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GRAIN INSPECTION, PACKERS AND STOCKYARDS
ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
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DON HANDBOOK
CHAPTER 8
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CHAPTER 8

ROMER - ACCUTOX™ DON TEST KIT

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8.1 GENERAL INFORMATION

The extraction solution and other materials used in the AccuTox™ test kit do not necessitate the use of a separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

8.2 EXTRACTION PROCEDURES

- a. Place 50-grams of ground sample into a clean plastic or glass container.
- b. Add 250 milliliters (ml) of distilled or deionized water.
- c. Seal or cover the mixing container and shake (by hand or mechanically) for 3 minutes.
- d. Allow the sample residue to settle (2 to 3 minutes) before obtaining a 0.5 ml test sample.

Or

- e. Unseal or remove the cover from the container and pour the extract through filter paper (standard coffee filters or Whatman No.1) into a sample jar labeled with the sample identification, before obtaining 0.5 ml test sample.

8.3 TEST PROCEDURES

- a. Sample Analysis.
 - (1) Allow reagents, antibody coated tubes, and sample extracts to reach room temperature (68 - 77° F) prior to running test (approximately 1 hour).
 - (2) Place the appropriate number of labeled antibody-coated tubes into the gripper tube rack. Reseal the unused tubes in a zip-lock bag with desiccant.
 - (3) Pipette 0.5 ml of the calibrators, and sample extracts directly into the bottom of the antibody coated tubes without touching the sidewalls, or splashing. Completely discharge the pipette by depressing the plunger with the thumb to the second stop (all the way down).

- (4) Pipette 0.5 ml of the enzyme conjugate into each tube. The conjugate must be pipetted without splashing. Start a timer set for 15 minutes upon completion.
- (5) Shake the rack using a circular motion for approximately 5 seconds.
- (6) At the completion of the 15-minute incubation period, dump the contents of the tubes into the appropriate waste container. Fill the tubes to overflowing and rinse with the wash solution. Invert and gently tap to empty the tubes of wash solution. Repeat this process three more times for a total of four washes. (It is very important not to under-wash the tubes, over washing will not affect the test).
- (7) Following the last wash, invert the tubes and forcibly tap onto absorbent paper several times to completely remove all of the wash solution. (It is important to remove as much wash solution as possible).
- (8) Pipette 0.5 ml of the substrate into each tube. The substrate must be pipetted (completely releasing the thumb after each addition) without splashing. Start a timer set for 5 minutes as soon as the substrate has been added to the first tube. Swirl the rack in a circular motion for approximately 5 seconds to mix. Solutions should all turn to a blue color after substrate has been added.
- (9) At completion of the 5 minute incubation period, add 0.5 ml of stop solution to each tube, pipetting without splashing. Swirl the rack in a circular motion for approximately 5 seconds to mix. All solutions should turn to a yellow color after adding the stop solution.
- (10) Make sure that the spectrophotometer is set at 450 nm. Run a blank with a clean, unscratched test tube filled with fresh distilled or deionized water.
- (11) Zero the spectrophotometer prior to reading the tubes. Make sure that there are no air bubbles in the blank tubes before zeroing.
- (12) Wipe each tube with a lint free towel before reading and allow a few seconds for the spectrophotometer reading to stabilize before printing the absorbance level reading.
- (13) Read and record absorbance levels of the calibrator, control, and samples.
- (14) Calculate results using log/logit data computer program with user curve generated during test run.

b. Spectrophotometer Calibration Procedure.

- (1) Ensure that the spectrophotometer is connected to a computer.
- (2) Turn on the spectrophotometer by using the "ON/OFF" key. After turning on the power, the spectrophotometer goes through a self-check

Note: The lid must be closed.

- (3) Using the dial on the right side of the spectrophotometer, dial in 450 nm.
- (4) The spec will then display "Enter Program #." At this time press "0" and the "Enter" button.
- (5) "P O" will then be displayed. Take the blank tube filled half way with distilled or deionized water and place into the well. Cover the tube with the small cylinder cover supplied.
- (6) Press the key labeled "Zero."
- (7) Open the HachLink™ program or start a new file. Enter the operator name and sample ID for the first reading (ppm standard).
- (8) Insert the 0 ppm standard and press "SHIFT" then "PRINT/4". The value will appear in the HachLink™ software.
- (9) Insert the next tube, enter the sample ID, information, and press "SHIFT", then "PRINT/4". Repeat until all standards and samples have been read.
- (10) Go to the Edit menu of the software, and press "Copy All" to capture the entire data field.
- (11) Go to the AccuTox® Excel Spreadsheet and place the cursor on the field labeled DATE in Section 2. Paste the copied field from the HachLink™ into the Excel spreadsheet.
- (12) The appropriate standard values from the user-generated curve must be entered into the proper calibration fields in Section 1. Verify that the calibration curve meets a minimum correlation coefficient [r] of 0.99 or greater.

8.4 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

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

8.5 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

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

$$\begin{aligned} \text{In this example:} \quad \text{True DON Value} &= (15 \div 5) \times 3.1 \text{ ppm} \\ &= 3 \times 3.1 \text{ ppm} = 9.3 \text{ ppm} \end{aligned}$$

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the controls provided with the kits.

8.6 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

8.7 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain. Discard solid material in the trash can for routine disposal.

8.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits:

- (1) Antibody coated tubes.
- (2) Tubes labeled “Blank” (reusable).
- (3) Conjugate.
- (4) Substrate.
- (5) Calibrator standards.
- (6) Stop solution.
- (7) Wash solution.

b. Materials Required but not Provided:

- (1) Hach Spectrophotometer.
- (2) HachLink™ software.
- (3) 500 µl pipettor with tips.
- (4) Gripper test tube rack.

- (5) Timer - 15 minute capacity.
- (6) Plastic squirt bottle for wash solution.
- (7) Sealable plastic bags or plastic/glass containers with tight fitting lids.
- (8) Distilled or deionized water.
- (9) 100 ml graduated cylinder.
- (10) Filter paper (standard coffee filters or Whatman No.1).
- (11) Sample grinder.
- (12) Balance.

8.9 STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions.

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

b. Precautions.

- (1) Do not open the desiccant canister until ready to use.
- (2) Do not use the test kits beyond the noted expiration date.
- (3) Prolonged exposure to high temperatures may adversely affect test results.