




FumoniTest and FumoniTest WB

Instruction Manual

VICAM®

A Waters Business

34 MAPLE STREET, MILFORD MA 01757 USA
TEL: 800.338.4381, 508.482.4935
FAX: 508.482.4972 EMAIL: VICAM@VICAM.COM

TABLE OF CONTENTS

1.1 INTENDED USER

FumoniTest™ and FumoniTest WB are quantitative methods for the detection of Fumonisin B_1, B_2 and B_3 in a variety of commodities. The FumoniTest and FumoniTest WB methods described in this manual are sample preparations for HPLC quantitation. The methods are intended for customers with HPLC equipment and experience.

1.2 PRINCIPLE

Fumonisins are mycotoxins produced by the fungi *Fusarium verticilliodes (= F. moniliforme)* and *F. proliferatum. Fusarium* species are a frequent, almost universal, inhabitant of corn. Fumonisins are present in most corn samples tested. Fumonisin is thought to cause equine leukoencephalomalacia in horses, swine pulmonary edema, and human esophageal cancer.

To measure fumonisin levels, samples are prepared by mixing with an extraction solution, followed by blending and filtering. The extract is then applied to the FumoniTest™ or FumoniTest™ *WB* columns, which contains specific antibodies to the fumonisins. At this stage, the fumonisins bind to the antibody on the column. The columns are then washed to rid the immunoaffinity column of impurities. By passing an eluting solution through the column, the fumonisins are removed from the antibody. The eluting solution of FumoniTest™ or FumoniTest™ *WB* can then be injected into an HPLC system. These steps are outlined in section 1.7, FumoniTest™ HPLC Procedure Overview, and 1.8 FumoniTest™ *WB* HPLC Procedure Overview.

1.3 APPLICABILITY

FumoniTest™ has been optimized for quantitative measurement of fumonisins in beer, corn, milo and poultry feed. FumoniTest *WB* has been optimized for Corn. Assistance in using the FumoniTest™ and FumoniTest™ *WB* for 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 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:

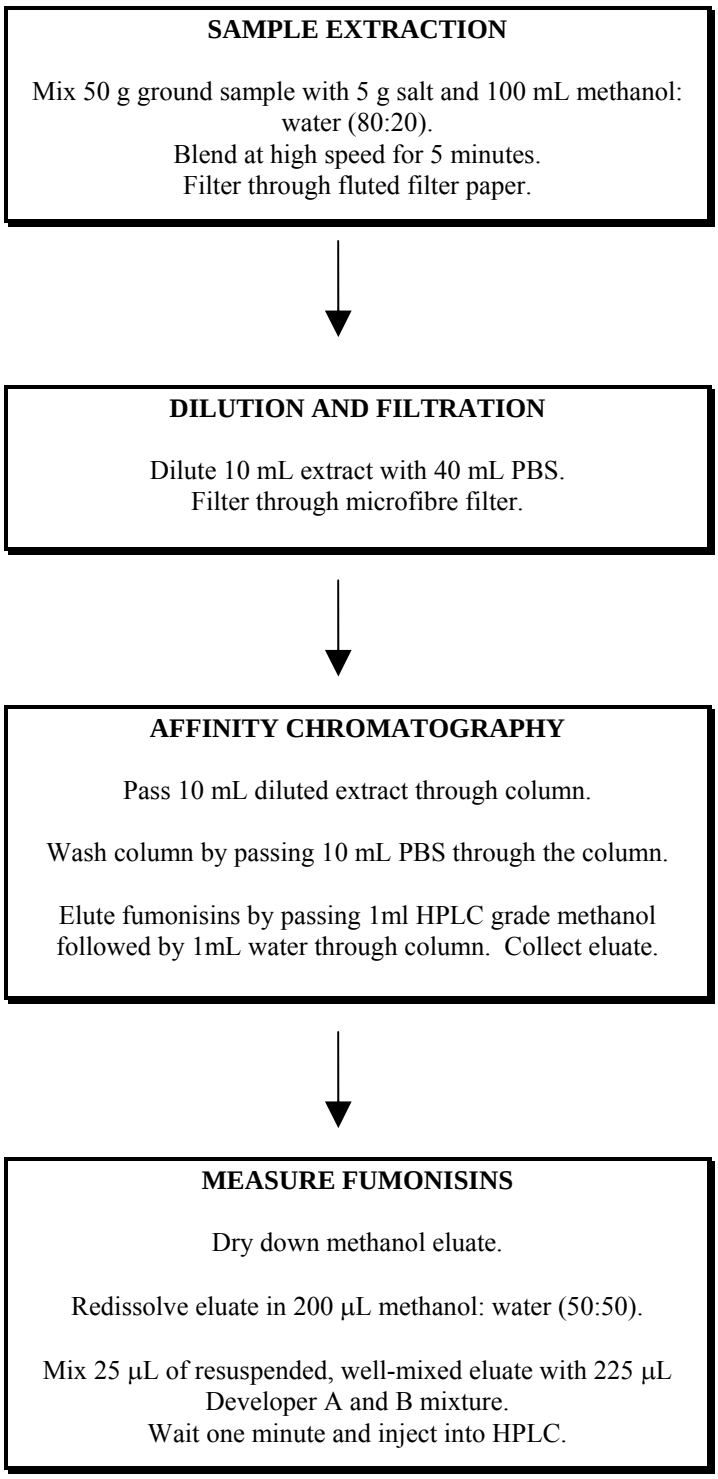
www.gipsa.usda.gov. Click on "Federal Grain Inspection" then "Publications" on the left.

1.6 SHELF LIFE AND STORAGE CONDITIONS

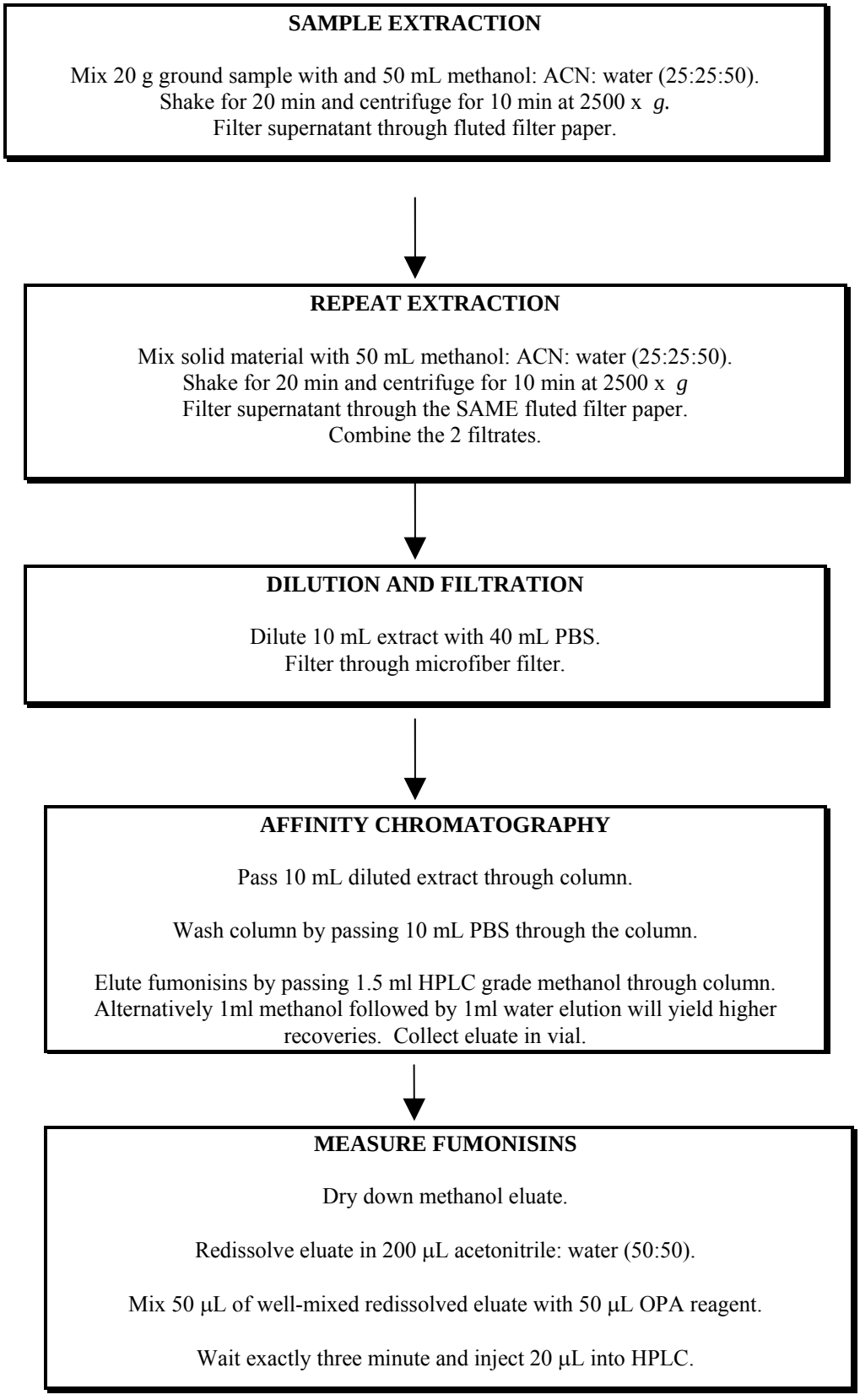
Store columns and reagents at refrigerated temperature (2 - 8°C) up until the expiration date on the box of columns or bottle of reagent. Columns and reagents are good for one year from production date. It is recommended that reagents and columns be at room temperature (18 – 22°C) for usage.

Developer A and Developer B should be kept tightly capped when not in use. Be sure to store developers at refrigerated temperature before use. Developer A and B mixture is stable for up to five days at room temperature.

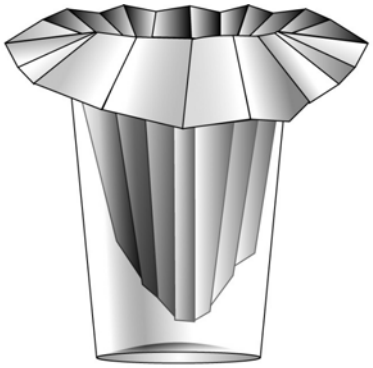
1.7 FUMONITEST™ HPLC PROCEDURE OVERVIEW



1.8 FUMONITEST™ WB HPLC PROCEDURE OVERVIEW



2.1 PREPARATION OF FILTRATION STEPS



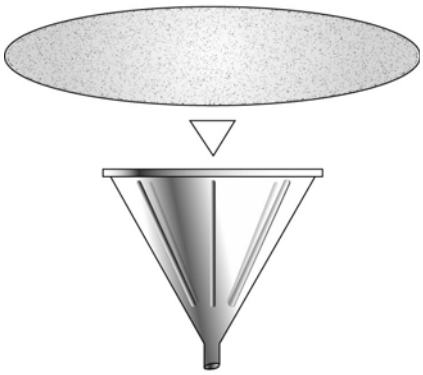
Fluted Filter

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 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.

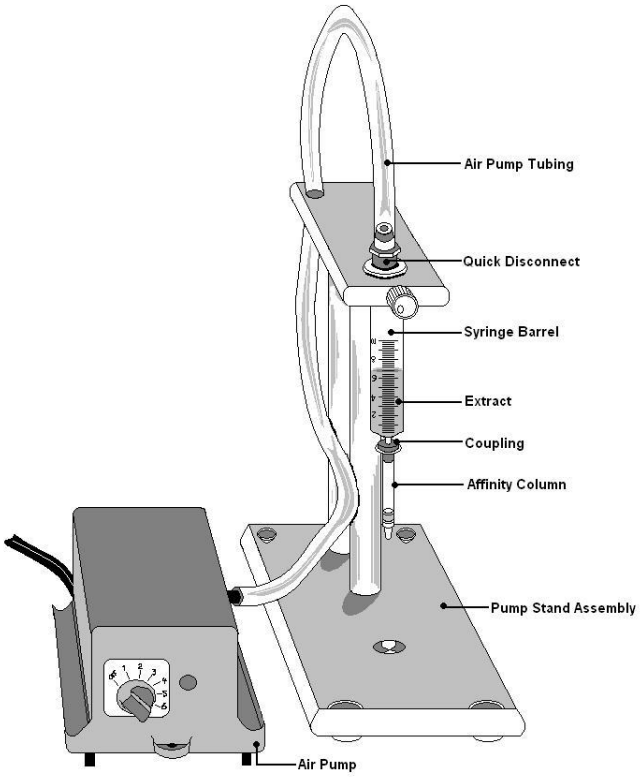
2. Place one microfibre filter gently into small funnel by pressing filter into funnel with index finger. Be careful not to rip or puncture the filter.

2.2 PUMP STAND SETUP

FumoniTest™ affinity chromatography is easily performed with the FumoniTest™ 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 (part #G4061 (110V) or #G4062 (220V)). Double (part # 21030), four-position (part #21045) and 12 position (part # G1103) pump stands are available for running multiple samples at one time. Alternatively, a vacuum manifold can be used to draw the extract through the column.

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 FumoniTest™ column. When using FumoniTest™ *WB* columns, order part # G1118 (WB column coupling).
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.
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/2 seconds or slower flow rate. The quick disconnect can be released so the methanol flows by gravity or pulsed to provide less pressure for the methanol elution.

Affinity Column Syringe Barrel Connection



2.6 CLEANING EQUIPMENT

Before Starting FumoniTest™ 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 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. Other pieces of equipment that need to be cleaned the same way before using are graduated cylinders, funnels and blender jars. Bottle dispensers need only to be rinsed thoroughly 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 dispensers with soap. Methanol dispenser needs only to be refilled with methanol.

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 large number of samples have been tested, the glass syringe barrel should be washed with a brush and mild detergent then rinsed 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.

FumoniTest™ developers will react with any protein. Keep developer solutions, column eluate and pipet tips used to measure elution solution and developer free from proteins (i.e. fingerprints).

3.1 PREPARATION OF EXTRACTION SOLUTION AND MOBILE PHASE

1. Use reagent grade (or better - i.e. HPLC grade) methanol and acetonitrile when preparing extraction solutions. Extraction solutions can be made at different total volumes than shown below as long as the ratio of methanol and water remains consistent.

Solution desired	Methanol (ml)	Acetonitrile (ml)	Purified Water (ml)	Total Volume (ml)
methanol: water (80:20)	800		200	1000 (1 liter)
acetonitrile: methanol: water (25: 25:50)	250	250	500	1000 (1 liter)

CAUTION: Extraction solvent is flammable. Keep container tightly capped when not in use.

Prepare extraction solution every week or as needed.

2. **PREPARATION OF MOBILE PHASE FOR HPLC CONDITION 2**

a. Dissolve 3.46g of sodium phosphate monobasic monohydrate (NaH_2PO_4-H_20 Fisher Scientific product S369-1) in 250mL of Milli-Q water to make 0.1M sodium phosphate monobasic.
b. Add 770mL Methanol to 230mL 0.1M sodium phosphate monobasic.
c. Adjust to approximately pH 3.3 with *o*-phosphoric acid.
d. Filter through a MAGNA nylon 0.22µm, 47mm filter. (Fisher # R02SP04700) and degas.

3.2 PREPARATION OF DILUTION/WASH SOLUTIONS

1. 2.5% sodium chloride, 0.5% sodium bicarbonate

2.5 g NaCl
0.5 g $NaHCO_3$
bring to 100 mL with purified water

2. 1X PBS

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

Alternatively, a 1X PBS solution can be prepared as follows:

8.0 g NaCl
1.2 g Na_2HPO_4
0.2 g KH_2PO_4
0.2 g KCl
dissolve in approximately 990 mL purified water
adjust pH to 7.0 with concentrated HCl

bring to 1 liter with purified water

3.3 PREPARATION OF DERIVATIZATION REAGENT

1. 0.1 M sodium tetraborate solution ($Na_2B_4O_7$)

   3.8 g $Na_2B_4O_7 \cdot 10\ HO_2$

   bring to 100 ml with purified water

2. OPA reagent

   Completely dissolve 40 mg *o*-Phthaldialdehyde in 1ml methanol
   Dilute with 5 ml 0.1 M $Na_2B_4O_7$ solution

   Add 50 µl 2-Mercaptoethanol

   Mix it well and store in the dark for up to one week at room temperature in a capped amber vial.

3.4 DERIVATIZATION (DEVELOPER) REAGENTS CAN BE PURCHASED FROM VICAM

Add 10 µl of Developer B (part# G5004) to Developer A (part# G5003). Keep Developer A and B mixture tightly capped and at room temperature when not in use. This mixture should be used for only five days and then discarded.

4.1 MATERIALS & EQUIPMENT REQUIRED FOR HPLC PROCEDURES

Consumables Required

Description	Part #
FumoniTest™ Columns, Fluorometer & HPLC (25 per box)	G1008
or *FumoniTest™WB* Columns (25/box)	G1060
or *FumoniTest™WB* Columns (50/box)	G1059
VICAM Fluted Filter Paper, 24 cm (100)	31240
Microfiber Filters, 1.5µm, 11 cm (100)	31955
FumoniTest™ Developer A HPLC (5 mL)	G5003
FumoniTest™ Developer B HPLC/Fluorometer (500 µL)	G5004
10X Concentrate of PBS, 150 ml	G1113
Disposable Cuvettes (250 per pack)	34000
Methanol, HPLC Grade (4 x 4 L)	35016
Disposable Plastic Beakers	36010
Distilled , reverse osmosis or deionized water	
Noniodized sodium chloride (salt, NaCl)	
Sodium bicarbonate (beer only)	
Acetonitrile	
HPLC Buffer	

Equipment Required

Description	Part #
Graduated Cylinder, 50 ml	20050
Graduated Cylinder, 250 ml	20250
Digital Scale with AC Adapter	20100
Commercial Blender with Stainless Steel Container	20200
Wash Bottle, 500 ml	20700
Cuvette Rack	21010
2-Position Pump Stand w/ Air Pump (10 mL)	21040
or 4-Position Pump Stand w/2 Air Pumps (10 mL)	21045
or 12-Position Pump Stand w/6 Air Pumps (10 mL)	G1104
Vortex Mixer	23040
500 mL Bottle Dispenser for Methanol (0-3 mL range)	20501
WB Column Coupling (6)	G1118
Filter Funnel, 65 mm (10 per pack)	36020
Filter Funnels, 105 mm (4 per pack)	36022
Adjustable micopipetters and tips	

4.2 FUMONITEST™ HPLC PROCEDURE FOR BEER

1.0 General Set Up

1.1 Prepare 2.5% sodium chloride, 0.5% sodium bicarbonate solution as described in section 3.2, Preparation of Dilution/Wash Solutions.

2.0 HPLC Set up: HPLC Condition 1.

3.0 Column Chromatography

3.1 Pass 5 ml beer completely through FumoniTest™ HPLC affinity column at a steady slow flow rate (gravity flow) until air comes through column.

3.2 Pass 1 mL 2.5% sodium chloride, 0.5% sodium bicarbonate solution through the affinity column until air comes through the column.

3.3 Pass 1 mL of purified water through the column at a rate of 2-3 drops per second.

3.4 Place glass cuvette (VICAM part # 34000) under FumoniTest™ column and add 1.0 ml HPLC grade methanol into glass syringe barrel. (Alternatively elution with 1mL methanol followed by 1mL water at a rate of 1 drop/2 seconds or slower (gravity) may yield higher recoveries.)

3.5 Elute FumoniTest™ column with 1 ml of methanol by gravity and collect all of the sample eluate in a glass vial.

3.6 Evaporate eluate to dryness under nitrogen on a hot plate at ~55°C.

3.7 Derivatize eluate using o-phthaldialdehyde/2-mercaptoethanol (OPA/MCE*) by adding 250 µL to the dried eluate and injecting 25 µL or 50 µL within 1 minute.

* OPA/MCE reagent

Completely dissolve 20 mg *o*-Phthaldialdehyde in 0.5 ml methanol
Dilute with 2 ml 0.05 M $Na_2B_4O_7$ solution
Add 25 µl 2-Mercaptoethanol

Mix it well and store in the dark for up to one week at room temperature in a capped amber vial.

4.0 Assay Range: not determined

5.0 Limit of Detection: 0.4 - 1.0 ng/ml

6.0 Recovery: Average of 71% at 2.0 µg/ml. Average of 79% at 5.0 µg/ml.

4.3 FUMONITEST™ HPLC PROCEDURE FOR CORN, MILO (SORGHUM) & 17% PROTEIN POULTRY FEED (1.0 g SAMPLE EQUIVALENT, 0 - 7 PPM)

1.0 General Set Up:

1.1 Prepare methanol: water (80:20) solution every week or as needed.

1.2 Prepare PBS solution as described in section 3.2, Preparation of Dilution/Wash Solutions.

1.3 Prepare developer A and B mixture every five days as described in section 3.4.

2.0 HPLC Set up: HPLC Condition 2.

3.0 Sample Extraction:

3.1 Place 50 g ground sample and 5 g salt into a blender jar.

3.2 Add to jar 100 ml methanol: water (80:20).

3.3 Cover blender jar and blend at high speed for 5 minutes.

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

4.0 Extract Dilution:

4.1 Transfer 10 ml filtered extract into another clean vessel.

4.2 Dilute extract with 40 mL PBS. Mix well.

4.3 Filter dilute extract through microfibre filter (VICAM part # 31955). Collect in a clean vessel.

5.0 Column Chromatography:

5.1 Pipet 10 ml (10 ml = 1 g sample equivalent) filtered extract and pass completely through FumoniTest™ affinity column at a rate of about 1 drop/second until air comes through column.

5.2 Pass 10 ml of PBS through the column at a rate of 1 -2 drops/second until air comes through the column.

5.3 Place glass cuvette (VICAM part # 34000) under FumoniTest™ column and add 1.0 ml HPLC grade methanol into glass syringe barrel.

5.4 Elute FumoniTest™ column by gravity (1 drop/2 seconds or slower) and collect all of the sample eluate in a glass cuvette.

5.5 Add 1mL purified water into glass syringe barrel and elute by gravity (1 drop/2 seconds or slower) and collect in the same glass cuvette.

5.5 Dry down combined methanol and water eluate, for example in a speed vac apparatus. Redissolve eluate in 200 µl methanol: purified water (50:50, v/v).

5.6 Transfer samples to 250 µl screw cap vials.

5.7 Set up autoaddition feature of the Waters Alliance system so that 45 µl of Developer A and B mixture is collected, followed by 10 µl of the sample, and another 45 µl of Developer A and B mixture. Set delay time to 1 minute and inject into HPLC.

5.8 For auto addition systems that cannot "sandwich" the 10 µL sample between 45 µL amounts of Developer A and B, consult your HPLC manufacturer to determine what modifications to your system will ensure that the sample and Developer mixture are well-mixed.

Notes : 1. Alternatively, fumonisins may also be derivatized by mixing 25 µL of the resuspended, well-mixed eluate with 225 µL Developer A and B mixture, waiting 1 minute and injecting 50 µL into the HPLC.
2. Thorough mixing of the sample and Developers A and B is critical to the derivatization reaction and obtaining high quality results.

6.0 Limit of Detection: 0.016 ppm

7.0 Recovery*: Greater than 80% recovery for fumonisins B_1, B_2 and B_3.

*****Recovery values listed above are for corn only.

4.4 FUMONITEST™ *WB* HPLC PROCEDURE FOR CORN, (0.4 g SAMPLE EQUIVALENT, 0 - 10 PPM)

1.0 General Set Up:

1.1 Prepare extraction solvent: acetonitrile: methanol: water (25: 25:50) solution every week or as needed.

1.2 Prepare PBS solution as described in section 3.2, Preparation of Dilution/Wash Solutions.

1.3 Prepare developer A and B mixture every five days as described in section 3.3.

2.0 HPLC Set up: HPLC Condition 3

3.0 Sample Extraction:

3.1 Weigh 20 g ground sample into 250 ml centrifuge bottle.

3.2 Add 50 ml extraction solvent into centrifuge bottle.

3.3 Cover and shake bottle for 20 minutes with orbital shaker.

3.4 Centrifuge for 10 min at 2500 x *g*.

3.5 Remove cover from bottle and pour supernatant into Whatman No. 4, 12 cm filter paper, avoiding transfer of solid material on filter. Collect filtrate in a clean vessel.

3.6 Again extract remaining solid material by adding 50 ml extraction solvent to the centrifuge bottle and shaking bottle for 20 minutes. Centrifuge for 10 min at 2500x *g*.

3.7 Filter extract through the same filter paper. Collect and combine the 2 filtrates.

4.0 Extract Dilution:

4.1 Transfer 10 mL filtered extract into another clean vessel.

4.2 Dilute extract with 40 mL PBS. Mix well.

4.3 Filter dilute extract through microfiber filter (VICAM part # 31955). Collect in a clean vessel.

5.0 Column Chromatography:

5.1 Pipet 10 ml (10 ml = 0.4 g sample equivalent) filtered extract and pass completely through FumoniTest™ *WB* affinity column at a rate of about 1 drop/second.

5.2 Pass 10 ml of PBS through the column at a rate of 1 -2 drops/second until air comes through the column.

5.3 Place vial under FumoniTest™ *WB* column.
Elute FumoniTest™ *WB* column by passing 1.5 ml HPLC grade methanol through the column at gravity flow rate (1 drop/2 seconds or slower). Collect all of the sample eluate in a 4 ml vial. Alternatively elution with 1mL methanol followed by 1mL water at gravity flow rate (1 drop/2 seconds or slower) will yield higher recoveries.

5.4 Dry down methanol eluate just to dryness under a stream of nitrogen at 60˚C. Redissolve eluate in 200 µl acetonitrile: water (50:50, v/v).

5.6 Transfer 50 µl aliquots to bottom of 1 ml test tube, and add 50 µL OPA reagent (see section 3.3). Mix solution for 30 seconds with vortex mixer.

5.7 Inject 20 µL derivatized solution (equivalent to 20mg matrix) into HPLC system exactly 3 minutes after adding OPA reagent.

4.5 REFERENCES AND OTHER PUBLISHED HPLC PROCEDURES FOR FUMONITEST

De Girolamo, A., et al. (2010). "Determination of fumonisins B_1 and B_2 in maize-based baby food products by HPLC with fluorimetric detection after immunoaffinity column clean-up." World Mycotoxin Journal **3**(2): 135-146.

De Girolamo, A., et al. (2011). "Comparison of methods and optimisation of the analysis of fumonisins B_1 and B_2 in masa flour, an alkaline cooked corn product." Food Additives and Contaminants **28**(5): 667-675.

Duncan, K., S. Kruger, et al. (1998). "Improved fluorometric and chromatographic methods for the quantification of fumonisins B_1, B_2 and B_3." Journal of Chromatography A **815**: 41-47.

Kim, E., et al. (2002). "Extraction of Fumonisins B_1 and B_2 fromWhite Rice Flour and Their Stability in White Rice Flour, Cornstarch, Cornmeal, and Glucose". Journal Of Agricultural And Food Chemistry **50**: 3614-3620.

Kim, E., Maragos, C.M., Kendra, D.F. (2004). "Liquid Chromatographic Determination Of Fumonisin B_1, B_2 And B_3 In Corn Silage." Journal Of Agricultural And Food Chemistry **52**:196-200.

Oh, K.S., et al. (2009). "Incomplete Recoveries of Fumonisins Present in Naturally Contaminated Corn Foods from an Immunoaffinity Column." Journal of AOAC International **92**(2): 496-501.

Oles, C.J. and M.W. Trucksess (2010). "Determination of Fumonisin B_1 in Botanical Roots by Liquid Chromatography with Fluorescence Detection: Single-Laboratory Validation." Journal of AOAC International **93**(4): 1155-1160.

Scott, P. M. and G. A. Lawrence (1995). "Analysis of Beer for Fumonisins." Journal of Food Protection **58**(12): 1379-1382.

Seo, E., et al. (2009). "Fumonisins B_1 and B_2 in Agricultural Products Consumed in South Korea: An Exposure Assessment." Journal of Food Protection **72**(2): 436-440.

Solfrizzo, M., et al. (2011). "Determination of Fumonisins B_1 and B_2 in Corn-Based Foods for Infants and Young Children by LC with Immunoaffinity Column Cleanup: Interlaboratory Validation Study." Journal of AOAC International **94**(3): 900-908.

Trucksess, M. W., M. E. Stack, et al. (1995). "Immunoaffinity Column Coupled with Liquid Chromatography for Determination of Fumonisin B_1 in Canned and Frozen Sweet Corn." Journal of AOAC International **78**(3): 705-710.

Visconti, A., M. Solfrizzo, et al. (2001). "Determination of Fumonisins B_1 and B_2 in Corn and Corn Flakes by Liquid Chromatography with Immunoaffinity Column Cleanup: Collaborative Study." Journal of AOAC **84**(6): 1828-1837.

Ware, G. M., P. P. Umrigar, et al. (1994). "Evaluation of FumoniTest Immunoaffinity Columns." Analytical Letters **27**(4): 693-715.

OFFICIAL METHODS

AOAC method 2001.04, Determination of Fumonisins B_1 and B_2 in Corn and Cornflakes by Liquid Chromatography and Immunoaffinity Column Cleanup [J. of AOAC Int. 84(6) 1828-1837, 2001]

CEN 14352 Determination of fumonisin B_1 and B_2 in maize based foods - HPLC method with immunoaffinity column clean-up

CEN 16187 Determination of fumonisin B_1 and fumonisin B_2 in processed maize containing foods for infants and young children - HPLC method with immunoaffinity column cleanup and fluorescence detection after precolumn derivatization

5.1 HPLC Conditions*

HPLC Condition 1:

- **1.1** Column: Ultratechsphere C18 (HPLC Technology Ltd.), 5 µm, 4.6 mm x 25 cm stainless steel column fitted with a Resolve C18 Guard-Pak (Waters).
- **1.2** Mobile phase: methanol: 0.05 M sodium dihydrogen phosphate (55:45 v/v) degassed
- **1.3** Flow rate: 1.0 ml/min.
- **1.4** Fluorescence detector: Kratos Spectroflow 980 fluorescence detector, excitation 335 nm, and emission 418 nm.)
- **1.5** Retention time: ~12 minutes for fumonisin B_1, ~14 minutes for fumonisin B_2.
- **1.6** Autosampler: Hewlett Packard Series 1050

HPLC Condition 2:

- **2.1** Column: Reverse phase C18 (Waters Nova-Pak®C18, 3.9 mm x 150 mm, 4 µm)
- **2.2** Mobile phase: methanol: 0.1 M NaH_2PO_4 (77:23, v/v) adjusted to approximately pH 3.3 with *o*-phosphoric acid. See section 3.1 for details.
- **2.3** Flow rate: 0.8 ml/min.
- **2.4** Fluorescence detector: Waters 474 scanning fluorescence detector, excitation 335 nm, and emission 440 nm.
- **2.5** Retention time: ~5.5 minutes for fumonisin B_1, ~11.5 minutes for fumonisin B_3, and ~12.5 minutes for fumonisin B_2.

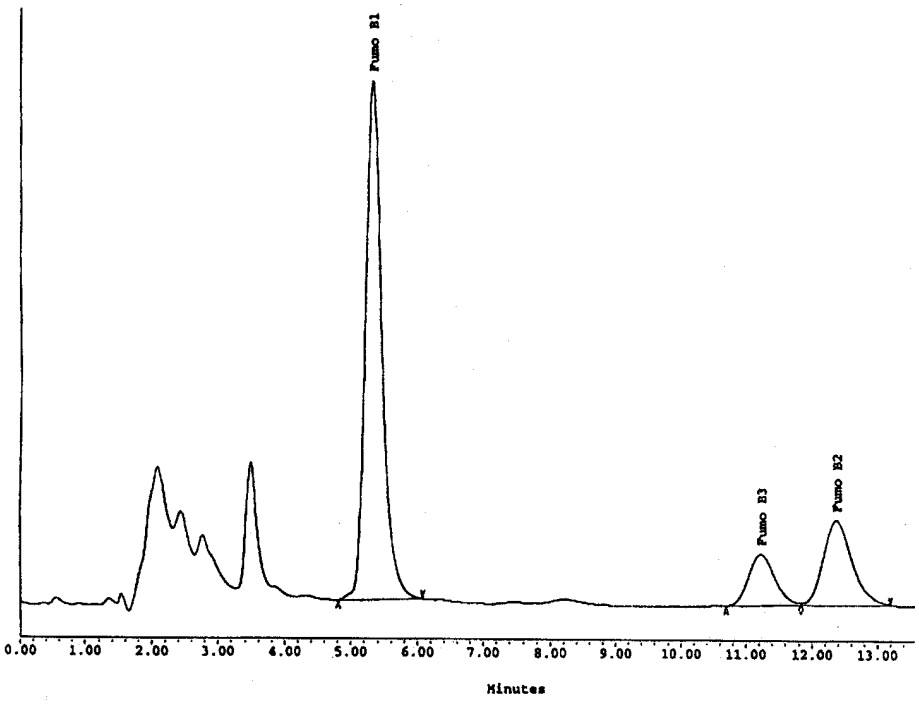
HPLC Condition 3:

- **3.1** Column: Reverse phase C18 (Rheodyne, 4.6 mm x 150 mm, 5µm)
- **3.2** Mobile phase: Methanol: 0.1 M NaH_2PO_4 (77:23, v/v) adjusted to approximately pH 3.35with phosphoric acid (H_3PO_4).
- **3.3** Flow rate: 1 ml/min.
- **3.4** Fluorescence detector: excitation 335 nm, and emission 440 nm.

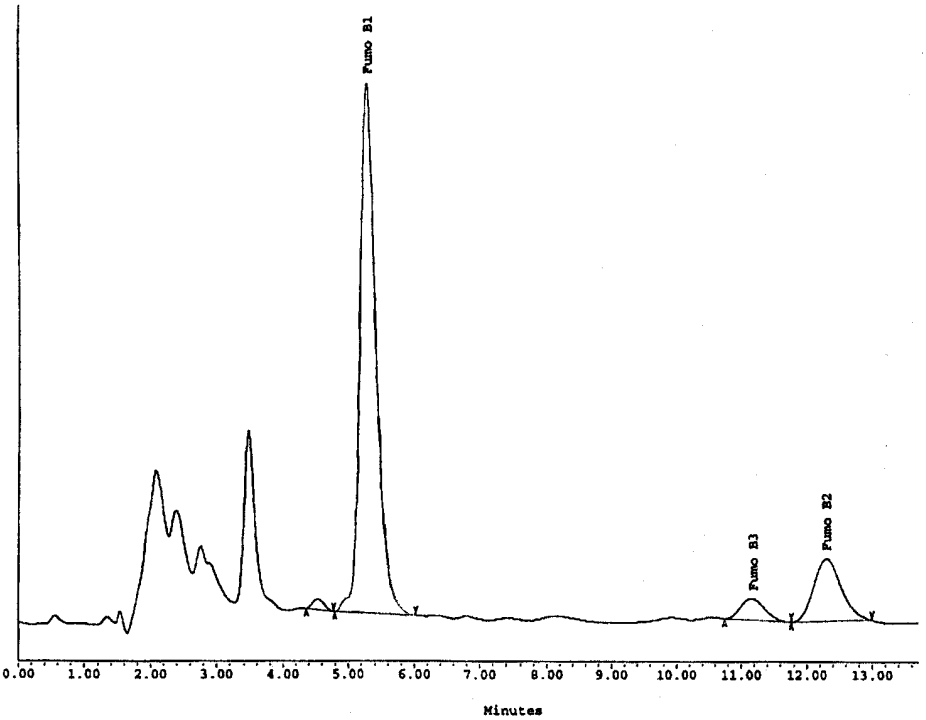
*****DISCLAIMER
Although specific equipment and HPLC columns are listed in this document, there are a number of equally suitable components that can also be used.

5.2 REPRESENTATIVE HPLC CHROMATOGRAMS (Using HPLC condition 2)

2.5 ppm fumonisin standard (5 B_1: 2 B_2: 1 B_3 ratio)



1.73 ppm fumonisin contaminated corn sample



5.3 HOW TO SPIKE A CORN SAMPLE WITH FUMONISIN

Fumonisin Standards can be ordered from PROMEC in South Africa (http://www.mrc.ac.za/promecsales/)

Fumonisin stock solution: Prepare stock solution containing 1mg/ml fumonisin B1 in acetonitrile–water (50:50, v/v), 1mg/ml fumonisin B2 in acetonitrile–water (50:50, v/v), and 1mg/ml fumonisin B3 in acetonitrile–water (50:50, v/v). Fumonisin stock solution is stable up to 6 months when stored at 4°C.

Mixed Fumonisins solution (MFS): To make 1.0mg/mL B1, B2, and B3 standard solution (5:2:1): mix 0.625mL of 1mg/mL B1 standard, 0.25mL of 1mg/mL B2 standard, and 0.125ml of 1mg/mL B3 standard.

1. To spike corn with Fumonisin at 0.25ppm and 1ppm level

0.20 ppm (µg/g) X 50 g corn = 10 µg
10 µg ÷ 1 µg/µl (MFS) = 10 µl
Add 10 µl Mixed Fumonisin Solution to 50 g corn

1 ppm (µg/g) X 50 g corn = 50 µg
50 µg ÷ 1 µg /µl (MFS) = 50 µl
Add 50 µl Mixed Fumonisin Solution to 50 g corn

2. To make standards for 4.3 FumoniTest HPLC corn, milo and 17% protein poultry feed procedure (10 ml = 1.0 g equivalent)

Fumonisin calibration solution (FCS) at 20 ng/µL : Add 20 µl MFS, 1.0 mg/mL (5:2:1) standard to 980 µl acetonitrile–water (50:50, v/v)

0.2 ppm (µg/g) X 1 g corn = 0.2 µg = 200 ng
200 ng ÷ 20 ng/µl (FCS) = 10 µl
Add 10 µl Fumonisin stock solution to a total 2mL methanol:water (50:50)

1.0 ppm (µg/g) X 1 g corn = 1.0 µg = 1000 ng
1000 ng ÷ 20 ng/µl (FCS) = 50 µl
Add 50 µl Fumonisin stock solution to a total 2mL methanol:water (50:50)

5.0 ppm (µg/g) X 1 g corn = 5.0 µg = 5000 ng
5000 ng ÷ 20 ng/µl (FCS) = 250 µl
Add 250 µl Fumonisin stock solution to a total 2mL methanol:water (50:50)

Dry the standard vials and the eluate vials from spiked or natural contaminated samples at the same time and derivatize together.

Best spiking is done with a Hamilton Syringe, but an adjustable pipetman with replaceable plastic tips can also be used. Spike the samples in a fume hood and let them dry.

Make a graph of ppm level of the standards vs peak area. The peak area of the unknown samples is then plugged into the equation of this line to calculate the ppm value of the samples. This calculation can be done with the software provided by an HPLC manufacturer. In addition, this calculation can be done using Microsoft EXCEL software.

6.0 GENERAL PRECAUTIONS FOR HPLC PROCEDURES

Do not elute column with acetonitrile.

Elute column slowly by gravity (~1 drop/2 seconds or slower).
Higher recoveries can be obtained by eluting with 1mL methanol followed by 1mL water or by eluting with 2 X 2mL 80% methanol rather than elution with only 100% methanol.

FumoniTest™ developers or OPA reagent will react with any protein. Keep developer solutions, OPA reagent ,column eluate and pipet tips used to measure elution solution and developer free from proteins (i.e. fingerprints).

If no peaks are seen on the chromatogram, check mobile phase composition. Mobile phase should be made with sodium phosphate monobasic monohydrate as described in section 3.1.

7.0 TECHNICAL ASSISTANCE

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

Phone:	800-338-4381	United States
	508-482-4935	All International and United States customers
Fax:	508-482-4972	
E-mail:	techservice@vicam.com	

8.0 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 FumoniTest™ and FumoniTest WB™ analytical procedures and products. VICAM makes no warranty of any kind, express or implied, other than that FumoniTest™ and FumoniTest 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 FumoniTest™ and FumoniTest WB™ products.

The foregoing notwithstanding, protocols and other products developed by VICAM are periodically improved and revised in order to maximize reliability and optimize customer use and satisfaction. When an improved, new or substitute version of a protocol and product is available, VICAM shall not be held liable or responsible for any earlier protocol or product, even

if use of earlier product or protocol be within the expiration date. Please inform yourself about any new protocols by e-mailing, faxing or phoning VICAM or your local VICAM distributor.

VICAM shall not be liable or responsible for any unsatisfactory or faulty results or performance involving the use of VICAM protocols or products if the testing or sampling in question is not conducted properly. The customer is solely and fully responsible for educating oneself about the proper testing and sampling procedures using VICAM protocols and products.

All VICAM products are protected by worldwide patents and trademarks.

9.0 ORDERING INFORMATION

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

Phone:	877-228-4244	United States
	417-725-6588	All International and United States customers
Fax:	417-725-6102	
E-mail:	vicam@vicam.com	

Instruction Manual # GN-MC9545-1