extracts.*
- **Bottle Dispensers:** Do not wash bottle dispensers with soap. Methanol bottle dispensers need only to be refilled with methanol.
- **Syringe Barrels:** 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.
- **Cuvettes:** 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.

* 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.

Reagent Preparation and Testing

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. 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.

Alternatively premixes methanol and water solutions can be purchased from VICAM:

Part number	Solution	Volume
100000211	Methanol:Water 80:20	20L Cubitainer
100000212	Methanol:Water 80:20	4L Cubitainer
100000213	Methanol:Water 60:40	20L Cubitainer
100000214	Methanol:Water 60:40	4L Cubitainer
100000217	Methanol:Water 70:30	4L Cubitainer

3.2 Preparation of AflaTest Developer Solution

To Prepare Dilute AflaTest Developer Solution:

1. Measure 5.0 mL AflaTest Developer concentrate and place in the 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 AflaTest Developer solution tightly capped when not in use. Do not use dilute AflaTest Developer solution more than 8 hours after preparation.

To use AflaTest Developer:
Dispense or pipet exactly 1 mL of dilute AflaTest 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 AflaTest Developer solution by pulling up and down on the top of the dispenser.

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

1. Make the dilute AflaTest Developer solution every 8 hours. If potency of dilute AflaTest Developer is in question, it is better to make up a new dilute solution from the AflaTest Developer concentrate.
2. Avoid contamination of the bottle of concentrated AflaTest Developer, glassware and bottle dispensers with dirt, dust and other liquids. Keep the bottle tightly capped when not in use. The stock solution of concentrated AflaTest 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 AflaTest Developer with the date on which it was first opened. Do not use more than 30 days after opening.
4. Test the dilute AflaTest Developer solution for background fluorescence. Put 2.0 mL dilute AflaTest 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 3.4, Reagent Check.

3.3 Preparation of Dilution and 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.

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)
20:80	200	800	1000 (1 liter)

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)

PBS (Phosphate Buffered Saline)

1. Dissolve the following into ~990 mL purified water
    - 8.0 g NaCl (Sodium Chloride)
    - 1.2 g Na_2HPO_4 (Disodium hydrogen phosphate)
    - 0.2 g KH_2PO_4 (Potassium hydrogen phosphate)
    - 0.2 g KCl (Potassium chloride)
2. Adjust pH to 7.0 with concentrated HCl (Hydrochloric Acid)
3. Bring the volume up to 1 liter with purified water

A 10X concentrate of PBS may also be purchased from VICAM (part # G1113). 10X PBS Concentrate should be diluted to 1X with purified water as needed - i.e. dilute 100 mL of 10X concentrate with 900 mL purified water.

0.01% Tween-20 Wash Buffer

0.1 mL Tween 20
1000 mL PBS

A 10X concentrate of 0.01% Tween-20 Wash Buffer may also be purchased from VICAM (part # G1114). The 10X Concentrate should be diluted to 1X with purified water as needed - i.e. dilute 100 mL of 10X concentrate with 900 mL purified water.

0.1% Tween-20 Wash Buffer

1 mL Tween 20
1000 mL PBS

A 10X concentrate of 0.1% Tween-20 Wash Buffer may also be purchased from VICAM (part # G1112). The 10X Concentrate should be diluted to 1X with purified water as needed - i.e. dilute 100 mL of 10X concentrate with 900 mL purified water.

2% Tween-20 Wash Buffer

20 mL Tween 20
1000 mL PBS

A 5X concentrate of 2% Tween-20 Wash Buffer may also be purchased from VICAM (part # G1105). The 5X Concentrate should be diluted to 1X with purified water as needed - i.e. dilute 100 mL of 5X concentrate with 400 mL purified water.

3.4 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 zero readout must be retested with a new cuvette. If the solution does not read zero, it should be discarded and a new solution prepared and tested.

If all three solutions tested give readouts above zero, 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 zero.

Fluorometer Procedures

4.1 Materials and Equipment

Basic Materials

Description	Part #
AflaTest® Columns (50/box)*	12022
Disposable Plastic Pipets (50)*	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
or AflaTest® Developer (25 mL)	G5002
Mycotoxin Calibration Standards*	33020
or AflaTest®-FGIS Calibration Standards	33030
or AflaTest®-M Calibration Standards	33040
Disposable Cuvettes (250)*	34000
Disposable Plastic Beakers (25)*	36010
Purified water	

Additional Materials

Description	Part #
Methanol, HPLC Grade (4 x 4 L)	35016
Methanol:Water 80:20 - 20L Cubitainer	100000211
Methanol:Water 80:20 - 4L Cubitainer	100000212
Methanol:Water 60:40 - 20L Cubitainer	100000213
Methanol:Water 60:40 - 4L Cubitainer	100000214
ACS Grade Salt (100 g) (noniodized salt, NaCl)	G1124
Tween-20 (50 mL) (for alfalfa, nutmeg, oregano, black pepper & turmeric)	33501
Microfibre Filters, 1.0µm, 9 cm (100) (for raw shelled peanuts)	G2005
0.22 micron nylon membrane syringe filters (for peanut hulls & corn gluten feed)	G2007
10X Concentrate of 0.1% Tween/PBS (150 mL) (for soy sauce)	G1112
10X Concentrate of 0.01% Tween/PBS (150 mL) (for figs & raisins)	G1114
AflaTest WB Columns (25/box) (for corn gluten feed)	G1024
5X Concentrate of 2% Tween/PBS (300 mL) (for corn gluten feed)	G1105

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

Basic Equipment

Description	**Part #**
Graduated Cylinder, 50mL*	20050
Digital Scale with AC Adapter*	20100
Commercial Blender with Stainless Steel Container*	20200
Graduated Cylinder, 250mL*	20250
Eberbach Glass Blender Jar. 500 mL*	20300
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 with air pump*	G4061
or Single Position Pump Stand with hand pump	21020
Filter Funnels, 65 mm (10 per pack)*	36020
Series 4EX Fluorometer*	FLSEREX

Additional Equipment

Description	**Part #**
Centrifuge capable of obtaining 2000g (for milk only)	G8200
Micro-pipette Tips for 1 mL Micro-pipettor (100)	20656
Vortex Mixer	23040
Filter Funnel, 105 mm (4 per pack)	36022
Micro-pipettor, 1 mL	G4033
2-Position Pump Stand with air pump	21040
4-Position Pump Stand with 2 air pumps	21045
12-Position Pump Stand with 6 air pumps	G1104
AflaTest FGIS Method Semi-Automated Pump Stand	G1122

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

4.2 AflaTest Fluorometer Procedure for Corn (0 - 100 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -1 — 27 — 13 ± 2 **Range:** 0 – 100 ppb **Limit of Detection:** 1ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50 g ground sample with 5 g NaCl and place in blender jar. 2. Add to jar 100 mL 80% methanol:20% water. 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL distilled water. Mix well. 3. Filter dilute extract through glass microfiber glass filter into a glass syringe barrel using markings on barrel to measure 10 mL.
4: Column Chroma-tography	1. Pass 10 mL of filtered extract completely through the AflaTest column at a rate of about 1 drop/second (10 mL = 1.0 g sample equivalent). 2. Wash the column with 10 mL of distilled water at a rate of 1-2 drops/seconds. 3. Repeat previous step once more until air comes though column. 4. Elute AflaTest column with 1 mL HPLC grade methanol at a rate of 1 drop/second or slower and collect all of the sample eluate in a glass cuvette. 5. Add 1 mL of freshly made AflaTest Developer solution to eluate in the cuvette. Mix well and measure fluorescence in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.3 AflaTest Fluorometer Procedure for Corn (0 - 500 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument / Green / Red / Yellow VICAM series 4 and 4EX / -2 / 110 / 54 ± 5 TorBex Model FX-100 series 3 / -2 / 120 / 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** 2 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50 g ground sample with 5 g NaCl and place in blender jar. 2. Add to jar 100 mL 80% methanol:20% water. 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL distilled water. Mix well. 3. Filter dilute extract through 1.5µm glass microfiber filter into a clean vessel.
4: Column Chroma-tography	1. Pass 2 mL filtered extract (2 mL = 0.2 g sample equivalent) completely through the AflaTest column at a rate of about 1 drop/second until air come through column. The 2 mL should be accurately measured with a handheld pipettor or a calibrated pipette. 2. Pass 5 mL or purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes though column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL of HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in the glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.4 AflaTest Fluorometer Procedure for Corn, Raw Peanuts and Peanut Butter Using AOAC Method (0 - 50 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow TorBex Model FX-100 series 3 — -1 — 24 — 12 ± 2 VICAM series 4 and 4EX — -1 — 22 — 11 ± 2 **Range:** 0 – 50 ppb **Limit of Detection:** The AOAC Collaborative study ran samples at a low level of 10 ppb. Other studies at VICAM showed detection possible at levels of 1 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (70:30 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25 g ground sample with 5 g NaCl and place in blender jar. 2. Add to jar 125 mL methanol:water (70:30). 3. Cover blender jar and blend at high speed for 2 minutes. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 15 mL filtered extract into a clean vessel. 2. Dilute extract with 30 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Pass 15 mL filtered diluted extract (15 mL = 1.0 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.5 AflaTest Fluorometer Procedure for Corn Using 50g Single Filtration Method (0 - 300 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2.0 — 110 — 54 ± 4 **Range:** 0 – 300 ppb **Limit of Detection:** 2 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50 g ground sample with 10 g salt (NaCl) and place in a 500 mL bottle. 2. Add to bottle 100 mL methanol:water (80:20). 3. Cover bottle and shake by hand for 1 minute (can also be shaken on a flat bed shaker).
3: Extract Dilution	1. Remove cover from bottle and add 250 mL purified water. 2. Replace cover on bottle and shake mixture vigorously for 5 to 10 seconds. 3. Filter diluted extract through a 1.5 µm microfibre filter (VICAM # 31955) into a clean vessel.
4: Column Chroma-tography	1. Pass 2 mL of filtered extract (2 mL = 0.29 g sample equivalent) completely through the AflaTest immunoaffinity column at a rate of about 1 drop/second until air comes through the column. The 2 mL should be accurately measured with a handheld pipettor or a calibrated pipette - do not use the markings on the syringe barrel to measure the 2 mL of extract. 2. Wash the column by passing 3 mL purified water through the column at a rate of 1-2 drops/second until air comes through column. 3. Repeat the previous step once more until air comes through the column. 4. Elute the column with 1 mL HPLC grade methanol at a rate of 1 drop/second or slower and collect all of the sample eluate in a glass cuvette. 5. Add 1 mL of freshly made AflaTest Developer solution to eluate in the cuvette. Mix well and measure fluorescence in a calibrated fluorometer. Read total aflatoxin concentration after 30 seconds.

4.6 AflaTest Fluorometer Procedure for Corn Using 25g Single Extraction Filtration Method (0 - 300 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument / Green / Red / Yellow VICAM series 4 and 4EX / -2 / 100 / 49 ± 4 TorBex Model FX-100 series 3 / -2 / 110 / 54 ± 4 **Range:** 0 – 300 ppb **Limit of Detection:** 2 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25 g ground sample with 5 g salt (NaCl) and place in a 250mL bottle. 2. Add to bottle 50 mL methanol:water (80:20). 3. Cover bottle and shake on a flatbed shaker for 1 minute. Alternatively this can be hand shaken.
3: Extract Dilution	1. Remove cover and add 125 mL purified water. 2. Place cover back on bottle and shake mixture vigorously for 10 seconds. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Pass 2 mL of filtered extract (2 mL = 0.29 g sample equivalent) completely through the AflaTest immunoaffinity column at a rate of about 1 drop/second until air comes through the column. The 2 mL should be accurately measured with a handheld pipettor or a calibrated pipette. 2. Pass 5 mL of purified water through the column at a rate of 1-2 drops/second. 3. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 4. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 5. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 30 seconds.

4.7 AflaTest Fluorometer Procedure for Corn Using Ethanol Extraction (0 – 100 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -3 — 180 — 88 ± 5 **Range:** 0 – 100 ppb **Limit of Detection:** Less than 3.0 ppb
1: Setup	1. Calibrate Fluorometer weekly. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare ethanol: water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL ethanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minutes. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel
4: Column Chroma-tography	1. Prepare an AflaTest column for use by removing both end caps and gently shaking the buffer solution from the top of the column. 2. Add 1 mL of filtered extract (1 mL = 0.167 g sample equivalent) to the top of the AflaTest column. Attach the column to a VICAM pump stand. Pass the filtered extract completely through the column at a rate of about 1 drop/second until air comes through column. 3. Wash the column with 1 mL of purified water at a rate of 1-2 drops/seconds. If column is attached to glass syringe barrel, remove column from syringe and place water directly into column headspace. 4. Repeat the previous step once more until air comes through column. 5. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade ethanol into the column headspace. Elute at a rate of 1 drop/second or slower and collect all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.8 AflaTest Fluorometer Procedure for Popped Popcorn (0 - 100 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -1 — 22 — 11 ± 2 TorBex Model FX-100 series 3 — -1 — 24 — 12 ± 2 **Range:** 0 – 100 ppb **Limit of Detection:** Interpolated to be 1 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Pass 20 mL filtered diluted extract (20 mL = 1.0 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.9 AflaTest Fluorometer Procedure for Corn Germ Meal (0 – 1000 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -3 — 160 — 79 ± 5 **Range:** 0 – 1000 ppb **Limit of Detection:** Less than 3 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Prepare an AflaTest column for use by removing both end caps and gently shaking the buffer solution from the top of the column. 2. Using a 1 mL micro-pipettor add 1 mL of filtered extract to the column headspace. Attach the column to a VICAM pump stand. Pass the filtered extract completely through the column at a rate of about 1 drop/second. 3. Repeat previous step once more until air comes through the column. The total amount of extract passed through the column is 2 mL. 4. Wash the column with 1 mL of purified water at a rate of 1-2 drops/seconds. If column is attached to glass syringe barrel, remove column from syringe and place water directly into column headspace. 5. Repeat the previous step once more until air comes through column. 6. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into the column headspace. Elute at a rate of 1 drop/second or slower and collect all of the sample eluate in a glass cuvette. 7. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.10 AflaTest Fluorometer Procedure for Brown Rice (0 – 1000 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -3 — 160 — 79 ± 5 **Range:** 0 – 1000 ppb **Limit of Detection:** Less than 3 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Prepare an AflaTest column for use by removing both end caps and gently shaking the buffer solution from the top of the column. 2. Using a 1 mL micro-pipettor add 1 mL of filtered extract to the column headspace. Attach the column to a VICAM pump stand. Pass the filtered extract completely through the column at a rate of about 1 drop/second. 3. Repeat previous step once more until air comes through the column. The total amount of extract passed through the column is 2 mL. 4. Wash the column with 1 mL of purified water at a rate of 1-2 drops/seconds. If column is attached to glass syringe barrel, remove column from syringe and place water directly into column headspace. 5. Repeat the previous step once more until air comes through column. 6. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into the column headspace. Elute at a rate of 1 drop/second or slower and collect all of the sample eluate in a glass cuvette. 7. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.11 AflaTest Fluorometer Procedure for Barley, Corn, Corn Meal, Corn Flour, Corn Screenings, Flaking Corn Grits, Corn/Soy Blend, Popcorn, Soybeans, Milled Rice, and Sorghum Using USDA-GIPSA Method (0 - 1000 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 160 — 79 ± 5 **Range:** 0 – 1000 ppb **Limit of Detection:** Less than 3 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Prepare an AflaTest column for use by removing both end caps and gently shaking the buffer solution from the top of the column. 2. Using a 1 mL micro-pipettor add 1 mL of filtered extract to the column headspace. Attach the column to a VICAM pump stand. Pass the filtered extract completely through the column at a rate of about 1 drop/second until air comes through column. 3. Wash the column with 1 mL of purified water at a rate of 1-2 drops/seconds. If column is attached to glass syringe barrel, remove column from syringe and place water directly into column headspace. 4. Repeat the previous step once more until air comes through column. 5. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into the column headspace. Elute at a rate of 1 drop/second or slower and collect all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.12 AflaTest Fluorometer Procedure for Wheat, Corn Bran, Rice Bran & Rough Rice Using USDA - GIPSA Method (0 - 1000 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 160 — 79 ± 5 **Range:** 0 – 1000 ppb **Limit of Detection:** Less than 3 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Prepare an AflaTest column for use by removing both end caps and gently shaking the buffer solution from the top of the column. 2. Using a 1 mL micro-pipettor add 1 mL of filtered extract to the column headspace. Attach the column to a VICAM pump stand. Pass the filtered extract completely through the column at a rate of about 1 drop/second. 3. Repeat previous step once more until air comes through the column. The total amount of extract passed through the column is 2 mL. 4. Wash the column with 1 mL of purified water at a rate of 1-2 drops/seconds. If column is attached to glass syringe barrel, remove column from syringe and place water directly into column headspace. 5. Repeat the previous step once more until air comes through column. 6. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into the column headspace. Elute at a rate of 1 drop/second or slower and collect all of the sample eluate in a glass cuvette. 7. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.13 AflaTest Fluorometer Procedure for Condensed Distillers Solubles Using USDA - GIPSA Method (0 - 1000 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 160 — 79 ± 5 **Range:** 0 – 1000 ppb **Limit of Detection:** Less than 3 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Pass 2 mL filtered diluted extract completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 6 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. The total amount of water washed through the column is 12 mL. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution 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 Dried Distiller Grain and Dried Distillers Grain with Solubles Usina USDA - GIPSA Method (0 – 1000 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 160 — 79 ± 5 **Range:** 0 – 1000 ppb **Limit of Detection:** Less than 3 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Prepare an AflaTest column for use by removing both end caps and gently shaking the buffer solution from the top of the column. 2. Using a 1 mL micro-pipettor add 1 mL of filtered extract to the column headspace. Attach the column to a VICAM pump stand. Pass the filtered extract completely through the column at a rate of about 1 drop/second. 3. Repeat previous step once more until air comes through the column. The total amount of extract passed through the column is 2 mL. 4. Wash the column with 1 mL of purified water at a rate of 1-2 drops/seconds. If column is attached to glass syringe barrel, remove column from syringe and place water directly into column headspace. 5. Repeat previous step five more times until air comes through column. The total amount of water washed through the column is 6 mL. 6. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into AflaTest column headspace. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 7. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.15 AflaTest Fluorometer Procedure for Alfalfa (0 - 500 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 110 — 54 ± 5 TorBex Model FX-100 series 3 — -2 — 120 — 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 2 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare 10% Tween 20 solution in water (by volume) every week or as needed. 5. Prepare AflaTest Developer solution every 8 hours. 6. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 5.0 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL of 10% Tween 20 solution. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second leaving column resin bed slightly wet. (Do not pass air through column.) 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.16 AflaTest Fluorometer Procedure for Corn Gluten Feed Using USDA-GIPSA Method (0 - 1000 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 160 — 79 ± 5 **Range:** 0 – 1000 ppb **Limit of Detection:** Less than 3 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (60:40 by volume) solution. 4. Prepare 2% Tween/PBS solution every month or as needed. 5. Prepare AflaTest Developer solution every 8 hours. 6. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (60:40). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel. 4. Pipet or pour 6 mL filtered extract into a clean 10 mL syringe barrel with a 0.22 micron nylon membrane syringe filter attached (VICAM part # G2007). 5. Using an air pump or a syringe piston, push extract through filter and collect in a clean vessel.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract completely through AflaTest WB column (VICAM part # G1024 – not an AflaTest column) at a rate of about 1 drop/second until air comes through column. 2. Remove the column from the glass syringe barrel and put 3 mL of 2% Tween/PBS directly into the column headspace. Attach the column to the glass syringe and put 7 mL of 2% Tween/PBS in the syringe barrel. Pass 2% Tween/PBS through the column at a rate of 1-2 drops/second until air comes through column. 3. Remove the column from the glass syringe barrel and put 3 mL of purified water directly into the column headspace. Attach the column to a second clean syringe barrel and put 7 mL of purified water in the syringe barrel. Pass purified water through the column at a rate of 1-2 drops/second. 4. Place glass cuvette (VICAM # 34000) under column and add 1 mL HPLC grade methanol into column headspace. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.17 AflaTest Fluorometer Procedure for Corn Gluten Meal Using USDA-GIPSA Method (0 – 1000 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 160 — 79 ± 5 **Range:** 0 – 1000 ppb **Limit of Detection:** Less than 3 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (60:40 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (60:40). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Pass 3 mL filtered diluted extract completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.18 AflaTest Fluorometer Procedure for Cottonseed Meal & Whole Cotton Seed (0 – 200 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -1 — 44 — 22 ± 2 Torbex Model FX-100series 3 — -1 — 48 — 24 ± 2 **Range:** 0 – 200 ppb **Limit of Detection:** Interpolated to be 1.0 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 5 minutes. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.
4: Column Chroma-tography	1. Pass 10 mL filtered diluted extract (10 mL = 0.5 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

Notes

- 25 g of sample with 5 g of salt and 100 mL methanol:water (80:20) can also be used at step 2:1 and 2:2. The rest of the procedure should be followed as written.
- Whole cottonseed sometimes will not blend well with 200 mL methanol:water (80:20) at step 2:2. 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 column at step 4:1. The extract dilution, fluorometer calibration settings, column washes and methanol elution should be followed as written.

4.19 AflaTest Fluorometer Procedure for Cottonseed Meal & Whole Cotton Seed (0 - 500 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 110 — 54 ± 5 Torbex Model FX-100series 3 — -2 — 120 — 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 2.0 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 5 minutes. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 5 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

Notes

- 25 g of sample with 5 g of salt and 100 mL methanol:water (80:20) can also be used at step 2:1 and 2:2. The rest of the procedure should be followed as written.
- Whole cottonseed sometimes will not blend well with 200 mL methanol:water (80:20) at step 2:2. 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 column at step 4:1. The extract dilution, fluorometer calibration settings, column washes and methanol elution should be followed as written.

4.20 AflaTest Fluorometer Procedure for Milo, Grains & Grain Based Feeds (0 – 100 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -1 — 22 — 11 ± 2 TorBex Model FX-100 series 3 — -1 — 24 — 12 ± 2 **Range:** 0 – 100 ppb **Limit of Detection:** 1 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.
4: Column Chroma-tography	1. Pass 10 mL filtered diluted extract (10 mL = 1.0 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution 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 Milo, Grains & Grain Based Feeds (0 – 500 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument / Green / Red / Yellow VICAM series 4 and 4EX / -2 / 110 / 54 ± 5 TorBex Model FX-100 series 3 / -2 / 120 / 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** 2 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Pass 2 mL filtered diluted extract (2 mL = 0.2 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. The 2 mL should be accurately measured with a handheld pipettor or a calibrated pipette. 2. Pass 5 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.22 AflaTest Fluorometer Procedure for Wheat Midds, Oats, Calf Mixing Pellets, Dried Distillers Grain, Canola Seed, Canola Meal, Safflower Seed, Safflower Meal and High Fiber Samples (0 – 200 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -1 — 44 — 22 ± 2 TorBex Model FX-100 series 3 — -1 — 48 — 24 ± 2 Range: 0 – 200 ppb Limit of Detection: Interpolated to be 1 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.
4: Column Chroma-tography	1. Pass 10 mL filtered diluted extract (10 mL = 0.5 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

Notes

- 25 g of sample with 5 g of salt and 100 mL methanol:water (80:20) can also be used at step 2:1 and 2:2. The rest of the procedure should be followed as written.

4.23 AflaTest Fluorometer Procedure for Wheat Midds, Oats, Calf Mixing Pellets, Dried Distillers Grain, Canola Seed, Canola Meal, Safflower Seed, Safflower Meal and High Fiber Samples (0 – 500 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument / Green / Red / Yellow VICAM series 4 and 4EX / -2 / 110 / 54 ± 5 TorBex Model FX-100 series 3 / -2 / 120 / 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 2 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 50g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 5 mL of purified water through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

Notes

- 25 g of sample with 5 g of salt and 100 mL methanol:water (80:20) can also be used at step 2:1 and 2:2. The rest of the procedure should be followed as written.

4.24 AflaTest Fluorometer Procedure for Corn, Raw Peanuts and Peanut Butter Using AOAC Method (0 - 50 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument / Green / Red / Yellow VICAM series 4 and 4EX / -1 / 22 / 11 ± 2 TorBex Model FX-100 series 3 / -1 / 24 / 12 ± 2 **Range:** 0 – 50 ppb **Limit of Detection:** The AOAC Collaborative study ran samples at a low level of 10 ppb. Other studies at VICAM showed detection possible at levels of 1 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (70:30 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25 g ground sample with 5 g NaCl and place in blender jar. 2. Add to jar 125 mL methanol:water (70:30). 3. Cover blender jar and blend at high speed for 2 minutes. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 15 mL filtered extract into a clean vessel. 2. Dilute extract with 30 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Pass 15 mL filtered diluted extract (15 mL = 1.0 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.25 AflaTest Fluorometer Procedure for Peanuts, Peanut Meal, Peanut Butter, Almonds, Pistachios, Apricot Nuts and Cashews (0 - 100 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -1 — 22 — 11 ± 2 TorBex Model FX-100 series 3 — -1 — 24 — 12 ± 2 **Range:** 0 – 100 ppb **Limit of Detection:** 1ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (60:40 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 125 mL methanol:water (60:40). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 20 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.
4: Column Chroma-tography	1. Pass 10 mL filtered diluted extract (10 mL = 1.0 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.26 AflaTest Fluorometer Procedure for Peanuts, Peanut Meal and Peanut Butter (0 – 200 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument / Green / Red / Yellow VICAM series 4 and 4EX / -1 / 44 / 22 ± 2 TorBex Model FX-100 series 3 / -1 / 48 / 24 ± 2 **Range:** 0 – 200 ppb **Limit of Detection:** 1ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (60:40 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 125 mL methanol:water (60:40). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 20 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 5 mL.
4: Column Chroma-tography	1. Pass 5 mL filtered diluted extract (5 mL = 0.5 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of about 1-2 drops/second. 3. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 4. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 5. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.27 AflaTest Fluorometer Procedure for Raw Shelled Peanuts, Low Limit of Detection (0 - 50 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -0.3 — 11 — 5 ± 1 **Range:** 0 – 50 ppb **Limit of Detection:** 0.5ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (70:30 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in 500 mL Eberbach blender jar (VICAM # 20300). Use of the 500 mL jar ensures proper blending. Larger volume jars may give less precise results. 2. Add to jar 125 mL methanol:water (70:30). 3. Cover blender jar and blend at high speed for 2 minutes. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 25 mL filtered extract into a clean vessel. 2. Dilute extract with 50 mL purified water. Mix well. 3. Filter dilute extract through 1.0 μm glass microfibre filter (VICAM cat. # G2005) into a clean vessel.
4: Column Chroma-tography	1. Pass 30 mL filtered diluted extract (30 mL = 2.0 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.28 AflaTest Fluorometer Procedure for Brazil Nuts (0 – 100 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -0.5 — 22 — 11 ± 1 **Range:** 0 – 100 ppb **Limit of Detection:** 0.5ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (60:40 by volume) solution. Prepare methanol:water (20:80 by volume) solution. 4. Make sure that 2 mL methanol:water (20:80) in a cuvette reads 0 ppb on a calibrated fluorometer. 5. Prepare AflaTest Developer solution every 8 hours. 6. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 125 mL methanol:water (60:40). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 20 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.
4: Column Chroma-tography	1. Pass 10 mL filtered diluted extract (10 mL = 1.0 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of methanol:water (20:80) through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.29 AflaTest Fluorometer Procedure for Peanut Hulls (0 - 500 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 110 — 54 ± 5 TorBex Model FX-100 series 3 — -2 — 120 — 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 2ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (60:40 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 10g salt (NaCl) and place in blender jar. 2. Add to jar 250 mL methanol:water (60:40). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 20 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel. 4. Pipet or pour 6 mL filtered extract into a clean 10 mL syringe barrel with a 0.22 micron nylon membrane syringe filter attached (VICAM part # G2007). 5. Using an air pump or a syringe piston, push extract through filter and collect in a clean vessel.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 5 mL of purified water through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.30 AflaTest Fluorometer Procedure for Pecans and Walnuts (0 – 100 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument / Green / Red / Yellow VICAM series 4 and 4EX / -1 / 22 / 11 ± 2 TorBex Model FX-100 series 3 / -1 / 24 / 12 ± 2 **Range:** 0 – 100 ppb **Limit of Detection:** Interpolated to be 2 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (60:40 by volume) solution. Prepare methanol:water (20:80 by volume) solution. 4. Make sure that 2 mL methanol:water (20:80) in a cuvette reads 0 ppb on a calibrated fluorometer. 5. Prepare AflaTest Developer solution every 8 hours. 6. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 125 mL methanol:water (60:40). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 20 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.
4: Column Chroma-tography	1. Pass 10 mL filtered diluted extract (10 mL = 1.0 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of methanol:water (20:80) through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.31 AflaTest Fluorometer Procedure for Nutmeg (0 - 500 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 110 — 54 ± 5 TorBex Model FX-100 series 3 — -2 — 120 — 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 2 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare a 15% Tween 20 solution in water (by volume) every week or as needed. 5. Prepare AflaTest Developer solution every 8 hours. 6. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 5 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL of 15% Tween 20 solution. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second leaving column resin bed slightly wet. (Do not pass air through column.) 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.32 AflaTest Fluorometer Procedure for Dried Onions (0 - 500 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 110 — 54 ± 5 TorBex Model FX-100 series 3 — -2 — 120 — 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 2 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.33 AflaTest Fluorometer Procedure for Oregano (0 - 500 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument / Green / Red / Yellow VICAM series 4 and 4EX / -2 / 110 / 54 ± 5 TorBex Model FX-100 series 3 / -2 / 120 / 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 2 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare a 10% Tween 20 solution in water (by volume) every week or as needed. 5. Prepare AflaTest Developer solution every 8 hours. 6. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 5 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL of 10% Tween 20 solution. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second leaving column resin bed slightly wet. (Do not pass air through column.) 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.34 AflaTest Fluorometer Procedure for Parsley (0 - 500 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument / Green / Red / Yellow VICAM series 4 and 4EX / -2 / 110 / 54 ± 5 TorBex Model FX-100 series 3 / -2 / 120 / 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 2 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 200 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel. Do not allow extract to sit for more than 15 minutes before proceeding to the next step.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 8 mL.
4: Column Chroma-tography	1. Pass 8 mL filtered diluted extract (8 mL = 0.2 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.35 AflaTest Fluorometer Procedure for Paprika, Chili Pepper and Red Pepper (0 - 500 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 110 — 54 ± 5 TorBex Model FX-100 series 3 — -2 — 120 — 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 2 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. Prepare methanol:water (20:80 by volume) solution. 4. Make sure that 2 mL methanol:water (20:80) in a cuvette reads 0 ppb on a calibrated fluorometer. 5. Prepare AflaTest Developer solution every 8 hours. 6. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract (4 mL = 0.2g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of methanol:water (20:80) through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute AflaTest column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.36 AflaTest Fluorometer Procedure for Black Pepper and Turmeric (0 - 500 PPB)

Calibration Settings	Use AflaTest FGIS calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 110 — 54 ± 5 TorBex Model FX-100 series 3 — -2 — 120 — 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 2 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare 10% Tween 20 solution in water (by volume) every week or as needed. 5. Prepare AflaTest Developer solution every 8 hours. 6. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 5 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL of 10% Tween 20 solution. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second leaving column resin bed slightly wet. (Do not pass air through column.) 2. Pass 10 mL of purified water through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.37 AflaTest Fluorometer Procedure for Figs and Raisins (0 - 100 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -0.60 — 22 — 11 ± 2 **Range:** 0 – 100 ppb **Limit of Detection:** 0.5ppb for raisins; 0.75ppb for figs
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare 0.01% Tween/PBS every month or as needed (VICAM part #G1114). 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample and place in blender jar (no salt is used). 2. Add to jar 30 mL purified water. Cover blender jar and blend at high speed for 1 minute. 3. Add to jar 70 mL HPLC grade methanol. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL of 0.01% Tween Wash Buffer. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel.
4: Column Chroma-tography	1. Pass 20 mL filtered diluted extract (20 mL = 1 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of 0.01% Tween Wash Buffer through the column at a rate of about 1-2 drops/second until air comes through column. 3. Pass 10 mL of purified water through the column at a rate of about 1-2 drops/second until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.38 AflaTest Fluorometer Procedure for Fluid Milk (0 – 2.0 PPB)

Calibration Settings	Use AflaTest-M calibration standards. Instrument / Green / Red / Yellow VICAM series 4 and 4EX / -0.10 / 2.0 / 1.0 ± 0.2 TorBex Model FX-100 series 3 / -0.10 / 2.2 / 1.1 ± 0.2 **Range:** 0 – 2.0 ppb **Limit of Detection:** 0.1 ppb
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. Prepare methanol:water (10:90 by volume) solution. 4. Make sure that 2 mL of methanol:water (80:20) in a cuvette reads 0 ppb on a calibrated fluorometer. 5. Make sure that 2 mL of methanol:water (10:90) in a cuvette reads 0 ppb on a calibrated fluorometer. 6. Prepare AflaTest Developer solution every 8 hours. 7. Make sure that reagent blank (1 mL methanol:water (80:20) + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Add 1gram salt to 50 mL fluid milk sample and mix well. 2. Centrifuge milk at 2000g* for 10 minutes. 3. Note: g* = centrifugation force, NOT rpm. The rpm value that corresponds to 2000g will vary depending on the centrifuge rotor. Use a nomogram to identify the rpm corresponding to 2000g for your centrifuge rotor. Nomograms are usually supplied from the manufacturer with the rotor. 4. 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. Immediately before affinity chromatography analysis, filter the skim sample through a 1.5μm glass microfibre filter.
3: Column Chroma-tography	1. Pass 10 mL filtered skim milk completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Remove column from the loading syringe barrel. 3. Fill column headspace with methanol:water (10:90) solution. 4. Place column on a second clean glass syringe barrel. Fill glass syringe barrel with 10 mL methanol:water (10:90) solution. 5. Pass 10 mL of methanol:water (10:90) through the column at a rate of 1-2 drops/second. 6. Repeat previous step once more until air comes through column. 7. Place glass cuvette (VICAM # 34000) under column and add 1 mL methanol:water (80:20) into glass syringe barrel. 8. Elute column at a rate of 1 drop/second or slower by passing the methanol:water through the column and collecting all of the sample eluate in a glass cuvette. 9. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.39 AflaTest Fluorometer Procedure for Soy Sauce (0 – 200 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -1.0 — 44 — 22 ± 2 **Range:** 0 – 200 ppb **Limit of Detection:** Interpolated to be 1 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (80:20 by volume) solution. 4. Prepare 0.1% Tween/PBS every month or as needed (VICAM part #G1112). 5. Prepare AflaTest Developer solution every 8 hours. 6. Make sure that reagent blank (1 mL methanol + 1 mL diluted Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g sample with 2.5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at HIGH speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution and Filtration	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5 µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 mL.
4: Column Chroma-tography	1. Pass 10 mL filtered diluted extract completely through AflaTest affinity column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of 0.1% Tween/PBS through the column at a rate of about 1-2 drops/second until air comes through the column. 3. Pass 10 mL of purified water through the column at a rate of about 1-2 drops/second until air comes through the column. 4. Repeat previous step once more until air comes through column. 5. Place glass cuvette under AflaTest column and add 1 mL HPLC grade methanol into glass syringe barrel. 6. Elute AflaTest column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 7. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.40 AflaTest Fluorometer Procedure for Tobacco (0 - 500 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -2 — 110 — 54 ± 5 TorBex Model FX-100 series 3 — -2 — 120 — 59 ± 5 **Range:** 0 – 500 ppb **Limit of Detection:** Interpolated to be 3ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Prepare methanol:water (80:20 by volume) solution. Prepare methanol:water (20:80 by volume) solution. 3. Make sure that 2 mL methanol:water (20:80) in a cuvette reads 0 ppb on a calibrated fluorometer. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g ground sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 100 mL methanol:water (80:20). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 10 mL filtered extract into a clean vessel. 2. Dilute extract with 40 mL purified water. Mix well. 3. Filter dilute extract through 1.5μm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 4 mL.
4: Column Chroma-tography	1. Pass 4 mL filtered diluted extract (4 mL = 0.2 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of methanol:water (20:80) through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a 7. calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.41 AflaTest Fluorometer Procedure for Vegetable Oil (0 - 200 PPB)

Calibration Settings	Use Mycotoxin calibration standards. Instrument — Green — Red — Yellow VICAM series 4 and 4EX — -1 — 44 — 22 ± 2 TorBex Model FX-100 series 3 — -1 — 48 — 24 ± 2 **Range:** 0 – 200 ppb **Limit of Detection:** Interpolated to be 1 ppb.
1: Setup	1. Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. 2. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. 3. Prepare methanol:water (60:40 by volume) solution. 4. Prepare AflaTest Developer solution every 8 hours. 5. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.
2: Sample Extraction	1. Weigh 25g sample with 5g salt (NaCl) and place in blender jar. 2. Add to jar 125 mL methanol:water (60:40). 3. Cover blender jar and blend at high speed for 1 minute. 4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.
3: Extract Dilution	1. Pipet or pour 20 mL filtered extract into a clean vessel. 2. Dilute extract with 20 mL purified water. Mix well. 3. Filter dilute extract through 1.5µm glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 5 mL.
4: Column Chroma-tography	1. Pass 5 mL filtered diluted extract (5 mL = 0.5 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column. 2. Pass 10 mL of purified water through the column at a rate of about 1-2 drops/second. 3. Repeat previous step once more until air comes through column. 4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel. 5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette. 6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

4.42 AflaTest Fluorometer Procedure Worksheet

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

Sample: _____

Calibration standards: _____ Fluorometer series: _____

Fluorometer Calibration:

Green =_____ Red =_____ Yellow =_____

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. Pass _____ mL filtered extract through the AflaTest column (= _____g sample).
11. Push the extract through the column slowly (about 1 drop/second).
12. Pass _____ mL distilled water into the column and push it through the column slowly (1-2 drops/second).
13. Repeat the column wash with another equal portion of distilled water _____ times.
14. Elute the aflatoxins from the AflaTest column with 1 mL HPLC grade methanol (1 drop/second) and collect eluate in a glass cuvette.
15. Add 1 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.

Additional Information

5.1 Spiking Samples with aflatoxin

We use the Supelco aflatoxin standard product # 4-6304 which comes in sealed ampules. The concentration of this aflatoxin standard solution is approximately 2.6ng/µl. This standard is prepared according to AOAC Official methods. The certificate of analysis will show the exact concentration of each of the 4 different aflatoxins. We use an opened ampule for up to two weeks.

Spike a 50g sample at 26ppb as follows:

26ppb (ng/g) X 50g sample = 1300ng

1300ng ÷ 2.6ng/µL standard concentration = 500µL aflatoxin standard

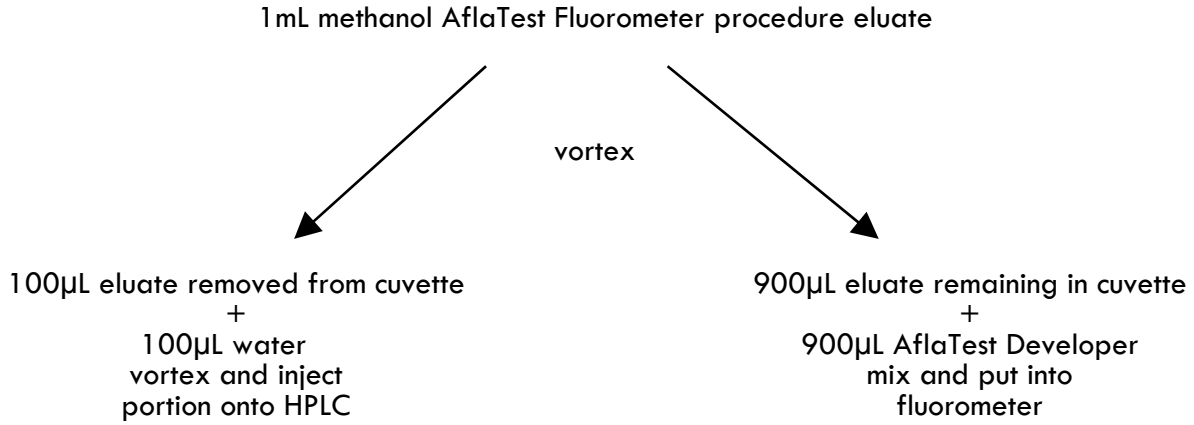
Add 500µL standard to 50g sample.

Spiking is preferentially done with a Hamilton syringe but an adjustable micro-pipettor with disposable plastic tips can also be used. Spike the samples in a fume hood and allow them to air dry for at least 30 minutes before assaying.

5.2 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.

5.3 General Precautions

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

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 the Reagent Check section. Cuvettes not purchased from VICAM may give background fluorescence and should never be used with VICAM's tests.

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.

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.

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

Do not exceed the flow rates recommended in the procedure.

Protect calibration standards from light and replace after the expiration date printed on the box.

Load sample on column immediately after microfibre filtration.

Perform test from beginning to end without interruptions.

5.4 Trouble Shooting

Problem:	False high readings
Solution:	• Check reagents. Make sure purified water, wash solution and methanol read 0. • If all three solutions tested give readouts above zero, 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. • 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 microfibre filtration. • Avoid contact of reagents with soft flexible plastic or rubber. Collect extract in a glass container or in a hard plastic beaker (VICAM # 36010). • 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. • Do not put AflaTest Developer solution through the AflaTest column.

Problem:	False low readings
Solution:	• Check to make sure method is followed correctly. • Make sure extraction solution is made correctly. • Maintain the recommended flow rates through the affinity column during sample passing, washing and elution. Do not pass sample through column or elute column faster than recommended flow rate.

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 after the expiration date printed on the box. • Calibrate fluorometer correctly for the procedure you are using. Correct calibration settings are listed in the procedure and may be different from the default settings in the fluorometer. • 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. Vicam sells reference materials for corn and peanuts. • 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. • Insert cuvette into fluorometer immediately after adding AflaTest Developer and mixing. Use a 60 second time delay.

VICAM Series 4 and 4EX Fluorometer

Problem:	Display problem
Solution:	Clear memory and reset the display using the following procedure for the **Series 4:** 1. Press “STOP” key. 2. At “VICAM VX.XX READY” type this number sequence: 8,3,1,1,5. 3. The display will show “CLEAR MEMORY?”, press ENTER. 4. The display will show “CONFIRM CLEAR?”, press 1. The memory is now cleared. 5. At the “VICAM VX.XX READY” display press these numbers once each time: 7,5,7,6,1,2. You won’t see any change in the display “VICAM VX.XX READY”. 6. For series 4 instruments with serial numbers greater than 177 and for series 4EX instruments, press the number 2. 7. For series 4 instruments with serial numbers of 177 or less, press the number 1. This will set the display to the latest revision. 8. The instrument is now ready for normal operation. **Series 4EX:** 1. Press “STOP” key. 2. Press number 8,3,1,1,5. 3. The display will show “CLEAR MEMORY?”, press ENTER. 4. The display will show “CONFIRM CLEAR?”, press 1. 5. The system will clear memory then display "POWER DOWN" and not respond to any action. 6. Turn fluorometer off then on. 7. The system will initialize then display "COLD START" instead of ready prompt. 8. Press ENTER. The display will read "Clear Summary Data"(line 1) "Press 1 for Yes" (line 2). 9. Press 1. The system will clear summary data, initialize pointers then display "Vicam 4EX Ready" 10. The system is now ready for use. 11. The COLD START will not appear again unless memory is cleared.

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

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.

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.

Problem:	False negatives.
Solution:	• Calibrate fluorometer with AflaTest Milk standards.

6.0 REFERENCES

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3. Hussain, I, Anwar, J, *Food Control*, A study on contamination of aflatoxin M1 in raw milk in the Punjab province of Pakistan, 19 (2008) 393-395.

4. Li, R et al, *Food Control*, Occurrence of four mycotoxins in cereal and oil products in the Yangtze Delta region of China and their food safety risks, 35 (2014) 117-122.

7.0 TECHNICAL ASSISTANCE

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

Phone:	+1-800-338-4381 +1-508-482-4935
Fax:	+1-508-482-4972
e-mail:	techservice@vicam.com

8.0 INDEX

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9.0 LIABILITY

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In the United States

Phone:	+1-877-228-4244 +1-417-725-6588
Fax:	+1-417-725-6102
e-mail:	vicam@vicam.com

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