

CHAPTER 16

AGRASTRIP TEST KIT

<u>Section Number</u>	<u>Section</u>	<u>Page Number</u>
16.1	GENERAL INFORMATION	16-1
16.2	PREPARATION OF EXTRACTION SOLUTION....	16-1
16.3	EXTRACTION PROCEDURES	16-2
16.4	TEST PROCEDURES	16-2
16.5	REPORTING AND CERTIFYING TEST RESULTS.....	16-5
16.6	CLEANING LABWARE	16-5
16.7	WASTE DISPOSAL	16-6
16.8	EQUIPMENT AND SUPPLIES	16-6
16.9	STORAGE CONDITIONS.....	16-7

16.1 GENERAL INFORMATION

The AgraStrip aflatoxin test kits from Romer Labs is a one-step lateral flow strip test that determines a qualitative level of total aflatoxin (B1, B2, G1, and G2) in a corn sample. Test kit #COKAS1000 has a 20 ppb level of detection. Test kit #COKAS1200 has a 10 ppb level of detection. Test procedures for both kits are identical.

16.2 PREPARATION OF EXTRACTION SOLUTIONS

The extraction solvents used in the AgraStrip test methods are

- a. Methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (Reagent grade or better) and 30 percent water
 1. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.
 2. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
 3. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.
 4. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

- b. Ethanol/water (distilled or deionized) mixture consisting of 50 percent ethanol (Reagent grade or better) and 50 percent water
 1. Using a graduated cylinder, measure 500 ml of ethanol and place it into a clean carboy with spigot.
 2. Add 500 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

3. Label the container stating the mixture (50 percent methanol and 50 percent water), date of preparation, and initials of technician who prepared the solution.
4. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 5 parts ethanol to 5 parts of deionized or distilled water.

16.3 EXTRACTION PROCEDURES

- a. Transfer 50 grams of ground sample into an extraction mixing jar.
- b. Add 100 ml of the extraction solvent (70/30 methanol/water or 50/50 ethanol/water).
- c. Cover the extraction jar and shake or blend on high speed for 1 minute.
- d. Remove the cover and filter the extract through Whatman #1 filter paper into a sample jar labeled with the sample identification.
- e. After collecting the filtrate dispose of the filter and ground material and set the filtrate aside for testing.

16.4 TEST PROCEDURES

- a. Sample Analysis.
 - (1) Break off one well per sample to be tested and place the microwells in a microwell holder. Reseal the unused microwells.
 - (2) Using a single channel pipette, add 50 microliters (μ l) of diluting solution (Blue capped bottle labeled: assay diluent) to each microwell.



- (3) Dissolve the coating conjugate in the microwell by pipetting the contents up and down 5 times.
- (4) Using a new tip for each sample, add 50 μ l of the sample extract to the corresponding microwell and mix the content by pipetting up and down 3 times.
- (5) Place a new aflatoxin test strip with the sample end down into the microwell.



- (6) Allow the strip to develop color for 5 minutes.
- (7) At the end of the 5 minute development period, remove the strip from the sample cup and interpret the results immediately.

b. Interpreting the Lateral Flow Test Strip.

Development of a Control Line within 5 minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded. A second preparation of the extract (using a fresh dilution) should be made and tested using another strip.

(1) Negative Result (\leq Level of detection).

A sample containing aflatoxin residues less than or equal to level of detection will develop 2 distinct lines, the Control Line and the Test Line, in the test area. A negative sample may be determined as soon as 2 lines are visible on the strip.



(2) Positive Result ($>$ Level of Detection).

A sample containing aflatoxin residues in excess of the level of detection will develop 1 distinct line, the Control Line.



(3) Invalid Test Result

Discard result and rerun with new sample extract.



16.5 REPORTING AND CERTIFYING TEST RESULTS

- a. Report results on the pan ticket and inspection log as being equal to or less than the level of detection for the kit (Example: ≤ 20 ppb or ≤ 10 ppb), or as exceeding the level of detection (Example: > 20 ppb or > 10 ppb), as applicable.
- b. Certify results as being equal to or less than the level of detection for the kit (Example: ≤ 20 ppb or ≤ 10 ppb), or as exceeding the level of detection (Example: > 20 ppb or > 10 ppb), as applicable.
- c. Verify level of detection by checking test kit part numbers.

10 ppb Test Kit Part # COKAS1200

20 ppb Test Kit Part # COKAS1000

- d. Refer to the Certification section of the handbook for more detailed certification procedures.

16.6 CLEANING LABWARE

- a. Negative Tests (≤ 20 ppb or ≤ 10 ppb.).

- (1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used extraction mixing jars, wash thoroughly, and then rinse with clean water before reusing.

- (2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb or > 10 ppb.).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used extraction mixing jars and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, and then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used test strips and pipettes cuvettes, for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

16.7 WASTE DISPOSAL

a. Negative Results.

If the test result is negative (either test kit), dispose of any remaining liquid filtrate in the chemical waste container. Discard the sample slurry (ground material) into a plastic garbage bag for disposal.

b. Positive Results.

If the result is positive (either test kit), the slurry (ground portion) remaining in the sample extraction jar must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, pour approximately 50 ml of bleach solution into the sample extraction jar and shake to mix with the sample slurry. After the slurry and bleach solution separate, handle the bleach rinse filtrate as a non-hazardous solution and dispose of by pouring the liquid down the drain. Discard the sample slurry (ground portion) paper into a plastic garbage bag for disposal.

16.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

- (1) 1 tube containing 24 aflatoxin test strips.

- (2) 1 tube containing 24 microtiter wells coated with antibody-particle complex.
- (3) 1 bottle of 1.8 ml assay diluent.

b. Materials Required but not Provided:

- (1) Timer (5 minute capacity).
- (2) 50 μ l pipettor with tips.
- (3) Microwell rack.
- (4) Balance.
- (5) Sample Grinder.
- (6) Methanol - ACS grade or better.
- (7) Ethanol – ACS grade or better.
- (8) Deionized or Distilled Water.
- (9) Blender with mixing jars.
- (10) Whatman #1 Filter Paper.

16.9 STORAGE CONDITIONS

a. Storage Conditions.

- (1) Store kit components at 36°- 77°F when not in use to assure full shelf life and do not use beyond the expiration date
- (2) Do not freeze test strip components.

b. Precautions.

- (1) Test strips and microwells must be kept inside their original tubes.
- (2) All reagents must be at room temperature (64-86 °F) before use.
- (3) The components in the test kit are quality control tested as a standard batch unit. Do not mix components from different lot numbers.