

CHAPTER 5

AFLACUP TEST KIT

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5.1 GENERAL INFORMATION

The AflaCup test kit is an enzyme linked immunosorbent assay (ELISA) developed by International Diagnostics Systems Corporation and marketed by Romer Labs. As the name implies, antibodies which react specifically with aflatoxins are contained in a cup. The test provides qualitative (less than or equal to a specified threshold) results.

5.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the AflaCup test method is a methanol/water (distilled or deionized) mixture consisting of 80 percent methanol (Reagent grade or better) and 20 percent water.

- a. Using a graduated cylinder, measure 800 ml of methanol and place it into a clean carboy with spigot.
- b. Add 200 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- c. Label the container stating the mixture (80 percent methanol and 20 percent water), date of preparation, and initials of technician who prepared the solution.
- d. 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 8 parts methanol to 2 parts of deionized or distilled water.

5.3 EXTRACTION PROCEDURES

- a. Transfer 50 grams of ground sample into an extraction mixing jar.
- b. Add 100 ml of the (80/20) methanol/water extraction solvent.
- c. Cover the extraction jar and blend on high speed for 1 minute.
- d. Remove the cover and funnel the extract through a Whatman No.1 filter or a coffee filter into a sample jar labeled with the sample identification.
- e. After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container.

5.4 REAGENT CHECK

a. Stabilization.

Prior to performing the test, allow one hour for all reagents to reach room temperature (73E- 84EF).

b. Testing Reagents.

Each day, before testing official samples, test at least one negative control cup to ensure that all reagents are functional.

Use the following procedures to test the control:

- (1) Apply 2 drops of negative control (green cap) to the center of the AflaCup.
- (2) Using a timer, allow the cup to set for a 1-minute reaction time.
- (3) Apply 2 drops of the aflatoxin enzyme (red cap) to the center of the cup.

Note: The enzyme solution may only be used with the antibody-coated cups contained in the same test kit.

- (4) Using a timer, allow the cup to set for a 1-minute reaction time.
- (5) Wash with 30 drops of the Wash Solution (white cap). When using more than one AflaCup, wash each cup with 3 series of 10 drops per cup.
- (6) Prepare fresh Substrate Solution in a small test tube by mixing 10 drops of Substrate Solution A (yellow cap) with 10 drops of Substrate Solution B (blue cap) for each AflaCup.

(Do not combine Substrate Solution A with Substrate Solution B more than 10 minutes before use.)

Note: If a blue color develops immediately after combining Substrates A and B, repeat this step. If the problem persists call Romer Labs for technical assistance.

- (7) Add the entire contents of the Substrate Mix from each test tube to each test cup in use.
- (8) Using a timer, allow the cup to set for a 1-minute reaction time.
- (9) Immediately read and interpret the result.

c. Interpreting Results.

A blue color indicates the reagents are functional. If the color remains white for at least one minute the reagents are not functional and must be replaced.

5.5 TEST PROCEDURES

a. Procedures for a 20 ppb cut-off.

- (1) Transfer 200 microliters (μl) of the dilution buffer to a culture tube (12x 75 mm) and then add 100 μl of the filtered extract.
- (2) Mix well and slowly apply 100 μl of the mixture to the center of the AflaCup.
- (3) Using a timer, allow the cup to set for a 1-minute reaction time.
- (4) Apply 2 drops of the aflatoxin enzyme (red cap) to the center of the cup.

Note: The enzyme solution may only be used with the antibody-coated cups contained in the same test kit.

- (5) Using a timer, allow the cup to set for a 1-minute reaction time.
- (6) Wash with 30 drops of the Wash Solution (white cap). When using more than one AflaCup, wash each cup with 3 series of 10 drops per cup.
- (7) Prepare fresh Substrate Solution in a small test tube by mixing 10 drops of Substrate Solution A (yellow cap) with 10 drops of Substrate Solution B (blue cap) for each AflaCup.

(Do not combine Substrate Solution A with Substrate Solution B more than 10 minutes before use.)

Note: If a blue color develops immediately after combining Substrates A and B, repeat this step. If the problem persists call Romer Labs for technical assistance.

(8) Add the entire contents of the Substrate Mix from each test tube to each test cup in use.

(9) Using a timer, allow the cup to set for a 1-minute reaction time.

b. Interpretation of Test Results.

(1) Negative (equal to or less than 20 ppb).

The sample is considered equal to or less than 20 ppb when the cup color changes to blue.

Note: Color will be concentrated in the center of the cup.

(2) Positive (Greater than 20 ppb).

The sample is considered greater than 20 ppb when the cup color remains white for at least one minute.

5.6 REPORTING AND CERTIFYING TEST RESULTS

- a. Report results on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold.
- b. Certify results as being equal to or less than a threshold.
- c. Refer to the Certification section of the handbook for more detailed certification procedures.

5.7 CLEANING LABWARE

a. Negative Tests (# 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, 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).

(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 glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, 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 columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

5.8 WASTE DISPOSAL

a. Negative Results (# 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

5.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits:

- (1) Cups with Aflatoxin Antibody attached.
- (2) Aflatoxin Enzyme, Dropper Bottle.
- (3) Negative Control Solution, Dropper Bottle.
- (4) Wash Solution, Dropper Bottle.
- (5) Substrate A, Dropper Bottle.
- (6) Substrate B, Dropper Bottle.
- (7) Dilution Buffer for Samples, Translucent.

b. Materials Required but not Provided:

- (1) Sample grinder.
- (2) Balance.

- (3) Methanol - Reagent grade or better.
- (4) Distilled or deionized water.
- (5) Blender with mixing jars.
- (6) Cuvette rack.
- (7) Pipettor and tips - 100 to 1000 μ l adjustable.
- (8) 100 ml graduated cylinder.
- (9) Funnel.
- (10) Timer.
- (11) Whatman No.1 Filter Paper or Coffee Filters.
- (12) Glass cuvettes (12 x 75 mm).

5.10 STORAGE CONDITIONS

Test kits should be refrigerated between 36E- 46EF.