

CHAPTER 5

NEOGEN - AGRISCREEN and VERATOX DON TEST KITS

<u>Section Number</u>	<u>Section</u>	<u>Page Number</u>
5.1	TESTING AREA	5-1
5.2	EXTRACTION PROCEDURES	5-1
5.3	PREPARATION OF SOLUTIONS	5-2
5.4	QUALITATIVE (SCREENING) TESTING	5-2
5.5	QUANTITATIVE TESTING	5-5
5.6	SUPPLEMENTAL ANALYSIS	5-10
5.7	CLEANING LABWARE	5-10
5.8	WASTE DISPOSAL	5-10
5.9	EQUIPMENT AND SUPPLIES	5-11
5.10	STORAGE CONDITIONS	5-12

5.1 TESTING AREA

The extraction solution and other materials used in the AgriScreen, and Veratox test kits does not necessitate the use of 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.

5.2 EXTRACTION PROCEDURES

a. Barley, Corn, Oats, and Wheat.

- (1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.
- (2) Label the collection container with the sample identification.
- (3) Thoroughly mix the ground sample and weigh a 50-gram portion.
- (4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.
- (5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.
- (6) Let material stand for 2 minutes to enable some of the sample to settle before filtering the extract.
- (7) Filter the extract by pouring at least 15 ml through the filter paper.

b. Malted Barley.

Follow step numbers 1-7 listed above, then pass 3 ml of the filtered extract through a Bond Elut SPE cartridge at a flow rate of 1 ml per minute.

5.3 PREPARATION OF SOLUTIONS

a. Conjugate.

- (1) Open one of the conjugate bottles and remove the rubber stopper.
- (2) Cut the tip off the enclosed squeeze tube and squeeze the tube contents into the bottle.
- (3) Replace the stopper and swirl contents until the pellet has dissolved.

ALLOW THE REHYDRATED CONJUGATE SOLUTION TO SET FOR 1 HOUR PRIOR TO USE.

Use the contents of the bottle until empty (**once rehydrated, contents must be used within 3 weeks**).

KEEP REFRIGERATED WHEN NOT IN USE.

b. Substrate.

Substrate is pre-activated, ready for use, and should be stored in the dark. Remove only one vial of substrate at a time from the foil pouch prior to use.

c. Stopping Reagents and DON Control.

Open the stopping reagents and the DON control bottles and set aside. Swirl to mix prior to use.

5.4 QUALITATIVE (SCREENING) TESTING

a. Testing Procedures.

NOTE: The AgriScreen kit is supplied with a 1 ppm control. Users must purchase another control to perform screening at a different level.

- (1) Remove the red-marked mixing well strip and break off the needed number of wells (one well for each sample and one well for control). Return the unused strip to the package.

NOTE: Do not run more than six wells (five samples plus one control) at a time unless using a multichannel pipettor.

- (2) Remove the antibody-coated well strip and break off the same number of wells. Return the unused strip to the package and tightly close the package opening.
- (3) Mark one end of the antibody-coated well strip with C for control so that you can identify the wells after washing.
- (4) Firmly place a pipette tip on the pipettor and add 100 microliters (μl) of conjugate to each mixing well. Discard the tip.

NOTE: 100 μl of liquid is the amount drawn into the pipette tip when the pipettor plunger is depressed and then released slowly.

- (5) Remove the stopper from the control bottle. Firmly place a new pipette tip on the pipettor and add 100 μl of the control to the first mixing well. Thoroughly mix by depressing the plunger five times. Discard the tip. Replace the rubber stopper on control bottle.
- (6) Firmly place a new pipette tip on the pipettor and add 100 μl from the sample collection tube to second well of the red-marked mixing strip. Thoroughly mix by depressing the plunger five times. Discard the tip.
- (7) Repeat step (6) for each additional sample.
- (8) Transfer 100 μl from each red-marked mixing well to the corresponding antibody-coated well. Use a new pipette tip for each well. Discard the red-marked wells.
- (9) Mix by sliding the wells back and forth on a flat surface in a manner to ensure adequate mixing (10 to 20 seconds) without splashing reagents from wells. **Wait 10 minutes** (begin time after mixing).
- (10) The initial reaction is now completed. Shake out the contents of antibody-coated wells.

- (11) Using a wash bottle, fill each antibody-coated well with distilled/deionized water and shake out. Repeat five times. Remove all water droplets by turning the wells upside down and vigorously tapping wells on a paper towel.
- (12) Firmly place a new pipette tip on the pipettor and add 100 μ l of substrate to each antibody-coated well. Discard the tip.
- (13) Mix as instructed in step (9) and **wait 10 minutes** (begin time after mixing).
- (14) Firmly place a new pipette tip on the pipettor and add 100 μ l of stop solution to each well. Discard the tip. Mix by tapping gently on the side of the antibody well strip.
- (15) Visually determine the levels at 1 ppm or 2 ppm only, or read the results in the Microwell Model EL 301 Strip Reader.

b. Reading the Results.

- (1) Make sure that the Microwell reader is on and allowed to warm up for a minimum of 15 minutes before using.
- (2) Remove the sample carriage and hit "Enter."
- (3) Insert the W2 filter (405 nm) and hit "Enter."
- (4) Insert the W1 filter (650 nm) and hit "Enter."
- (5) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.
- (6) Load the antibody-coated wells into the sample carriage so that the control is in position A1.
- (7) Load the sample carriage into the strip reader so that position A1 is under the reader.
- (8) Hit "Read" and record the value obtained for A1 (the control).

- (9) Slide the carriage to position A2 and hit "Read."
 - (10) If the value is **EQUAL TO** or **LARGER THAN** that recorded for A1, the sample is **LESS THAN** or **EQUAL TO** the control. If the value is **SMALLER THAN** that recorded for A1, the sample contains **MORE THAN** the control.
 - (11) Slide the carriage to the next sample and hit "Read."
 - (12) Repeat step (11) for each of the remaining samples.
- c. Reporting and Certifying Test Results.
- (1) Report results on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 2 ppm) or as exceeding the threshold.
 - (2) Certify results as being equal to or less than a threshold. (See the Certification Chapter of this handbook for detailed procedures and statements).

5.5 QUANTITATIVE TESTING

- a. Testing Procedures.
- (1) Remove the red-marked mixing well strip and break off the number of wells needed (five wells for controls and one for each sample) up to a maximum of twelve. Mark one end of red-marked mixing well strip with a 0 (zero) for the blank and the other end with an S for samples so that you can identify the wells. Place the wells in the well holder.
 - (2) Remove an equal number of antibody-coated wells. Mark one end of strip with a 0 (zero) for the blank and the other end with an S for samples and place the strip in the well holder with the 0 (zero) marked end on the left.
 - (3) Mix each reagent by swirling the reagent bottle prior to use.
 - (4) Firmly place a pipette tip on the 100 μ l pipettor and add 100 μ l of conjugate to each mixing well. Discard the tip.

- (5) Remove the cap from the 0 ppm control bottle. Firmly place a new pipette tip on the 100 μ l pipettor and add 100 μ l from the 0 ppm control bottle to the first (labeled 0 (zero)) mixing well. Discard the tip and replace the cap on the control bottle.
- (6) Remove the cap from the 0.5 ppm control bottle. Firmly place a new pipette tip on the 100 μ l pipettor and add 100 μ l from the 0.5 ppm control bottle to the second mixing well. Discard the tip and replace the cap on the control bottle.
- (7) Repeat step (6) with the remaining control standards placing 100 μ l amounts of these standards in the third, fourth, and fifth wells, respectively. A new pipette tip should be used for each standard solution.
- (8) Firmly place a new pipette tip on the 100 μ l pipette and add 100 μ l from the sample collection tube of the first sample to the sixth well. Discard the tip.
- (9) Repeat step (8) for each sample, placing 100 μ l of extract from each sample in a different well. Use a new pipette tip for each sample solution.
- (10) Using a 12-channel pipettor with new tips, mix the wells by pipetting the liquid up and down in the tips three times. Transfer 100 μ l to the antibody wells.
- (11) Mix by sliding the Microwell holder back and forth on flat surface in a manner to ensure mixing (10-20 seconds) without splashing reagents from wells. **Wait 10 minutes** (begin time after mixing). Discard the red-marked wells.
- (12) The initial reaction is now completed. Shake out the contents of antibody-coated wells.
- (13) Using a wash bottle, fill each antibody-coated well with distilled water and shake out. Repeat five times. Remove all water droplets by turning the wells upside down and vigorously tapping wells on a paper towel.
- (14) Pipette 3 ml of substrate into the reagent boat, and with new tips on the 12-channel pipettor, pipette 100 μ l of substrate into the wells and mix as instructed in step (11). **Wait 10 minutes** (begin time after mixing).

- (15) Discard the remaining substrate and rinse the reagent boat with water.
- (16) Pipette 3 ml of stop solution into the reagent boat. Using the same pipette tips as were used to dispense substrate, add 100 μ l red stop to each well and mix thoroughly as instructed in step (11). Discard the tips.

b. Reading the Results.

NOTE: Connect the Microwell reader to a computer system. For FGIS computers, the computer must have the necessary software installed on the C drive, subdirectory "DON." Perform the following (computer procedures may vary for official agencies depending on how the software is installed).

- (1) Allow the Microwell Model EL 301 Strip Reader to warm up for a minimum of 15 minutes before using.
- (2) Turn on the computer.
- (3) At the C:\> prompt, type in CD\DON and press the "Enter" key.
- (4) At the C:\DON> prompt, type LL and press the "Enter" key.

This will bring the MAIN MENU of the Log/Logit program on the computer screen.

- (5) Type A to select the "Run Log/Logit Program" option.
- (6) At "Please Enter the Number of Standards:" type 5 and press the "Enter" key.
- (7) At "Enter Standard Units:" type ppm and press the "Enter" key.
- (8) At "Standard 2 Concentration:" type the concentration level (e.g., 0.5) and press the "Enter" key.
- (9) At "Standard 3 Concentration:" type the concentration level (e.g., 1) and press the "Enter" key.

- (10) At "Standard 4 Concentration:" type the concentration level (e.g., 2) and press the "Enter" key.
- (11) At "Standard 5 Concentration:" type the concentration level (e.g., 6) and press the "Enter" key.
- (12) If all values are correctly entered, press the F1 key. If they are not, press the E key and follow the instructions on the screen to edit values. When all values are correct, hit the F1 key.

STOP! Do not use the computer keyboard until the samples have been read in step (21).

- (13) On the Microwell reader, remove the sample carriage and press the "Enter" key on the Microwell reader.
- (14) Insert the W2 filter (405 nm) and press the "Enter" key.
- (15) Insert the W1 filter (650 nm) and press the "Enter" key.
- (16) Press "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.
- (17) Load the antibody-coated wells into sample carriage so that the control labeled 0 (zero) is in position A1.
- (18) Load the sample carriage into the strip reader so that position A1 is under the reader.
- (19) Press "Read" and an absorbance value for A1 should appear in the screen on the Microwell reader.
- (20) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear.
- (21) Repeat step (20) until absorbance values have been obtained for all controls and samples.
- (22) Return to the computer keyboard and type in "R." A message appears that tells you to "press data out on reader now!"

- (23) Press the "Data Out" key on the Microwell reader. This will cause the collected data to be transferred to the computer.
- (24) Enter a sample number for each sample and press the "Enter" key.
- (25) When the last sample number is entered, hit the "Enter" key and the calculated ppm for each standard and sample will appear on the screen.
- (26) Record the results for each sample along with the correlation coefficient, slope, and y-intercept data on a data sheet.

NOTE: The correlation coefficient values must read .98 or higher to ensure accurate results. If the correlation value is less than .98, rerun the test. In addition, contact Neogen if the correlation coefficient is consistently below .98. Moreover, the slope value must read -2.0 (+ OR - 0.5).

If the slope value consistently reads outside these tolerances, contact Neogen as soon as possible to report these findings. Do not certify results if the correlation coefficient is less than 0.98 or the slope value is out of tolerance.

c. Reporting and Certifying the 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.

5.6 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.

5.7 CLEANING LABWARE

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

5.8 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.

5.9 EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits:

- (1) monoclonal antibody-coated microwells.
- (2) red-marked mixing wells.
- (3) yellow-labeled bottle(s): DON control.
- (4) blue-labeled bottles: conjugate solution.
- (5) squeeze tubes: hydration solution.
- (6) green labeled bottle: substrate solution.
- (7) red-labeled bottle: stop solution.

b. Materials Required but not Provided:

- (1) Mixing Bags - 18-ounce Nasco Whirlpack bags; Fisher Scientific No. 01-812-6C, or similar type of sealable plastic bag.
- (2) Nalgene funnels - 80 mm top I.D., stem 30 mm, stem O.D. 18 mm; American Scientific Products No. F7465-2.
- (3) Plastic beakers - 250 ml plastic.
- (4) Cylinders - Polypropylene, graduated, 250 ml capacity.
- (5) Carboy - Nalgene, polyethylene, with spigot, 2 gallon capacity; Fisher Scientific No. 02-936-6A.
- (6) Filter paper - 24 cm diameter; Whatman No. 1, or equivalent.
- (7) Timer - 10-minute capacity.
- (8) Markers - Sharpie or equal (permanent ink that will not wash off).
- (9) Absorbent material - Kim wipes or paper towels.

- (10) Wash Bottle 250 ml plastic squeeze bottle.
- (11) Microwell Strip Reader Model EL301.
- (12) IBM Compatible Computer.
- (13) Multichannel Pipettor - TiterTek 12 channel or equivalent.
- (14) Pipettor and Pipette Tips (100 μ l) - Pipetteman, MLA or equivalent.
- (15) Pipettor and Pipette Tips (1ml) - Pipetteman, MLA or equivalent.
- (16) Microwell Holder.
- (17) Deionized or distilled water.
- (18) Strand Sizer or similar type shaking device.
- (19) Whirlpack Bag Rack; Fisher Scientific No. 01-812-5E, or equivalent.
- (20) Bond Elut SPE Cartridge, CN-E, 100 mg/1ml; Varian Sample Preparation Products No. 1210-2007 (For malted barley only).
- (21) Vac Elut 10 with collector rack for 16 x 100 mm test tubes; Varian Sample Products No. 1223-4039 (For malted barley only).
- (22) Balance.
- (23) Sample Grinder.

5.10 STORAGE CONDITIONS

Test kits should be refrigerated at temperatures between 36° F and 46° F.