





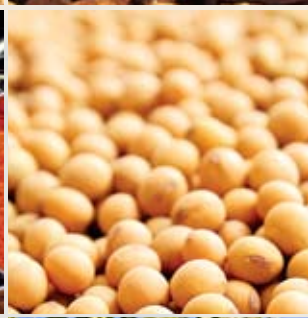
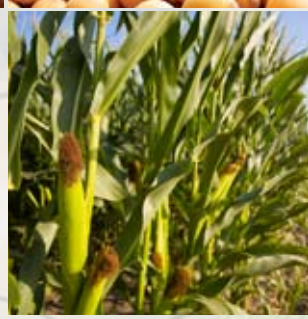

AflaTest WB

Instruction Manual

VICAM®

A Waters Business

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1.1 INTENDED USER

AflaTest®WB is a quantitative method for the detection of aflatoxin in many commodities. VICAM's advanced biotechnology permits the measurement of all the major aflatoxins (including AFB1, AFB2, AFG1, AFG2 and AFM1) without the use of toxic solvents like chloroform or methylene chloride. *AflaTest WB* 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 WB* 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 WB* 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. These steps are outlined in section 1.7, *AflaTest WB* Overview.

1.3 APPLICABILITY AND APPROVALS

AflaTest WB has been validated for quantitative measurement of aflatoxins in corn. Other aflatoxin immunity column methods (as listed in the AflaTest Instruction Manual) can also be used with this column. Assistance in measuring aflatoxin in commodities not listed in this manual can be obtained by contacting our Technical Assistance Department.

AflaTest WB meets the aflatoxin immunoaffinity column requirements in AOAC® official methods 991.31 and 999.07.

1.4 LIMITATIONS

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

1.5 SAMPLING

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

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

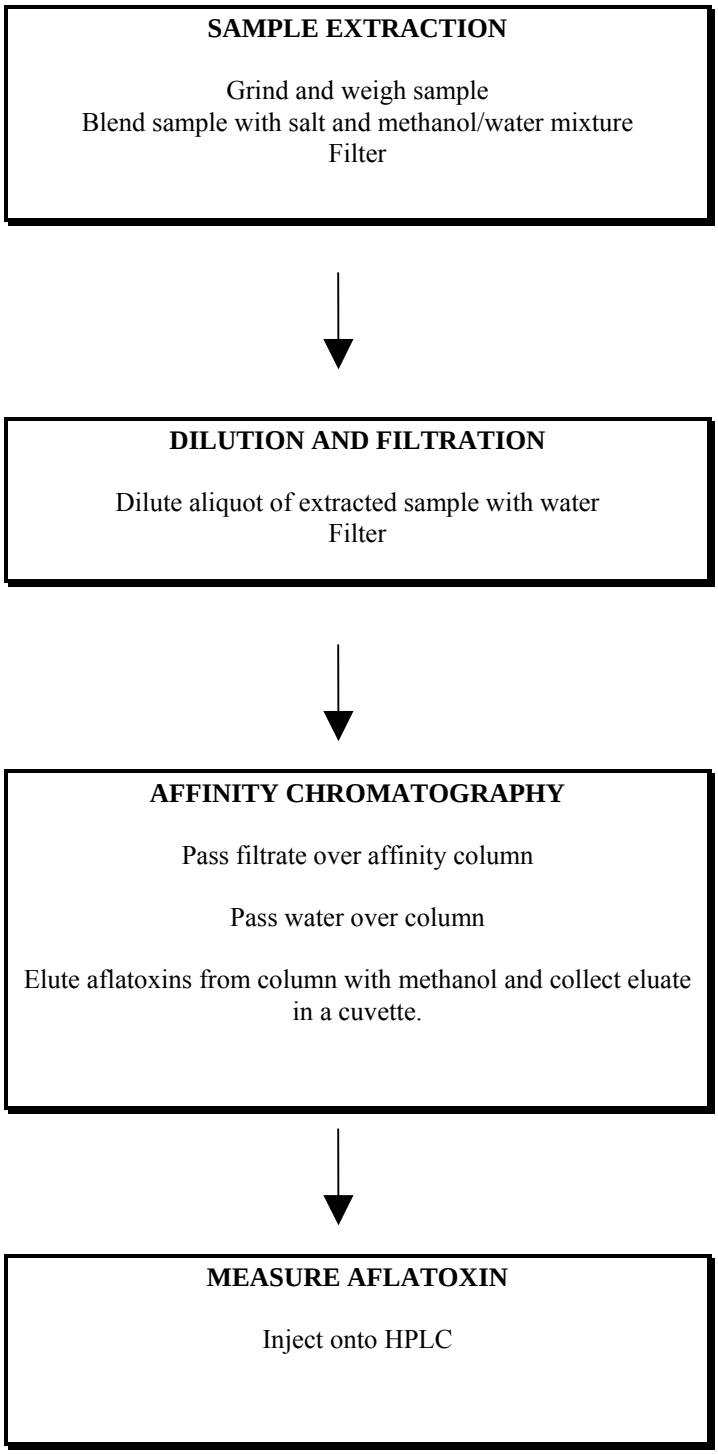
These can be viewed online at http://www.usda.gov/gipsa/reference-library/handbooks/hb.htm. Limited quantities of these publications are available free, upon request. Send requests to:

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

1.6 SHELF LIFE AND STORAGE CONDITIONS

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

1.7 *AFLATEST®WB* OVERVIEW



2.1 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 in to 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 microfiber filter. This removes any precipitates in the extract and assures that the extract will easily pass through the affinity column. Microfiber filtration is performed just prior to affinity chromatography.

2.2 PUMP STAND SETUP

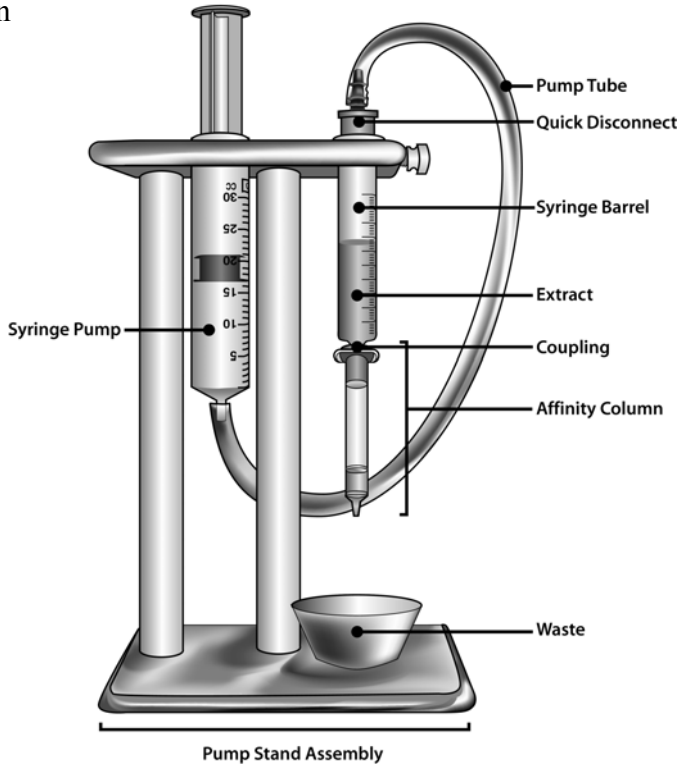
AflaTest WB affinity chromatography is easily performed with the *AflaTest WB* affinity column attached to a pump stand (part # 21020). The stand has a 10 mL glass syringe barrel that serves as a reservoir for the column. A large plastic syringe with tubing and coupling provides air pressure to manually push liquids through the column. An adjustable air pump (part #20650) can be attached to the pump tube instead of the large pump syringe barrel to operate without using hand pressure. Double position pump stand (part # 21030), four-position pump stand with aquarium pumps (part #21045) and 12 position pump stand with pumps (part #G1104) are available for running multiple samples at one time. Alternatively, a vacuum manifold can be used to pull liquid through the *AflaTest WB* column.

When using a pump stand:

1. Remove large top cap from column.
2. VICAM part G1118 HPLC Column Coupling provides a reusable coupling for attaching the column to the syringe barrel reservoir.
3. Pour extract after microfiber filtration into glass syringe barrel reservoir.
4. Pull up on the plastic syringe piston.
5. Insert coupling on end of tube into syringe barrel. Remove column bottom cap.
6. Apply pressure to piston of plastic syringe to push liquid through the column. Maintain a flow rate of 1-2 drops per second. Push all liquid through the column. Repeat for wash and elution steps (see procedures).

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

Affinity Column Syringe Barrel Connection



2.3 CLEANING EQUIPMENT

Wash blender jar, blender blade assembly, funnel and gasket with soap and hot water. Rinse thoroughly with cold tap water and then dry completely.

3.0 REAGENT PREPARATION

Extraction Solution

The A*flaTest WB* procedure uses a methanol/water solution to extract aflatoxin out of the sample. 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)
70:30	700	300	1000 (1 liter)
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 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.

Phosphate Buffered Saline (pH 7.4)

0.20 g KCl
0.20 g KH_2PO_4
2.92 g $Na_2HPO_4 \bullet 12H_2O$
8.00 g NaCl

Dissolve in 900 mL purified water. Adjust to pH 7.4 with 0.1M HCl or 0.1M NaOH and dilute to 1000 mL. Commercial buffered saline tablets may also be used.

4.1 MATERIALS AND EQUIPMENT REQUIRED FOR HPLC PROCEDURES

Materials Required

Description	Part #
AflaTest®WB Columns (25/box)	G1024
AflaTest®WB Columns (50/box)	G1025
VICAM Fluted Filter Paper, 24 cm (100)	31240
Microfibre Filters, 1.5µm, 11 cm (100)	31955
Disposable Cuvettes (250 per pack)	34000
Methanol, HPLC Grade (4 x 4 L)	35016
Disposable Plastic Beakers (25 per pack)	36010
Distilled, reverse osmosis or deionized water	
Noniodized sodium chloride (salt, NaCl)	

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 (or vacuum manifold)	21020
Filter Funnel, 65 mm (10 per pack)	36020
HPLC System as specified in procedure	

Suggested but not required

Description	
Micro-pipettor, 1 mL	G4033
Micro-pipette tips for 1 mL Micro-pipetter (100)	20656

4.2 *AFLATEST®WB* HPLC PROCEDURE FOR CORN

1.0 HPLC Set up:

1.1 Column: 4.6 mm x 25 cm, 5µm , C18
1.2 Mobile phase: water:acetonitrile:methanol (3:1:1) degassed. Add 2 mL H_3PO_4 and 120 mg KBr per liter.
1.3 Flow rate: 1.0 mL/min.
1.4 Fluorescence: excitation 360 nm, emission 450 nm
1.5 Post column derivatization: Kobra cell
1.6 Dilution of raw extract and immunoaffinity cleanup is performed by automated equipment (Aspec XL by Gilson Abimed)

2.0 Sample Extraction:

2.1 Place 25g ground sample with 5g salt (NaCl) into blender jar.
2.2 Add to jar 100 mL methanol:water (80:20).
2.3 Cover jar and blend at high speed for 3 minutes.
2.4 Remove cover and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution

3.1 Pipet 3.1 mL filtered extract into a clean vessel.
3.2 Dilute extract with 9.9 mL of PBS. Mix well.
3.3 Filter dilute extract through glass microfibre filter into a clean vessel.

4.0 Column Chromatography

4.1 Remove two end caps from *AflaTest WB* affinity column.
4.2 Attach column to outlet of 10 mL reservoir on pump stand or put in automated system.
4.3 Pass 12.6 mL filtered diluted extract completely through *AflaTest WB* affinity column at a rate of about 1-2 drops/second until air comes through column.
4.4 Pass 20 mL of purified water through the column at a rate of about 2 drops/second.
4.5 Elute affinity column by passing 1.5 mL HPLC grade methanol through column at a rate of 1-2 drops/second and collecting all of the sample eluate (1.5 mL) in a glass cuvette.
4.6 Add 1.5 mL of purified water to eluate. Inject 20-100 µL onto HPLC.

6.0 Limit of Detection and Recovery:

	Limit of Detection	Recovery
B1	**0.03 µg/kg**	**103%**
B2	**0.05 µg/kg**	**100%**
G1	**0.05 µg/kg**	**95%**
G2	**0.11 µg/kg**	**101%**

4.3 *AFLATEST®WB* ALTERNATIVE HPLC PROCEDURE FOR CORN, RAW PEANUTS, PEANUT BUTTER (AOAC METHOD)

1.0 HPLC Set up:

1.1 Column: 4.6 mm x 25 cm, 5µm , C18 (Rainin)

1.2 Mobile phase: water:acetonitrile:methanol (3:1:1) degassed

1.3 Flow rate: 1.0 mL/min.

1.4 Fluorescence detector: Kratos 950 fluorescence detector, excitation 360 nm, emission >420 nm cut off emission filter

1.5 Post column:
Post column iodine: 100 g Iodine dissolved in 2 mL methanol, then add 200 mL water, stir for 1 hour and filter through 0.45 µm filter.
Flow rate: 0.3 mL/min.
Reaction temperature: 70°C (FIAtron FH-40 heater & FIAtron TC-50 controller)
Reaction time: ~1 minute.

2.0 Sample Extraction:

2.1 Place 25g ground sample with 5g salt (NaCl) into blender jar.

2.2 Add to jar 125 mL methanol:water (70:30).

2.3 Cover jar and blend at high speed for 2 minutes.

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

3.0 Extract Dilution

3.1 Pipet or pour 15 mL filtered extract into a clean vessel.

3.2 Dilute extract with 30 mL of purified water. Mix well.

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

4.0 Column Chromatography

4.1 Remove two end caps from *AflaTest®WB* affinity column.

4.2 Attach column to outlet of 10 mL reservoir on pump stand.

4.3 Pass 15 mL filtered diluted extract (15 mL = 1g sample equivalent) completely through *AflaTest®WB* affinity column at a rate of about 1-2 drops/second until air comes through column.

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

4.5 Repeat with another 10 mL of purified water.

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

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

4.3 REPRESENTATIVE CHROMATOGRAMS

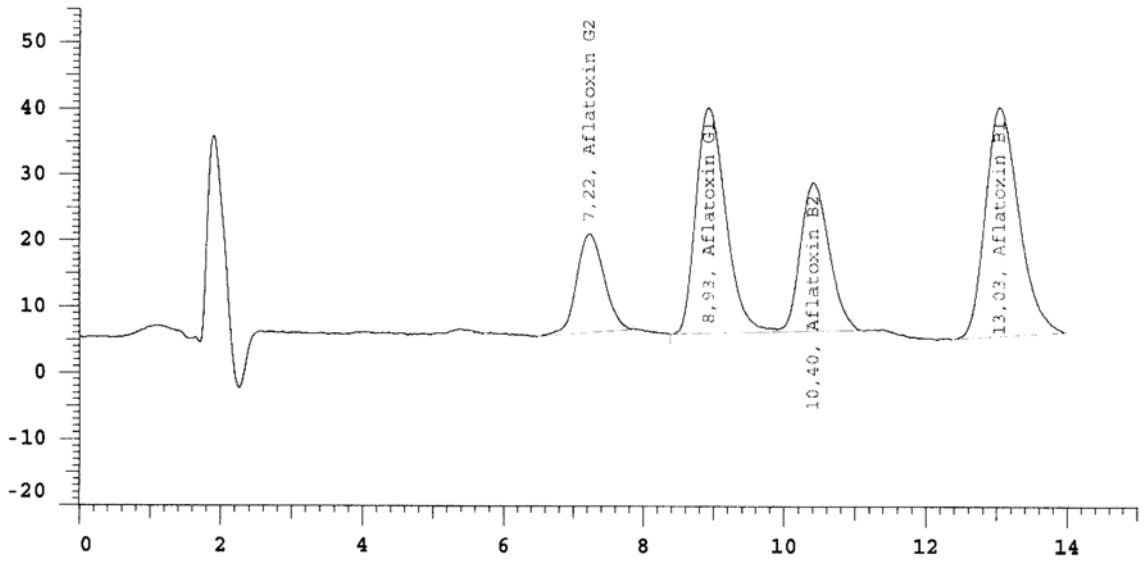


figure 1: sample chromatogram of an aflatoxin standard for a level of 1.3 µg/kg

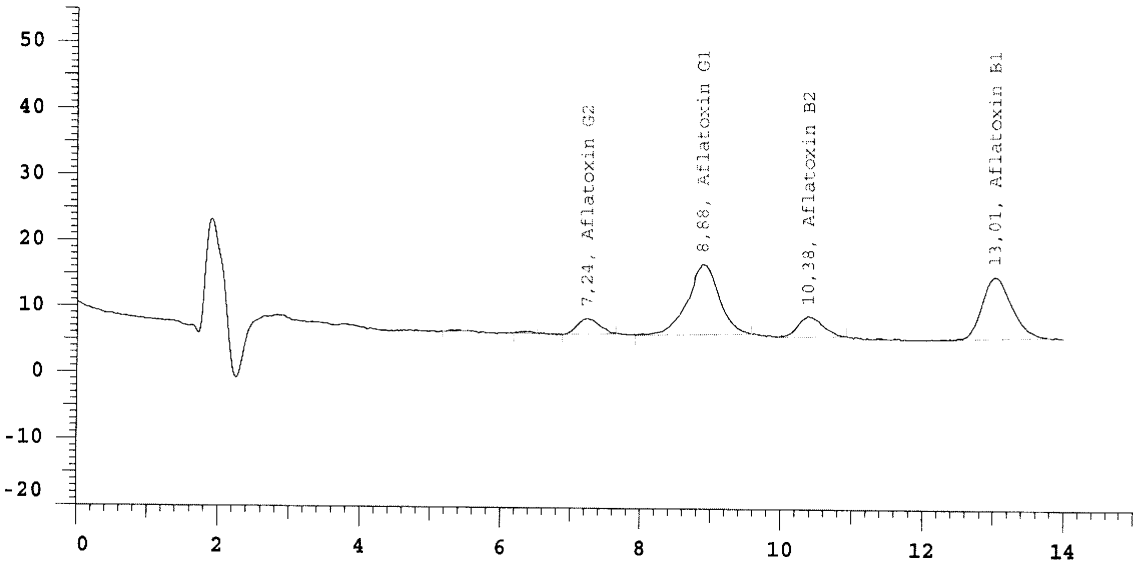


figure 2: sample chromatogram of a corn sample with a level of 0.3 µg/kg

5.0 GENERAL PRECAUTIONS FOR HPLC PROCEDURES

Absorbance detection is possible at 365 nm without post column iodine. This detection is less sensitive than fluorescence detection with KOBRA cell or post column iodine. For greater sensitivity, add 100 µl 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 derivatization with iodine.

6.0 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	

7.0 LIABILITY

The analytical methods described above have been developed by VICAM to be used exclusively with the reagents in this test. The user assumes all risk in using *AflaTest WB* analytical procedures and products. VICAM makes no warranty of any kind, express or implied, other than that *AflaTest WB* products conform to VICAM's printed specifications and quality control standards. VICAM will at its option repair or replace any product or part thereof which proves to be defective in workmanship or material. VICAM's undertaking to repair or replace such products is exclusive and is in lieu of all warranties whether written, oral expressed, or implied, including any implied warranty of merchantability or fitness for a particular purpose. VICAM shall have no liability for anticipated or lost profits or any loss, inconvenience or damage whether direct, incidental, consequential or otherwise, to person or property, or for strict liability or negligence arising from or in connection with the use of these assay procedures or *AflaTest WB* product.

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8.0 REFERENCES

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

2 Kok, W. T. (1994*). Journal of Chromatography* "Derivatization reactions for the determination of aflatoxins by liquid chromatography with fluorescence detection." (B. 659): 127-137.

3. Stroka, J. and Anklam, E, *Journal of AOAC International*, "Immunoaffinity column cleanup with liquid chromatography using post-column bromination for determination of aflatoxins in peanut butter, pistachio paste, fig paste, and paprika powder: collaborative study", 83 (2) (2000) 320-340.

9.0 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	