




Afla B

Instruction Manual

VICAM®

A Waters Business

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1.1 INTENDED USER

Afla B™ is a quantitative method for the detection of aflatoxin in many commodities. VICAM's advanced biotechnology permits the measurement of aflatoxin B_1 and B_2 without the use of toxic solvents like chloroform or methylene chloride. Afla B aflatoxin testing can be 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. Many countries regulate only aflatoxin B_1. Using Afla B, aflatoxins B_1 and B_2 can be isolated, and B_1 measured by HPLC. In fluorometer measurements, total B_1 and B_2 is reported, and it is known that B_1 accounts for 94% of total B_1 and B_2 measured in corn[1]. B_1 accounts for 82.5% of total B_1 and B_2 measured in peanuts[1].

Samples are prepared by mixing with an extraction solution, blending and filtering . The extract is then applied to the Afla B™ column bound with specific antibodies to aflatoxin. At this stage, the aflatoxin binds to the antibody on the column. The column is then washed with water to rid the immunoaffinity column of impurities. By passing methanol through the column, the aflatoxin is removed from the antibody. This methanol solution can then be injected into an HPLC system or measured in a fluorometer. These steps are outlined in section 1.6, Afla B™ Overview.

1.3 APPLICABILITY AND APPROVALS

Afla B has been optimized for quantitative measurement of aflatoxins in corn, grains and feeds. Assistance in measuring aflatoxin in other commodities can be obtained by contacting our Technical Assistance Department.

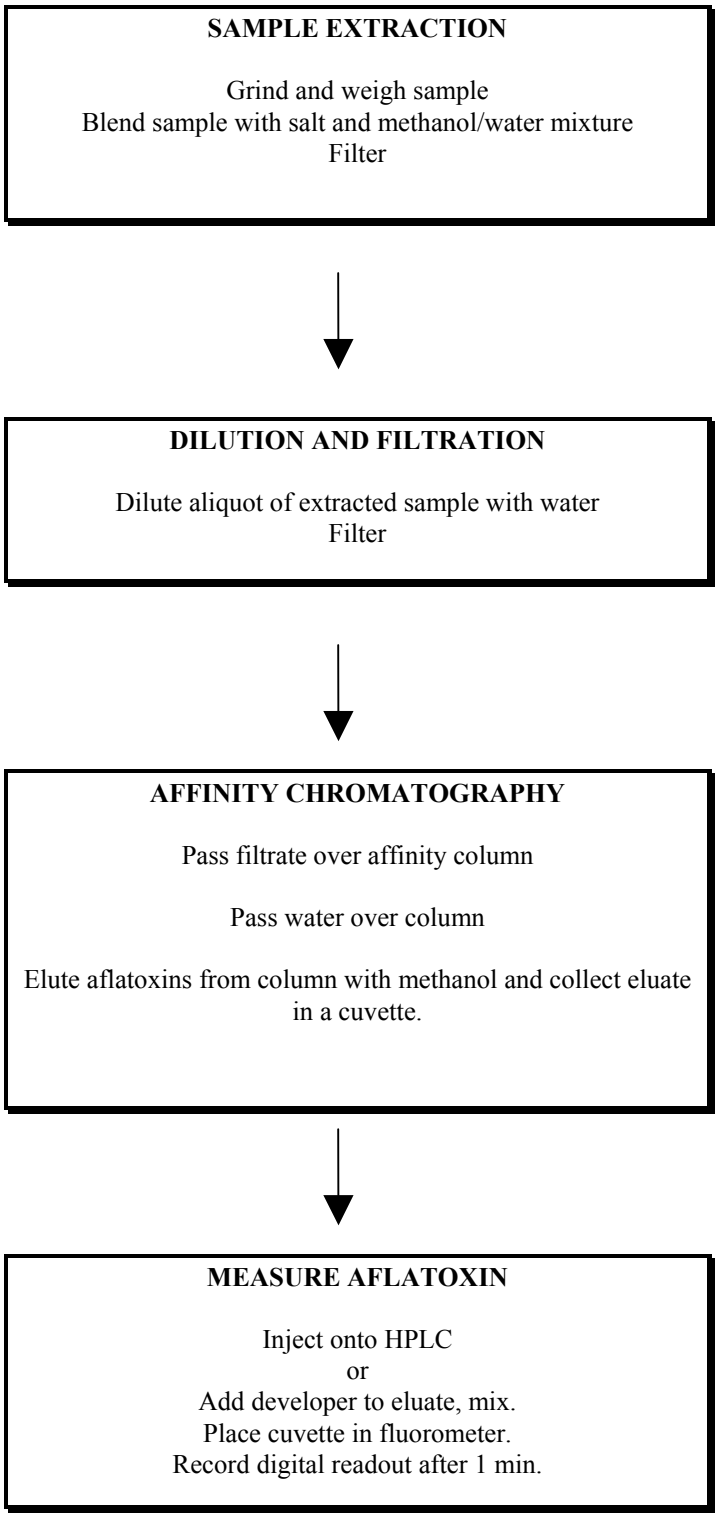
1.4 LIMITATIONS

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

1.5 SHELF LIFE AND STORAGE CONDITIONS

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

1.6 AFLA B™ OVERVIEW



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

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

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

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

3. Use only Mycotoxin Calibration standards.

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

5. Turn the span knob all the way clockwise.

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

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

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

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

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

10. Remove the RED standard from the chamber.

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

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

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

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

2.2 FLUOROMETER CALIBRATION - TORBEX MODEL FX-100 SERIES 3

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

A. Starting the Fluorometer

1. Turn power ON using the on/off switch on the rear panel.

2. The model FX-100 will identify itself on the LCD then proceed through a series of self tests. If any error messages appear consult the operator's manual.

3. The fluorometer will then proceed through a printer check. If any error messages appear consult the operator's manual.

4. Set Date and Time

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

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

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

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

   One is selected and changed using the numerical keypad.

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

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

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

5. Set Test Delay Time

The following message will be displayed:

TEST DELAY TIME
60 SEC PRESS ENTER

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

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

6. Set Answer Format

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

SELECT ANSWER FORMAT
INTEGERS DECIMALS

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

7. Select measurement units

The following message will be displayed:

SELECT MEASUREMENT UNITS
PPM PPB

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

B. Calibration Procedure

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

START CALIBRATION
CHECKING GAINS. WAIT

Then the message changes to read:

INSERT RED VIAL

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

CALIBRATION VALUE
24.0 PPB PRESS ENTER

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

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

MEASURING CALIBRATOR

When calibration is completed the following message will appear:

REMOVE RED VIAL

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

INSERT GREEN VIAL
CONTINUE CALIBRATION

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

BLANK VALUE
-1.0 PPB PRESS ENTER

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

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

MEASURING BLANK

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

MEASURING BLANK
REMOVE GREEN VIAL

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

PUSH ENTER TO CONTINUE
RECAL <SOFT KEYS> RETEST

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

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

READY TO START TESTING
INSERT SAMPLE TO MEASURE

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

C. Sample Measurement

1. To measure aflatoxin concentration in sample:

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

   TEST DELAY IN PROGRESS
   _ _ _ SECS TILL MEASUREMENT

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

   PERFORMING MEASUREMENT

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

   TEST RESULTS _ _ _ PPB
   REMOVE SAMPLE VIAL

2. Re-Measurement Options

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

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

PREV RESULTS _ _ _ PPB
INSERT NEXT SAMPLE

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

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

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

b. Repeat Measurement

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

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

d. Re-calibrate

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

2.3 FLUOROMETER CALIBRATION FOR VICAM V1 SERIES 4

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

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

3. The fluorometer will read:

START CALIBRATION... OPEN THE LID
INSERT RED VIAL

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

4. The display will read:

HIGH CAL 22 PPB

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

5. The display will read:

READING HIGH CAL... SAVING HIGH INTENSITY

and then read:

OPEN THE LID
INSERT GREEN VIAL

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

6. The display will read:

LOW CAL - 1.0 PPB

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

7. The display will read:

READING LOW CAL... SAVING LOW INTENSITY

and then read:

OPEN THE LID

Open the lid and remove the green vial.

8. The display will read:

VICAM V1.1 READY

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

Press S**ELECT TEST**. The display will read:

AFLATEST

Press E**NTER**.

9. The display will read:

START RUN TEST OPEN THE LID

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

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

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

CALIBRATE TEST

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

AFLATEST

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

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

AFLATEST (or the desired test)

Then press **ENTER**.

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

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

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

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

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

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

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

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

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

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

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

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

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

10. **READY** light will illuminate.

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

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

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

14. The red **OVER HIGH CAL** light will turn on when the toxin concentration is higher than the red vial concentration (exceeds the top value in the assay range).

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

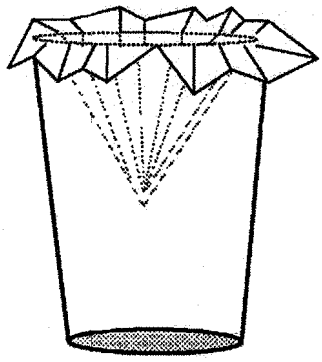
2.5 COMPARISON OF FLUOROMETER CALIBRATION SETTINGS

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

	Sequoia-Turner Model 450		Torbex FX100	VICAM V1	MF-2000™
	series 1	series 2	series 3	Series 4	Overlay
Gram equivalent of method	Red/green (yellow)	Red/green (yellow)	Red/green (yellow)	Red/green (yellow)	
1.0	20 / 0 (10 ± 2)	26 / 0 (13 ± 2)	24 / -1 (12 ± 2)	22 / -1 (11 ± 2)	AflaTest® 1.0g

2.6 PREPARATION OF FILTRATION STEPS

Fluted Filter



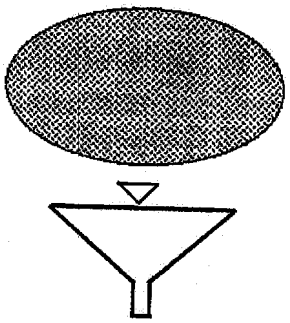
Fluted Filter Assembly

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

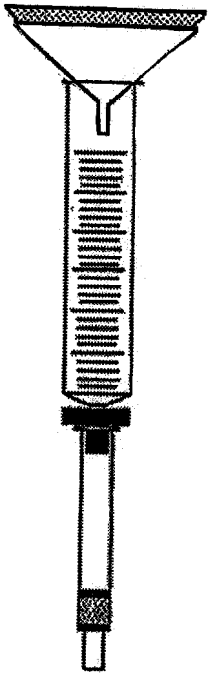
1. Open one fluted filter carefully and insert into clean container. Optional: a funnel (part # 36022) may be used to hold the filter.

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

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



Microfibre Filter



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

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

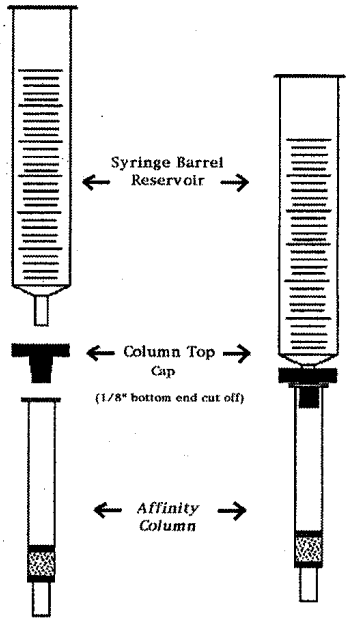
2. Place one microfibre filter gently into small funnel by pressing filter into funnel with index finger. Be careful not to rip or puncture the filter.

2.7 PUMP STAND SETUP

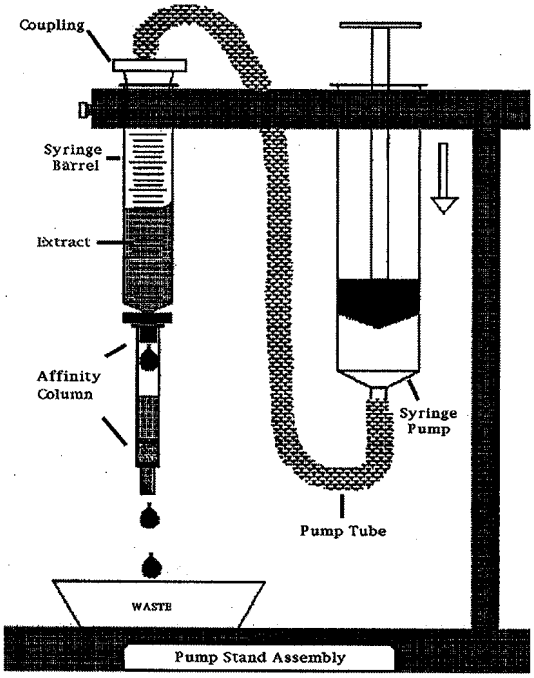
Afla B affinity chromatography is easily performed with the Afla B affinity column attached to a pump stand. The stand has a 10 mL glass syringe barrel that serves as a reservoir for the column. A large plastic syringe with tubing and coupling provides air pressure to manually push liquids through the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

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

Note: Avoid pulling up on plastic syringe piston while coupling is attached to glass



Affinity Column Syringe Barrel Connection



2.8 CLEANING EQUIPMENT

Before Starting Afla B™ Testing

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

Before using the equipment, it should be washed with a mild detergent solution and then rinsed thoroughly with purified water. This includes the glass syringe barrels used for sample reservoirs. The syringe barrels are treated with a lubricant for use with a piston plunger. The lubricant needs to be washed off before using the syringe barrel for Afla B. 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:

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

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

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

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

Other Important Precautions

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

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

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

3.1 PREPARATION OF EXTRACTION SOLUTIONS

The Afla B procedure uses 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)

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

3.2 PREPARATION OF AFLATEST® DEVELOPER SOLUTION

To Prepare Dilute AflaTest Developer solution:

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

2. Add 45.0 mL purified water and mix well.

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

To use Developer:

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

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

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

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

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

4. Test the dilute Developer solution for background fluorescence. Put 2.0 mL dilute Developer into a cuvette. Place the cuvette in a calibrated fluorometer. The fluorometer digital display should be 0. If readout does not equal 0, see Section 3.4, Reagent Check.

3.3 PREPARATION OF HPLC SOLUTIONS

1. HPLC Mobile Phase

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

Solution should be filtered and degassed before use.

2. Iodine solution (0.05%)

0.5 g Iodine
100 mL Methanol
900 mL purified water

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

3.4 REAGENT CHECK

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

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

To Check Reagents and Cuvettes:

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

***** IMPORTANT *****

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

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

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

4.1 MATERIALS AND EQUIPMENT REQUIRED FOR FLUOROMETER PROCEDURES

Consumables Required

Description	Part #
*Disposable Pipets(50 per pack)	20652
*VICAM Fluted Filter Paper, 24 cm (100)	31240
*Microfibre Filters, 1.5µm, 11 cm (100)	31955
*Kim Wipes Tissues (1 box)	31967
*AflaTest Developer (50 mL)	32010
*Mycotoxin calibration Standards	33020
*Disposable Cuvettes (250 per pack)	34000
Methanol, HPLC Grade (4 x 4 L)	35016
*Disposable Plastic Beakers (25 per pack)	36010
Afla B™ Columns, Fluorometer & HPLC	G1004
Distilled, reverse osmosis or deionized water	
Noniodized sodium chloride (salt, NaCl)	

Equipment Required

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

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

Suggested but not required

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

4.2 AFLA B™ FLUOROMETER PROCEDURE FOR CORN, GRAINS & FEEDS (1.0 GRAM SAMPLE EQUIVALENT, 0 - 50 PPB)

1.0 Calibration **Settings:** use Mycotoxin calibration standards

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

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

2.0 Set up:

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

3.0 Sample Extraction:

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

4.0 Extract Dilution

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

5.0 Column Chromatography

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

6.0 Limit of Detection: 1 ppb

5.1 MATERIALS AND EQUIPMENT REQUIRED FOR HPLC PROCEDURES

Consumables Required

Description	Part #
Disposable Pipets(50 per pack)	20652
VICAM Fluted Filter Paper, 24 cm (100)	31240
Microfibre Filters, 1.5µm, 11 cm (100)	31955
Disposable Cuvettes (250 per pack)	34000
Methanol, HPLC Grade (4 x 4 L)	35016
Disposable Plastic Beakers (25 per pack)	36010
Afla B™ Columns, Fluorometer & HPLC	G1004
Distilled, reverse osmosis or deionized water	
Noniodized sodium chloride (salt, NaCl)	

Equipment Required

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

Suggested but not required

Description
Adjustable Pipettor, 1 mL handheld

5.2 AFLA B™ HPLC PROCEDURE FOR CORN (1.0 GRAM SAMPLE EQUIVALENT, 0 - 50 PPB)

1.0 HPLC Set up:

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

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

1.3 Flow rate: 1.0 mL/min.

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

1.5 Post column:
Photochemical reactor[2]: Aura Industries, Staten Island, N.Y. or use post column iodine[3].
Post column iodine: 0.05% Iodine solution, see Section 3.3, Preparation of HPLC Solutions.
Flow rate: 0.2 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

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

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

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

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

3.0 Extract Dilution

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

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

3.3 Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography

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

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

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

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

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

Commodity	Aflatoxin	Limit of Detection (ppb)
Corn	$7B_1:1B_2:3G_1:1G_2$ 0.1	
Corn B	$_1$ 0.05	
Corn B	$_2$ 0.02	
Corn G	$_1$ *ND	
Corn G	$_2$ ND	

***Not determined**

Note: Limit of detection is better with post column iodine vs. post column photochemical reactor.

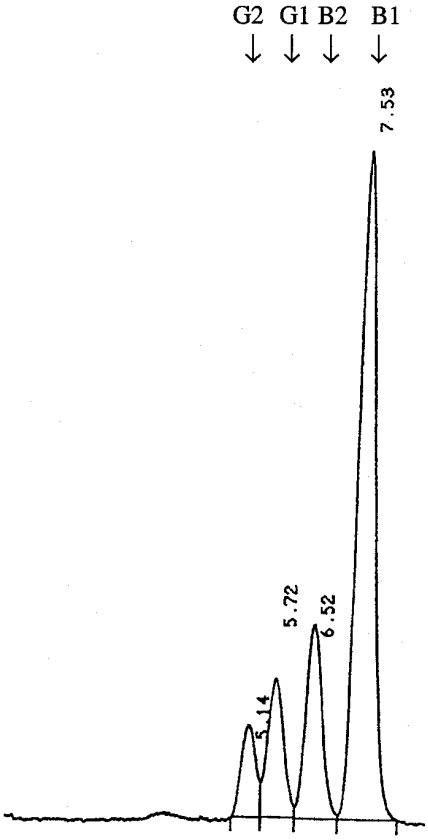
6.0 Recovery

Commodity Aflatoxin		% Recovery
Corn	$7B_1:1B_2:3G_1:1G_2$ *71	
Corn B	$_1$ 69	
Corn B	$_2$ 73	
Corn G	$_1$ 13	
Corn G	$_2$ 0	

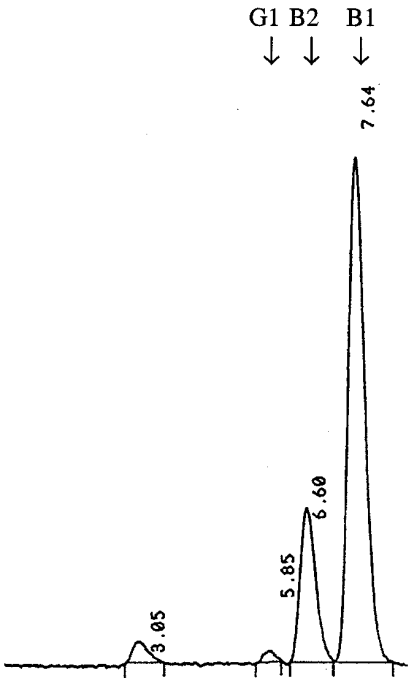
***Average of the percentage recoveries from Aflatoxin B_1 and B_2**

5.3 REPRESENTATIVE CHROMATOGRAMS

10 ppb standard



10 ppb sample



6.1 GENERAL PRECAUTIONS FOR FLUOROMETER PROCEDURES

Make sure to add salt to sample before extraction. Make sure salt has no additives.

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

Always use good, clean equipment and reagents (HPLC grade methanol for sample elution and purified, reverse osmosis or deionized water). Check reagents for background fluorescence as described in section 3.4, Reagent Check. Cuvettes not purchased from VICAM may give background fluorescence and should never be used with VICAM's tests.

Do not exceed the flow rates recommended in the procedure.

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

Protect calibration standards from light and replace every year.

Load sample on column immediately after microfibre filtration.

Perform test from beginning to end without interruptions.

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

6.2 TROUBLESHOOTING FOR FLUOROMETER PROCEDURES

1. Problem: False high readings

Solution:

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

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

2. Problem: False low readings

Solution:

Check to make sure method is followed correctly.

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

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

3. Problem: Inconsistent readings

Solution:

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

Protect calibration standards from light and replace every year.

Calibrate fluorometer correctly for the procedure you are using.

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

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

Mix filtrate well after diluting.

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

Collect all of the sample eluate in the cuvette.

Use a 60 second time delay.

TorBex Model FX-100 Series 3 Troubleshooting

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

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

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

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

3. Problem: Printer prints nonsense.

Solution: Check dip switch settings.

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

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

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

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

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

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

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

4. Problem: Fluorometer is not holding calibration

Solution:

The testing room should be maintained at a consistent temperature.

Check that calibration vials are clear.

Gently blow out sample chamber with canned air.

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

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

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

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

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

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

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

VICAM Series 4 Fluorometer Troubleshooting

1. Problem: Display problem

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

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

2. Problem: Results vary from 0 to 270ppb 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.

6.3 GENERAL PRECAUTIONS FOR HPLC PROCEDURES

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

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

7.0 TECHNICAL ASSISTANCE

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

Phone: 800-338-4381 Canada, Mexico and the United States

508-482-4935 All International and United States customers

Fax: 508-482-4972

e-mail: techservice@vicam.com

8.0 LIABILITY

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9.0 REFERENCES

1. Data supplied by D. M. Wilson, (unpublished), Department of Plant Pathology, Coastal Plain Experimental Station, University of Georgia, Tifton, GA 31793, USA.

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

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

10.0 ORDERING INFORMATION

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

Phone:	877-228-4244	Canada and the United States
	800-338-4381	Mexico
	417-725-6588	All International and United States customers
Fax:	417-725-6102	
e-mail:	vicam@vicam.com	