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DON HANDBOOK
CHAPTER 6
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CHAPTER 6

NEOGEN - VERATOX 5/5 DON TEST KIT

PART NUMBER 8331

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6.1 GENERAL INFORMATION

The extraction solution and other materials used in the Veratox 5/5 test kit (P/N 8331) does 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.

6.2 EXTRACTION PROCEDURES

- a. Standard Extraction Procedure - Testing Wheat, Oats, Barley, Malted Barley, Corn, and Rice at the 0.5 minimum to 5 ppm maximum Conformance Limit.
- (1) Place a sheet of filter paper (Whatman #1 or equivalent) into a clean funnel mounted over a collection container.
 - (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 a 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 the extract sit for 2 minutes to enable some of the sample residue to settle before filtering the extract.
 - (7) Filter the extract by pouring through the filter paper into the labeled sample jar. Collect a minimum of 15 ml of the extract.

OR

Use a filtering syringe and push 1 - 2 ml of the extract through the syringe and collect the filtrate in a clean cuvette.

- (8) Dilute the sample extract 1: 2 (1+1) with deionized or distilled water. For example, add 1.0 ml of extract to 1.0 ml of deionized or distilled water.

- (9) Mix well.
 - (10) Proceed to test analysis steps.
- b. Optional Extraction Procedure - Testing Wheat, Oats, Barley, Malted Barley, Corn, and Rice at the **0.5 minimum to 5 ppm maximum** Conformance Limit.
- (1) Place a sheet of filter paper (Whatman #1 or equivalent) into a clean funnel mounted over a test tube or collection container.
 - (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 suitable size extraction container.
 - (5) Add 500 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 through the filter paper into the labeled sample jar. Collect a minimum of 15 ml of the extract.

OR

Use a filtering syringe and push 1 - 2 ml of the extract through the syringe and collect the filtrate in a cuvette.

- (8) Proceed to the test analysis procedures.

6.3 TEST PROCEDURES

a. Analysis Procedure.

- (1) Allow reagents, antibody coated wells, mixing wells, and sample extracts to reach room temperature (64 – 86° F) prior to running a test. When a test kit is removed from refrigerated storage wait approximately 1 hour before use.
- (2) Remove five red-marked wells to be used for controls, and one red-marked mixing well for each sample to be tested. Place these wells in the microwell holder.

NOTE: The maximum number of test samples that can be run at one time is 19. Using two strips (24 wells), designate 5 wells for the controls and the remainder of the wells for test samples.

- (3) Remove an equal number of antibody-coated wells. Immediately return antibody wells that will not be used to the foil pack with desiccant. Fold down ends of the pack and seal with tape to protect the antibody wells.
- (4) Mark one end of the strip so that the wells can be identified after washing.
- (5) Mix each reagent by swirling the reagent bottle prior to use.
- (6) Using a new pipette tip for each, transfer 100 µl of conjugate from the blue-labeled bottle into each mixing well.
- (7) Using a new pipette tip for each, transfer 100 µl of each control and sample extract into the mixing wells as shown below:

Well #	1	2	3	4	5	6	7	8	9	10	11	12
Sample	C 0	C 0.5	C1.0	C 2.0	C 6.0	S1	S2	S3	S4	S5	S6	S7

Where C 0 is the zero control, C 0.5 is the 0.5 ppm control, C 1.0 is the 1.0 ppm control, C 2.0 is the 2.0 ppm control, and C 6.0 is the 6.0 ppm control. S1 is sample 1, S2 is sample 2, etc.

- (8) Using a 12- channel pipettor, mix the wells by pipetting the liquid up and down in the tips 3 - 4 times. Transfer 100 μ l to the antibody wells and mix by sliding the microwell holder back and forth on a flat surface for 10 – 20 seconds without splashing reagents from the wells. Incubate for **5 minutes** at room temperature (64 –86° F). Discard the red-mixing wells.
- (9) Dump the contents of the antibody wells. With a wash bottle or a running stream, fill each antibody well with deionized or distilled water and then dump the water out. Repeat this step 5 times, then turn the wells upside down and tap out on a paper towel until the remaining water has been removed.
- (10) Pour the needed volume of substrate from the green-labeled bottle into a designated-labeled reagent boat. Attach 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. Incubate for **5 minutes**. Discard the remaining substrate and rinse the reagent boat with water.
- (11) Pour the Red Stop solution from the red-labeled bottle (same volume as prepared for substrate) into a designated-label reagent boat. Eject the excess substrate from the 12-channel pipettor, prime the tips, and pipette 100 μ l of the Red Stop to each well. Mix by sliding back and forth on a flat surface without splashing reagents. Discard the tips.
- (12) Wipe bottom of microwells with a dry cloth or towel and read in the Awareness Stat-Fax Model 321 PLUS Reader using a 650 nm filter, or the Biotek EL 301 Microwell Reader. All 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 the Results with the Stat-Fax Model 321 PLUS Microwell Reader.

To begin from the "Ready" prompt, press Menu, key in test number 99, and then press Enter. The preprogrammed test menu will print. Use the test number listed for the DON 5/5 method. For example, if the DON 5/5 method is listed as menu number 5, press Menu, key in the test number 5, and then press Enter. Verify the calibration constants by running a strip. The constants should read:

C 1	0.0
C 2	0.5
C 3	1.0
C 4	2.0
C 5	6.0

If no test for the DON 5/5 is present or the calibrators are not correct, contact Neogen Corporation.

- (1) 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.
- (2) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph.

Press "No" (0) to skip this feature.
- (3) 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.
- (4) If a diluted sample extract (see Standard Extraction Procedure) is being analyzed, the reader value for the extract will need to be modified to adjust for the dilution of the extract.

- (5) If the original extract was diluted 1+1 with water (this is an actual 1:2 dilution), the sample results are multiplied by 2. If the original extract was diluted 1+3 with water (this is an actual 1:4 dilution), the sample results are multiplied by 4.

NOTE: If the correlation coefficient is less than 0.98 or if the slope exceeds $-2.0 (\pm 0.5)$, the 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.

- (c) Reading the Results with the Biotek EL 301 Microwell Reader.
 - (1) Make sure that the microwell reader is on and allowed to warm-up for a minimum of 15 minutes before using.
 - (2) Remove sample carriage and hit "Enter."
 - (3) Insert W2 filter and hit "Enter."
 - (4) Insert 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 microwells into sample carriage so that the first control labeled 0 is in position A1.
 - (7) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.
 - (8) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.
 - (9) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.
 - (10) Repeat step (9) until absorbance values have been obtained for all controls and samples. Record the values.

- (11) Use the data reduction software provided by Neogen to convert the absorbance values into concentration values.

6.4 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.

6.5 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** (5 ppm) 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 value at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value. Dilute 1 ml of the original extract from 6.2 a (8) as applicable, with 2 ml of the extraction solution (distilled/deionized water). The total volume is 3 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end).

- (4) 1 Blue-Labeled Bottle - DON-HRP Conjugate Solution.
- (5) 1 Green-Labeled Bottle - 24 ml K-Blue Substrate Solution.
- (6) 1 Red-Labeled Bottle - 32 ml Red Stop Solution.

b. Materials Required but not Provided:

- (1) Extraction Materials: Whirlpack Bags -18 oz., or equivalent sealable sample bag.
- (2) Microwell Strip Reader: Awareness Technology STAT-FAX Model 321 PLUS Reader, or Biotek El-301 Microwell Reader with a 650 nm filter.
- (3) 12-Channel multi-channel pipettor and pipette tips.
- (4) 100 μ l pipettor and pipette tips.
- (5) 100 ml pipettor and pipette tips.
- (6) Deionized or Distilled Water.
- (7) Absorbent Materials: Paper Towels, Kay Dry paper or equivalent.
- (8) Neogen Filter Syringe.
- (9) Microwell holder.
- (10) Timer: 3-channel minimum.
- (11) Waterproof marker: Sharpie or equivalent.
- (12) Wash Bottle.
- (13) Reagent Boats.
- (14) Sample grinder.
- (15) Balance.

6.9 STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions.

Store test kits in a dedicated refrigerator between 2 - 8° C (36-46° F) when not in use.

b. Precautions.

- (1) Do not use test kit components beyond expiration date.
- (2) Do not mix reagents from one kit lot number with reagents from a different kit lot number.
- (3) Do not run more than 24 wells (2 strips) at one time.
- (4) Kits should be brought to room temperature (18-30° C, 64-86° F) prior to use.
- (5) Avoid prolonged storage of test kits at ambient temperatures.
- (6) Do not freeze test kits.