

VICAM AFLACHECK

QUALITATIVE AFLATOXIN TEST

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GENERAL INFORMATION

VICAM’s AflaCheck rapid lateral flow strip test is a competitive immunoassay that detects aflatoxins in corn at a detection threshold of 20 ppb. The lateral flow strip test membrane is inserted into diluted sample extract. The sample pad at the bottom of the strip contains aflatoxin-specific antibodies conjugated to colloidal gold particles. If aflatoxin is present, it will bind with the antibody-particle conjugates. Through capillary action, the membrane wicks the moisture on the pad to a test zone with binding sites for capturing unbound antibody-particle conjugates. If the level of aflatoxin in the extract is below the threshold, the proportion of antibody-conjugated gold particles that attach to these sites will be high enough to produce a red or pink test line. The absence of a test line indicates a contamination level at or above the test threshold. The remaining antibody-particle conjugates form a red or pink control line in the upper section of the strip, confirming that the particles flowed along the membrane as intended.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	VICAM, A Waters Business (508) 482-4935
Test Kit Name:	VICAM AflaCheck Test Kit
Product Number:	100000173
Effective Date of Instructions:	07/15/2015
Instructions Revision Number	0
Type of Service	Qualitative
Detection Threshold	20 ppb
Supplemental Analysis:	N/A
Approved Commodities:	Corn
Extraction method:	Shake 50-gram sample with 200 milliliters (mL) 70% methanol/30% distilled or deionized water (v/v) for 1 minute.
Test Format:	Lateral flow strip
Detection Method:	Visual

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

a. Preparation of Extraction Solvent: 70% methanol (MeOH)/30% water (v/v)

- (1) Use a graduated cylinder to measure and pour 700 mL of ACS reagent-grade or higher methanol into a carboy with a spigot.
- (2) Add 300 mL of deionized or distilled water to the methanol and shake until completely mixed.
- (3) Label the container with a description of its contents (70% methanol/30% water), date of preparation and initials of technician who prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container.
- (5) To prepare smaller or larger amounts of solution maintain the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

Note: Premixed 70% MeOH/30% water extraction solvent may be purchased from VICAM part # 100000217.

b. Paper rack:

The paper rack is nested upside-down in the kit box. Turn it over, and place it back in the box. The opening in the top of the rack will serve as a holder for the dilution tube.

EXTRACTION PROCEDURES

a. Extraction procedure for corn:

- (1) Weigh 50 g of sample and add to 500 mL glass or hard plastic vessel with a cap.
- (2) Measure 200 mL of the 70/30 methanol/water (v/v) extraction solution in a graduated cylinder, and pour the solution into the vessel.
- (3) Cap the vessel, and shake vigorously for 1 minute.
- (4) Allow the sample suspension to settle for 3 minutes

TEST PROCEDURES

a. AflaCheck Strip Test

- (1) Place the dilution tube in the opening in the paper rack.
- (2) Pipette 250 μ L of distilled water into the dilution tube, using the technique outlined below.
 - (a) Squeeze the top bulb of a 250 μ L strip test pipettor.

- (b) Draw up enough water to fill the pipettor barrel to the point where one or two drops of water spill over onto the lower bulb. The barrel should be completely full. Make sure none of the liquid drips out of the pipettor before dispensing.
 - (c) Dispense the contents of the pipettor into the dilution tube by squeezing the top bulb until the barrel is completely empty.
- (3) Uncap the vessel containing the sample extract and insert a new strip test pipettor into the clear liquid above the solid corn material. Using the same technique described in step 2, pipette 250 μ L of sample extract into the dilution tube.
 - (4) Cap the dilution tube and shake it by hand to mix the contents.
 - (5) Insert an AflaCheck test strip (with the arrows pointing down) into the dilution tube.
 - (6) Allow the test to develop for 5 minutes. (Negative results may become visible sooner.)
 - (7) Interpret the results immediately.

b. Interpretation of results:

- (1) The control line should be visible in the upper section of the strip. This line indicates the test is working properly.
- (2) A second colored line (the test line) in the mid-section of the strip indicates the test is negative (i.e., the sample contains less than 20 ppb of aflatoxin). The control line and the test line may appear in as little time as 3 minutes.

Negative Results:

Sample contains < 20 ppb of aflatoxin.

(Any visible test line indicates a negative result.)



- (3) If no test line appears after 5 minutes, the result is positive (i.e., the level of aflatoxin in the sample equals or exceeds 20 ppb).

Positive Results:

Sample contains \geq 20 ppb of aflatoxin.



- (4) If no control line appears, the test is invalid. Repeat the procedure with a new test strip.

Invalid Results: Test is invalid.

Repeat with new Strip Test.



SUPPLEMENTAL ANALYSIS

N/A

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@usda.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage conditions:

Store test kits refrigerated at 4° to 8°C (39° to 46°F) when not in use.

b. Precautions:

- (1) Strip tests and sample extract should be at room temperature (64° to 86° F) when used.
- (2) Follow the test instructions issued by GIPSA.
- (3) Do not run test in a location where air from an air conditioner, heater, or window will blow directly on the strips.
- (4) A control line must be visible on the strip regardless of the test result.
- (5) The color of the test line and/or control line may vary in intensity. Regardless of whether the line is light or dark, it should be interpreted the same way.
- (6) Store test strips in the original tube capped when not in use.

EQUIPMENT AND SUPPLIES

a. Materials supplied with the kit (VICAM part # 100000173):

- (1) 25 AflaCheck Strip Tests (VICAM part # 100000175)

- (2) 50 Strip Test pipettors (VICAM part # 600000812)
- (3) 25 Strip Test dilution tubes (VICAM part # 600000813)
- (4) 1 paper test rack

b. Materials required but not supplied with the kit:

- (1) Wide mouth bottles, 500 mL (Lab Safety Supply part # 9HUH9 or equivalent)
- (2) Graduated cylinder, 250mL (VICAM part # 20250)
- (3) Digital Timer (VICAM part # G4036)
- (4) Pre-mixed 70% methanol solution (VICAM part # 100000217) **or** ACS reagent-grade or HPLC grade methanol for making 70% methanol solution
- (6) Distilled water
- (7) Digital Scale (VICAM part # 20100)

REVISION HISTORY

Revision 0 (07/15/2015)