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FGIS Issuance Change

CHANGE TO

☐ DIRECTIVE ☐ MANUAL ☒ HANDBOOK

CHANGE NO:	**TO** *(No.)*	**TITLE:**	**DATE:**
3		Don (Vomitoxin) Handbook	8-30-04

PURPOSE OF CHANGE: The DON (Vomitoxin) Handbook has been revised to clarify the procedures for performing a supplemental DON analysis using the RIDASCREEN® Fast DON test method, and to make minor editorial changes.

FILING INSTRUCTIONS

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Chapter 3	5-20-02	Chapter 3	8-30-04
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Retain this issuance sheet as an aid in verifying handbook contents.

/s/ David Orr

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CHAPTER 3

SAMPLE PREPARATION

3.1 SAMPLE SIZE AND PREPARATION

A sample of approximately 200 grams, with dockage and stones removed, is required for the DON testing and file sample (100 grams work portion, 100 grams file portion). An additional sample may be required if subsequent review inspections are requested. A similar sample size is recommended for submitted samples.

Obtain samples according to the guidelines in the Grain Inspection Handbook, Book I, "Grain Sampling." From the 100-gram ground work portion, divide (using a Boerner divider) out a portion of 50 grams for DON testing and weigh on an FGIS-approved type scale with a minimum division size of 0.1 gram.

3.2 GRINDING SAMPLES

Grind approximately 100 grams (dockage and stone free) of grain using a Romer Mill-Model 2a, Udy Grinder, Perten Falling Number Mill, Bunn Commercial Coffee Grinder, or an equivalent device that meets FGIS' performance requirements.

SAFETY NOTE: OPERATOR MUST OBSERVE SAFETY PRECAUTIONS AND WEAR EYE PROTECTION WHEN OPERATING THE GRINDER. SEE THE OPERATOR'S MANUAL FOR MORE SAFETY TIPS.

The grinding apparatus must be adjusted to produce a particle size that is sufficiently fine enough to obtain a homogeneous blend. Generally, a sufficiently coarsely ground sample of wheat resembles whole wheat flour, while a sample that is too coarsely ground has the appearance of bulgur or semolina. Avoid over-grinding or pulverizing a sample because it produces an excessively powdery mix that will slow down the filtration process.

a. <u>Procedures for Checking the Performance of the Grinder.</u>

To check the performance of equipment used for grinding **small grains (e.g., wheat and barley),** use the following procedures:

(1) Grind a sample portion of approximately 100 grams of relatively dry wheat (i.e., 13 percent or less moisture).

(2) Weigh the entire portion that was ground.

(3) Sieve the portion across a standard No. 20 wire woven sieve.

(4) Weigh the portion that passed through the sieve.

(5) Determine the percent of fine material, by weight, as follows:

Fines = weight from step (4) divided by the weight from step (2) X 100.

For locations that perform mycotoxin testing on coarse (e.g., corn) and small grains, perform the check using a 100-gram sample portion of corn having a moisture content of 14 percent or less.

b. Optimum Particle Size.

The optimum range for particles of coarse and small grain passing through the No. 20 sieve is between 60 and 75 percent. Whenever the ground particles appear to be too coarse, or the results of a grinder check indicate that less than 50 percent of the ground portion passes through the No. 20 sieve, the grinder should be adjusted or repaired to meet the optimum range requirements.

Grinding apparatuses must be checked periodically to determine whether they are producing a final product that meets the particle size requirements as listed above. Official personnel shall determine the frequency of the checks based on a number of items that include visual observation of the ground product, number of samples ground since last check, and time (number of days) since the last check was performed. Record all particle check results in a convenient location for future reference purposes.

9.1 GENERAL INFORMATION

FGIS has approved two separate Diagnositx test kits for quantitative DON testing. The EZ-Quant DON Plate test kit (part number 600312) is used for testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm. The EZ-Quant 0.5 PPM DON Plate test kit (part number 600313) is used for testing barley and malted barley from 0.5 to 2.5 ppm.

9.2 TESTING AREA

The extraction solution and other materials used in the Diagnostix EZ-Quant DON Plate test kits (part numbers 600312 and 600313) do 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 the handbook to ensure a safe and efficient work environment.

9.3 EXTRACTION PROCEDURES

The extraction procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

a. Place 50 grams of ground sample into a clean plastic bag.

b. Add 250 ml of distilled or deionzed water and seal/close the bag securely to prevent spillage.

c. Shake vigorously (by hand or mechanically) for three minutes.

d. Let the extract sit for 2-3 minutes to allow for some settling of the slurry.

e. Filter a minimum of 15 ml of the extract through Whatman #4 filters (or equivalent) into a clean container that is labeled with sample ID number.

9.4 PREPARATION OF SOLUTIONS

The procedures listed below are applicable for the EZ-Quant DON test kit (part number 600312) and the EZ-Quant 0.5 ppm DON Plate test kit (part number 600313).

a. To prepare the Wash Solution, transfer the contents of the Wash Concentrate vial to a 500-ml container and add 475 ml of distilled or deionized water.

b. Swirl to mix.

9.5 TEST PROCEDURES

a. EZ-Quant DON Plate Test Kit (Part Number 600312) - Testing Wheat, Barley, Malted Barley, and Corn from 0.5 to 5 ppm.

(1) Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one-hour).

(2) Place the appropriate number of red mixing wells and clear test wells into a microwell holder. Do not run more than two strips at a time. Be sure to re-seal unused wells in the zip-lock bag with desiccant.

NOTE: The maximum number of test samples that can be run at one time is 19. Using two strips of 12 wells, designate 5 wells for the calibrators and the remainder of the wells for test samples.

(3) Dispense 100 µl of Enzyme Conjugate into each red mixing well.

(4) Dispense 100 µl of the appropriate calibrators and samples into the appropriate red mixing wells as illustrated below.

NOTE: Use a clean pipette for each addition.

Wells	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
First Strip	**0**	**0.5**	**1.0**	**2.0**	**6.0**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Second Strip	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**

Key : S= Sample, 0= 0 ppm calibrator, 0.5= 0.5 ppm calibrator, etc.

(5) Using a 12-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips 5 times in the mixing wells.

(6) Using a 12-channel pipette, transfer 100 µl of the reaction mixture into the corresponding clear test wells and tap the holder several times to mix. Discard the red mixing wells into an appropriate waste container.

(7) Incubate the clear test wells for 10 minutes.

(8) Dump the contents of the wells into an appropriate waste container and carefully shake out any residue solution.

(9) Using a wash bottle filled with wash solution, fill each well to overflowing then dump the contents and shake out any residue solution. Repeat four times for a total of 5 washes.

(10) After the final wash, tap the strips repeatedly onto absorbent paper to remove excess wash. After tapping, check for large bubbles, which should be burst with a clean pipette tip and tapped out again.

(11) Using a 12-channel pipette, dispense 100 µl of Substrate into each well and tap the holder several times to mix the contents.

(12) Using a paper towel to block out the light reflectance, cover the wells and incubate for 5 minutes.

(13) Using a 12-channel pipette, dispense 100 µl of Stop Solution into each well.

(14) Within 10 minutes, read and record the absorbance of each well at 450 nm using the Bio-Tek EL 301™ Microwell Strip Reader equipped with a 450 nm filter.

b. <u>EZ-Quant 0.5 PPM DON Plate Test Kit (Part Number 600313) - Testing Barley and Malted Barley from 0.5 to 2.5 ppm</u>.

(1) Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one-hour).

(2) Place the appropriate number of red mixing wells and clear test wells into a microwell holder. Do not run more than two strips at a time. Be sure to re-seal unused wells in the zip-lock bag with desiccant.

NOTE: The maximum number of test samples that can be run at one time is 19. Using two strips of 12 wells, designate 5 wells for the calibrators and the remainder of the wells for test samples.

(3) Dispense 100 µl of Enzyme Conjugate into each red mixing well.

(4) Dispense 100 µl of the appropriate calibrators and samples into the appropriate red mixing wells as illustrated below.

NOTE: Use a clean pipette for each addition.

Wells	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
First Strip	**0**	**0.2**	**0.5**	**1.0**	**2.5**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Second Strip	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**

Key : S= Sample, 0= 0 ppm calibrator, 0.5= 0.5 ppm calibrator, etc.

(5) Using a 12-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips 5 times in the mixing wells.

(6) Using a 12-channel pipette, transfer 100 µl of the reaction mixture into the corresponding clear test wells and tap the holder several times to mix. Discard the red mixing wells into an appropriate waste container.

(7) Incubate the clear test wells for 10 minutes.

(8) Dump the contents of the wells into an appropriate waste container and carefully shake out any residue solution.

(9) Using a wash bottle filled with wash solution, fill each well to overflowing then dump the contents and shake out any residue solution. Repeat four times for a total of 5 washes.

(d) 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.

(3) Hyperion Microreader™ 3 Model 4027-002 Microwell Reader.

(a) After the power is turned on the instrument will proceed through a calibration mode then advance to the "Main Menu" setting.

(b) When prompted to "Run a test", select yes, select the appropriate test number, then press "Enter".

(c) At the "Run XXX test?" prompt select yes, select the number of wells (e.g., 8, 12, 16, 24) then press "Enter".

(d) At the "Insert strip" prompt insert the test well strip and press "Y" to continue.

(e) The reader will read the optical density of the wells and print a report.

(f) After the report is printed a "Continue test" prompt will appear. To continue testing select yes and follow the to the instrument prompts as indicated above.

(g) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

10.5 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.

10.6 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 value 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 diluted filtrate (obtained from step f., section 10.2) 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:

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

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

10.7 CLEANING LABWARE

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