

CHAPTER 17

VERATOX AFLATOXIN TEST KIT

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17.1 GENERAL INFORMATION

The Veratox aflatoxin test is for the quantitative analysis of aflatoxin in select grains and commodities. **The test kit is limited to providing aflatoxin measurements between 5 – 50 ppb.** Accurate aflatoxin measurements above 50 ppb can be obtained by performing a supplemental analysis involving a diluted extract.

17.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the Veratox test is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

- a. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.
- b. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- c. Label the container stating the mixture (70 percent methanol and 30 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 7 parts methanol to 3 parts of deionized or distilled water.

17.3 EXTRACTION PROCEDURES

- a. Transfer 50 grams of ground sample into an extraction mixing jar.
- b. Add 250 ml of the (70/30) methanol/water extraction solvent.
- c. Cover the extraction jar and blend on high speed for 2 minutes.
- d. Pour the extract through filter paper or a filtering syringe into a sample jar labeled with the sample identification. Collect a minimum of 5 ml of the extract.

17.4 TEST PROCEDURES

a. Sample Analysis.

- (1) Allow reagents, microwells, and sample extracts to reach room temperature prior to running the test.
- (2) Remove one red-marked mixing well for each sample to be tested, plus four red-marked wells to be used for controls. Place these wells in the microwell holder.
- (3) Remove an equal number of antibody-coated wells. Immediately return antibody wells that will not be used to the foil pack with desiccant. Reseal the foil pack to protect the antibody. Mark one end of the strip so that the wells can be identified after washing.
- (4) Mix each reagent by swirling the reagent bottle prior to use.
- (5) Place 100 μ l of conjugate from the blue-labeled bottle in each red-marked mixing well.
- (6) Using a new pipette tip for each, transfer 100 μ l of controls and sample extracts into the red-marked mixing wells as shown below:

Test Strip

Mixing Well #	1	2	3	4	5	6	7	8	9	10	11	12
Antibody Well #	1	2	3	4	5	6	7	8	9	10	11	12
Sample	C0	C5	C15	C50	S1	S2	S3	S4	S5	S6	S7	S8

Where C 0 is the zero control, C 5 is the 5 ppb control, C 15 is the 15 ppb control, and C 50 is the 50 ppb control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

NOTE: Do not run more than 20 samples per set of control standards.

- (7) Using a 12-channel pipettor, mix the liquid in the wells by pipetting the liquid up and down in the tips 3 times.

- (8) Transfer 100 μ l to the antibody coated wells.
- (9) Mix by sliding the microwell holder back and forth on a flat surface for 10-20 seconds without splashing reagents from the wells.
- (10) Incubate for **2 minutes** at room temperature 18-30°C (64-86°F). Discard the red-marked mixing wells.
- (11) Shake out the contents of the antibody wells.
- (12) Fill each antibody well with deionized or distilled water and then dump the water out. Repeat this step 5 times.
- (13) Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.
- (14) Pour the needed volume of substrate from the green-labeled bottle into the green-labeled reagent boat. With new tips on the 12-channel pipettor, prime and pipette 100 μ l of substrate into the wells and mix by sliding back and forth on a flat surface for 10-20 seconds.
- (15) Incubate for **3 minutes**. Discard the remaining substrate and rinse the reagent boat with water.
- (16) Pour the stop solution from the red-labeled bottle (same volume as prepared for substrate) into the red-labeled reagent boat.
- (17) Eject the excess substrate from the 12-channel pipettor, prime the tips, and pipette 100 μ l of the stop solution to each well. Mix by sliding back and forth on a flat surface for 10-20 seconds. Discard the pipette tips.
- (18) Wipe the bottom of the microwells with a dry cloth or towel and read in the Stat-Fax 321 Plus or BioTek EL301 reader using a 650-nm filter. Air bubbles should be eliminated, as they could affect analytical results.

(Results should be read within 20 minutes of completion of the test.)

b. Reading Results with the Microwell Reader.

(1) Biotek EL 301 Microwell Reader.

- (a) Make sure that the microwell reader is on and allowed to warm-up for a minimum of 15 minutes before using.
- (b) Remove sample carriage and hit "Enter."
- (c) Insert W2 filter and hit "Enter."
- (d) Insert W1 filter (650 nm) and hit "Enter."
- (e) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.
- (f) Load microwells into sample carriage so that the first control labeled 0 is in position A1.
- (g) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.
- (h) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.
- (i) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.
- (j) Repeat step (i) until absorbance values have been obtained for all controls and samples. Record the values.
- (k) Use the data reduction software provided by Neogen to convert the absorbance values into concentration values.

(2) Stat-Fax Model 321 Plus Microwell Reader

- (a) To begin from the "Ready" prompt, press Menu, key in the test number, and then press Enter. For aflatoxin, the Veratox test number is 2.
- (b) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

- (c) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test.

- (d) Record the results for each sample along with the correlation coefficient, slope, and y-intercept on the data sheet.

NOTE: If the correlation coefficient is less than 0.98 or if the slope exceeds -2.0 ± 0.5 , the Stat-Fax reader will print "Invalid Calibration" and no results will be reported. If the slope value consistently reads outside these tolerances, contact Neogen as soon as possible to report these findings.

17.5 REPORTING AND CERTIFYING TEST RESULTS

- a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.
- b. Sample results over 50 ppb are reported as >50 ppb unless a supplemental analysis is performed.
- c. Refer to the Certification section of the handbook for more detailed certification procedures.

17.6 SUPPLEMENTAL ANALYSIS

- a. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 50 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 50 ppb, the sample extract must be diluted so that a value between 5 and 50 ppb is obtained.

The sample value must then be multiplied by the appropriate dilution factor so that the true concentration of the original sample is obtained.

For example, if the extract is diluted 1:2 with 70% methanol/water (1.0 ml of extract added to 2.0 ml of 70% methanol/water), the dilution factor is 3. Similarly, if the extract is diluted 1:3 with 70% methanol/water (1.0 ml of extract added to 3.0 ml of 70% methanol/water), the dilution factor is 4.

b. Example.

If the original analysis reported the aflatoxin value at 70 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

- (1) Dilute 5 ml of the original extract (section 17.3, step d) with 5 ml of the extraction solvent mixture. The total volume is 10 ml. This is a 1 to 2 dilution (compares volume in the beginning with the total volume in the end).
- (2) Proceed to sample analysis.
- (3) Multiply the analytical results obtained by 2 to obtain the actual aflatoxin concentration. For example, if 34 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 68 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

$$\begin{aligned} \text{True Aflatoxin Value} &= (10 \div 5) \times 34 \text{ ppb} \\ &= 2 \times 34 \text{ ppb} = 68 \text{ ppb} \end{aligned}$$

17.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, 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, 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 the liquid down the drain and place the materials in a garbage bag and discard.

17.8 WASTE DISPOSAL

a. Negative Results (\leq 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the syringe into a plastic garbage bag for disposal.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the remaining ground portion must be decontaminated, using bleach, prior to disposal. Discard the filter syringe and remaining 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.

17.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

(1) 48 antibody coated microwells.

(2) 48 red-marked mixing wells.

- (3) 4 yellow-labeled bottles each containing 1.5 ml aflatoxin controls. Control bottles are 0, 5, 15, and 50 ppb.
- (4) 1 blue-labeled bottle of 7 ml aflatoxin-HRP conjugate solution.
- (5) 1 green-labeled bottle of 24 ml K-Blue substrate solution.
- (6) 1 red-labeled bottle of 32 ml Red Stop solution.

b. Materials Required but not Provided:

- (1) Methanol - ACS grade or better.
- (2) Deionized or Distilled Water.
- (3) 250 ml graduated cylinder.
- (4) 125 ml container.
- (5) Whatman #1 filter paper, Neogen filter syringes, or equivalent.
- (6) Filter funnel.
- (7) Sample collection tubes.
- (8) High-speed blender with a one liter jar.
- (9) Sample grinder.
- (10) Balance.
- (11) Biotek EL 301 or Stat-Fax Model 321 Plus Microwell reader equipped with a 650-nm filter.
- (12) 12-channel pipettor.
- (13) 100 μ l Pipettor and pipette tips.
- (14) Paper towels, Kaydry paper or equivalent absorbent material.
- (15) Microwell holder.
- (16) Waste receptacle.

- (17) Timer: 3 channel minimum.
- (18) Waterproof marker, Sharpie or equivalent.
- (19) 250 ml plastic squeeze wash bottle.
- (20) 2 reagent boats for 12-channel pipettor.

17.10 STORAGE CONDITIONS

The test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 35° F and 46° F. **(DO NOT FREEZE)**