

CHAPTER 18

CHARM SCIENCES, INC. - ROSA® AFLATOXIN P/N TEST KIT

<u>Section Number</u>	<u>Section</u>	<u>Page Number</u>
18.1	GENERAL INFORMATION	18-1
18.2	PREPARATION OF EXTRACTION SOLUTION.....	18-1
18.3	PREPARATION OF TESTING MATERIALS.....	18-2
18.4	EXTRACTION PROCEDURES.....	18-3
18.5	TEST PROCEDURES.....	18-4
18.6	REPORTING AND CERTIFYING TEST RESULTS.....	18-8
18.7	CLEANING LABWARE	18-8
18.8	WASTE DISPOSAL	18-9
18.9	EQUIPMENT AND SUPPLIES	18-10
18.10	STORAGE CONDITIONS.....	18-11

18.1 GENERAL INFORMATION

The ROSA® Aflatoxin P/N test kit uses lateral flow test strip technology that provides qualitative (equal to or less than a specified threshold) results. This kit can be used to screen at 10 ppb or 20 ppb aflatoxin.

18.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the ROSA® Aflatoxin P/N test method is either a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (Reagent grade or better) and 30 percent water or 50 percent ethanol (Reagent grade or better) and 50 percent water.

NOTE: When screening at the 10 ppb level only Methanol/Water may be used as the extraction solution.

a. Methanol/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

- (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 ethanol and shake vigorously until it is completely mixed.
- (3) Label the container stating the mixture (50 percent ethanol 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 1 part ethanol to 1 part deionized or distilled water.

18.3 PREPARATION OF TESTING MATERIALS

NOTE: A Negative and Positive Control should be run periodically using the Performance Monitoring Mode (see section 18.5 d.) to verify performance of equipment and test strips (daily, weekly, bi-weekly, or monthly, based on internal quality assurance standards).

a. Negative Control.

Add 100 µl of 70% methanol or 50% ethanol solution to 1.0 ml of AFQ Buffer to prepare Negative Control Diluted Extract.

b. Positive Control (20 ppb).

- (1) Reconstitute with 3.0 ml dilution buffer and mix for 30 seconds.
- (2) Add 300 µl 70% methanol or 50% ethanol and mix for 30 seconds. Mix again before use.

NOTE: Store at 32-45 °F for up to one week, or freeze at -4 °F for 2 months.

c. Equipment Preparation.

- (1) Incubator must be at 45±1°C (temperature indicator is green).
- (2) Incubator must be clean and level.

d. AFQ Dilution Buffer.

- (1) Predispense 1.0 ml of AFQ Dilution Buffer into a micro-centrifuge tube for each sample to be tested.
- (2) Use this solution at room temperature.
- (3) Store AFQ Dilution Buffer and any unused predispensed tubes at 32-45 °F.

e. Test Strips.

- (1) Remove ROSA® Aflatoxin P/N moisture resistant container from the refrigerator and allow it to reach room temperature to limit condensation.
- (2) Remove only the number of strips to be used and return container to 32-45 °F storage. Strips are stable at room temperature for at least 12 hours.

NOTE: If blue desiccant packets turn white or pink, performance test the strips with Negative and Positive Controls before continued use.

18.4 EXTRACTION PROCEDURES

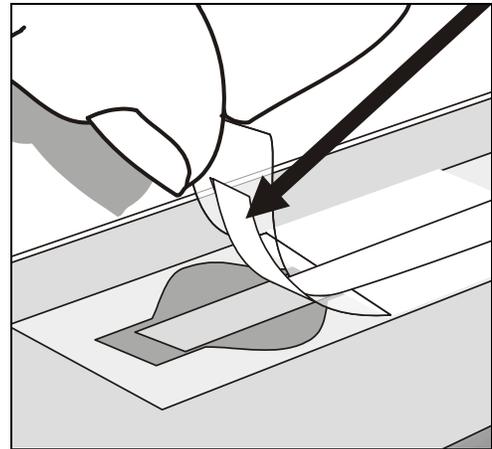
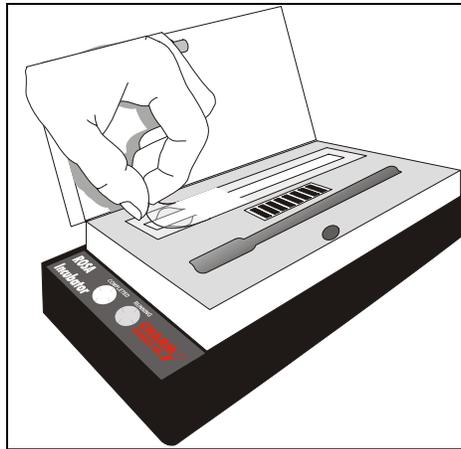
- a. Transfer 50grams of ground sample into a clean extraction container.
- b. Add 100 ml of the (70/30) methanol/water or (50/50) ethanol extraction solvent.
- c. When screening at the 20 ppb level shake for at least 60 seconds. Allow sample to settle for 1 minute to obtain sample extract.

NOTE: If particles are present after settling, filter or centrifuge to clarify sample extract. **To Filter:** pour the extract through Whatman 2V (or equivalent) filter paper and filter into a labeled collection container. **To Centrifuge:** transfer 1.0-1.5 ml of sample extract to a labeled micro-centrifuge tube and centrifuge for 10 seconds. Clarified extract is now ready for testing.

18.5 TEST PROCEDURES

a. Sample Preparation.

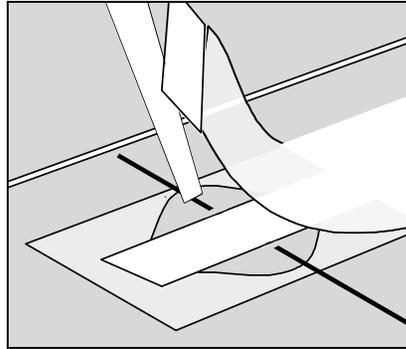
- (1) Pipet 100 μ l of clarified extract to a predisposed (1.0 ml AFQ Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract.
- (2) Label the test strip to identify sample.
- (3) Open the incubator lid and place test strip in the ROSA-M Incubator with the flat side facing upward.
- (4) While holding the strip flat on the incubator, use tab to peel tape back to the indicated line exposing the sample pad. Avoid bending back the white wick and sponge under the tape.



b. Sample Analysis.

- (1) Pipet 300 μ l of diluted extract into the side of sample compartment at the position indicated by the silver line on the incubator.

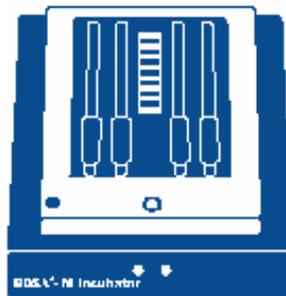
NOTE: Pipet very slowly.



- (2) Reseal the tape over the sample pad compartment. When testing multiple samples, complete the peel, pipet, and reseal steps on each strip before going to the next strip.

NOTE: Add diluted extract to all strips within 1 minute. If a quad incubator is used, 4 samples can be incubated simultaneously.

- (3) Close lid on the incubator and tighten the latch. The solid red timer light will automatically start when the lid is closed.



LF-INC4-45D: Quad incubator, 3-minute timer with display, set for 45° C for Test Strips

- (4) Incubate for 3 minutes. After the incubation step is complete, a beeper will sound and the yellow “test complete” light will begin to flash.
- (5) Remove strips and interpret the results. **Strips must be removed from the incubator and read within 2 minutes of incubation completion.** After strip removal, lower but do not latch the incubator lid.

c. Visually Interpreting the Lateral Flow Test Strip.

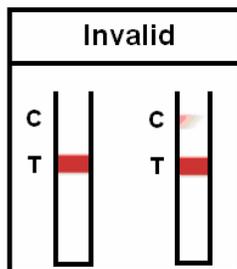
Development of a Control Line indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded. A second preparation of the extract (using a fresh dilution) should be made and tested using another strip.

Note: When screening at the 10 ppb level only the ROSA-M reader must be used.

Note: The examples shown below depicting invalid, negative, and positive results is for illustration purposes only. Do not use these color bars as actual intensity measurement for determining if the sample is positive or negative.

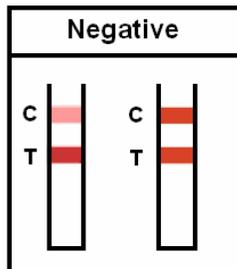
(1) Invalid Result.

A test is invalid if a Control Line is missing, smeared, or uneven, or if the Test Line is uneven. It is invalid if the diluted extract is obscuring either the Control (C) or Test Line (T).



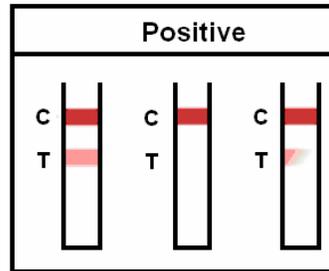
(2) Negative Result.

A sample containing aflatoxin residues less than or equal to 20 ppb will develop a Test Line that is darker or equal in intensity to the Control Line in the test area.



(3) Positive Result.

A sample containing aflatoxin residues in excess of 20 ppb will develop a Test Line that is lighter in intensity than the Control Line.



d. Interpreting the Lateral Flow Test Strip using the ROSA-M Reader.

NOTE: Periodically enter Performance Mode in reader by selecting AFLA channel and pressing ESC and then 5. Follow reader prompts. Run each of the calibration strips to verify reader performance. Strips must test within +/- 2 ppb of the average listed on the strips.

- (1) Insert a clean valid test strip into the ROSA-M Reader. Slide the strip into the slot, with the sample compartment in the up position, until it stops.



LF-ROSA READER-M: ROSA-M Reader supplied with calibrators.

- (2) Read result on **AFLA** Channel (2-Line Mode) of the ROSA-M Reader with **Matrix 01** for screening level 10 ppb and **Matrix 00** for screening level 20 ppb. If desired, enter **Sample** and/or **Operator**. Press **ENTER** to read.
- (3) **Result:** The ROSA-M Reader interprets the strip and displays either **NEGATIVE** or **POSITIVE**.

18.6 REPORTING AND CERTIFYING TEST RESULTS

- a. Report results on the pan ticket and inspection log as being equal to or less than the screening level (Example: ≤ 20 ppb or ≤ 10 ppb), or as exceeding the screening level (Example: > 20 ppb or > 10 ppb), as applicable.
- b. Certify results as being equal to or less than the screening level (Example: ≤ 20 ppb or ≤ 10 ppb), or as exceeding the screening level (Example: > 20 ppb or > 10 ppb), as applicable.
- c. Refer to the Certification section of the handbook for more detailed certification procedures.

18.7 CLEANING LABWARE

- a. Negative Tests (≤ 20 ppb when screening at 20 ppb or ≤ 10 when screening at 10 ppb).
 - (1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used extraction mixing jars, 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 (Exceeds screening level - Example: > 20 ppb when screening at 20 ppb and > 10 when screening at 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, 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, for at least 5 minutes.

Pour off the liquid down the drain and place the materials in a garbage bag and discard.

18.8 WASTE DISPOSAL

a. Negative Results (\leq Screening Level).

If the test result is negative (equal to or less than screening level), 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 ($>$ Screening Level).

If the result is positive (more than screening level), 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.

18.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits.

Kits can be purchased that contain 20, 100, or 500 strips and include Control and AFQ Dilution Buffer.

(1) LF-APN-20 –

- (a) 1 package containing 20 ROSA® Aflatoxin P/N strips packed in a moisture-resistant container.
- (b) 1 Aflatoxin B1 20 ppb Control.
- (c) 1 AFQ Dilution Buffer

(2) LF-APN-100 –

- (a) 1 package containing 100 ROSA® Aflatoxin P/N strips packed in a moisture-resistant container.
- (b) 1 Aflatoxin B1 20 ppb Control.
- (c) 1 AFQ Dilution Buffer.

(3) LF-APN-500 –

- (a) 5 packages containing 100 ROSA® Aflatoxin P/N strips packed in a moisture-resistant container.
- (b) 5 Aflatoxin B1 20 ppb Controls.
- (c) 5 AFQ Dilution Buffers.

b. Materials Required but not Provided:

- (1) Sample grinder.
- (2) Balance.
- (3) Methanol or Ethanol - Reagent grade or better.

- (4) Deionized or Distilled water.
 - (5) Sample extraction containers.
 - (6) 1.0 ml pipettor and pipette tips.
 - (7) 300 µl pipettor and pipette tips.
 - (8) 100 µl pipettor and pipette tips.
 - (9) 100 ml graduated cylinder.
 - (10) 1.5 ml micro-centrifuge tubes.
- c. Optional Equipment and Supplies:
- (1) Mini-centrifuge.
 - (2) Whatman 2V filter paper or equivalent.
 - (3) Filter funnel.

18.10 STORAGE CONDITIONS

a. Storage Conditions.

Test kits should be refrigerated between 32°- 45°F.

b. Precautions.

- (1) Do not use the test kits beyond the noted expiration date.
- (2) Prolonged exposure to high temperature and/or high humidity may adversely affect the test results.
- (3) Do not open the desiccated canister until ready to use the strips.