

# Program Notice

FGIS-PN-11-09

09-01-2011

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## CHARM SCIENCES ROSA® DON P/N TEST METHOD

### 1. PURPOSE

This Program Notice is issued to notify official locations of the re-evaluation of the Charm ROSA® P/N test method for official mycotoxin testing of wheat and corn at screening level thresholds 0.5, 1, 2, and 5 parts per million (ppm).

### 2. BACKGROUND

The Policies Procedures and Market Analysis Branch (PPMAB), along with the Technology and Science Division (TSD) have an ongoing evaluation of new or revised procedures for mycotoxin test methods. In order to offer official use of TSD-approved new or revised mycotoxin test methods before revisions to handbooks are available, program notices communicate updated instructions. The following test procedures are approved for use by the Federal Grain Inspection Services and Official Agency Personnel.

**NOTE:** TSD has also granted a temporary extension of the Charm ROSA®P/N test method Certificate of Conformance (COC) expiration date for official testing of barley at 1 ppm only until further notice.

### 3. TEST INSTRUCTIONS

#### a. General Information.

The ROSA® DON P/N test kit, product number LF-DONPN uses lateral flow test strip technology that provides qualitative (equal to or less than a specified threshold) results in wheat, corn, and barley. Screening level thresholds (**0.5, 1, 2, and 5**) parts per million (ppm) are approved testing levels for wheat and corn. The approved screening level threshold for barley is **1 ppm** only. All screening levels are available with this kit based on the extract volume used to perform official testing.

The extraction solution, and other materials used with this test method does not necessitate the use of a separate FGIS-approved laboratory space. Federal Grain Inspection Service (FGIS) employees must comply with all applicable safety and sanitation requirements as listed in the DON handbook to ensure a safe and efficient work environment.

| Approved Test Kit Information                                    |            |                                    |                 |                              |                       |
|--|------------|------------------------------------|-----------------|------------------------------|-----------------------|
| Test Kit Vendor  |            | Charm Sciences Inc. 1-978-687-9200 |                 |                              |                       |
| Test Kit Name  |            | Charm ROSA® DON P/N Test Method    |                 |                              |                       |
| Product Number   | QAC Number | Conformance Limits                 | Type of Service | Extraction Solution          | Supplemental Analysis |
| LF-DON PN  | VOMI       | Thresholds                         | Qualitative     | Distilled or Deionized Water | No                    |
|  |            | 0.5, 1, 2, 5 ppm                   |                 |                              |                       |
| <b>Grain/Commodities Approved for and testing basis.</b>         |            |                                    |                 |                              |                       |
| Wheat-dockage free, Corn-bcfm included, and Barley-dockage free. |            |                                    |                 |                              |                       |

b. Preparation of Extraction Solution.

**NOTE: A Negative and Positive Control should be run periodically using the Performance Monitoring Mode to verify performance of equipment and test strips (daily, weekly, bi-weekly, or monthly, based on internal quality assurance standards).**

(1) Negative Control.

- (a) Use the DON Dilution Buffer for the Negative Control.
- (b) To run Negative Control, use 300 µl and follow test procedures found in section (i), Sample Analysis.

(2) Positive Control.

- (a) Prepare/reconstitute the 0.5 ppm DON Positive Control by adding 6.0 ml of DON Dilution Buffer and mix well for 30 seconds.
- (b) Allow to stand for 10 minutes at room temperature. Mix again before use.
- (c) To run Positive Control use 300 µl and follow test procedures found in section (i), Sample Analysis.
- (d) A valid positive test result must be received before official testing. The reconstituted Positive Control is equivalent to Diluted Extract prepared from (500 ppb or 0.5 ppm) DON in wheat.

**NOTE: Store reconstituted Positive Control refrigerated for up to 1 week or aliquot, and freeze within 6 hours of reconstitution at 15° C (4° F) or below for up to 2 months. Thaw slowly (overnight in the refrigerator or with cool water) and shake well before use. Keep/store thawed Positive Control refrigerated and use within 24 hours of thawing. DO NOT REFREEZE.**

c. Equipment Preparation.

- (1) Incubator must be at  $45 \pm 1^{\circ}$  C (temperature indicator is green).
- (2) Incubator must be clean and level.

d. DON Dilution Buffer.

- (1) Predisperse 1.0 ml of DON Dilution Buffer into a micro-centrifuge tube for each sample to be tested.
- (2) Use this solution at room temperature.
- (3) Store DON Dilution Buffer bottle and any unused predispersed micro-centrifuge tubes at 32 - 45° F.

e. Test Strips.

- (1) Remove ROSA® DON P/N moisture resistant container from the refrigerator and allow it to reach room temperature to limit condensation.
- (2) Remove only the number of strips to be used and return container to 32 - 45° F storage. Strips are stable at room temperature for at least 12 hours.

**NOTE: Inspect desiccant indicator. Beads inside desiccant packet should be blue. Discard test strips if desiccant beads turn purple or pink.**

f. Extraction Procedures.

- (1) Procedures for Extraction of wheat, barley, and corn.
  - (a) Transfer 50 grams of ground sample into a clean extraction container.
  - (b) Add 250 ml of deionized or distilled water.
  - (c) Blend or shake for 1 to 2 minutes. Allow sample to settle for 1 minute to obtain a sample extract.
  - (d) Prepare additional sample extracts (up to 4 for quad incubator) following steps a – c.
  - (e) Continue to Sample Preparation section.

**NOTE: If particles are present after settling, filter or centrifuge to clarify sample extract. To Filter: funnel the extract through Whatman 2V (or equivalent) filter paper into a labeled collection container. To Centrifuge: transfer 1.0-1.5 ml of sample extract to a labeled micro-centrifuge tube and centrifuge for 10 seconds. The clarified extract is now ready for testing.**

g. Sample Preparation.

To prepare Diluted Extracts for the appropriate Screen Levels select from the following:

(1) **Sample Preparation for 0.5 ppm Screening Level for Wheat and Corn.**

- (a) Pipet 100  $\mu$ l of clarified extract to a predisposed (1.0 ml DON Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract #1.
- (b) Repeat for additional samples or proceed to the next section.

(2) **Sample Preparation for 1.0 ppm Screening Level for Wheat, Corn and Barley.**

- (a) Pipet 50  $\mu$ l of clarified extract to a predisposed (1.0 ml DON Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract #2.
- (b) Repeat for additional samples or proceed to the next section.

(3) **Sample Preparation for 2.0 ppm Screening Level for Wheat, Corn.**

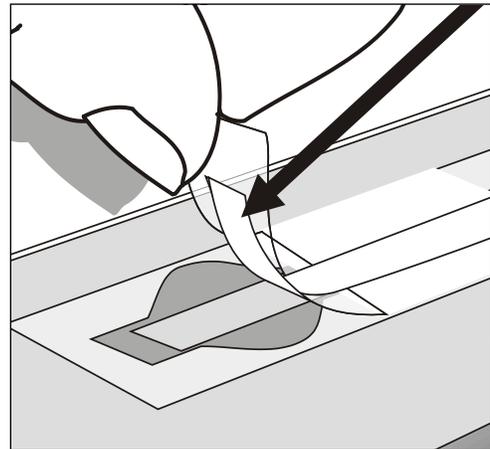
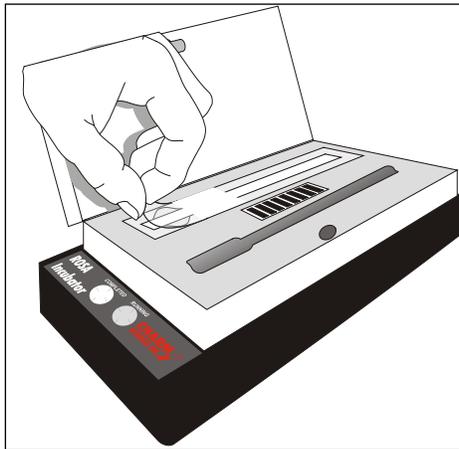
- (a) Pipet 300  $\mu$ l of diluted extract #1 to a predisposed (1.0 ml DON Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract #3.
- (b) Repeat for additional samples or proceed to the next section.

(4) **Sample Preparation for 5.0 ppm Screening Level for Wheat and Corn.**

- (a) Pipet 100  $\mu$ l of diluted extract #1 to a predisposed (1.0 ml DON Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract #4.
- (b) Repeat for additional samples or proceed to the next section.

h. Test Strips and Equipment Preparation.

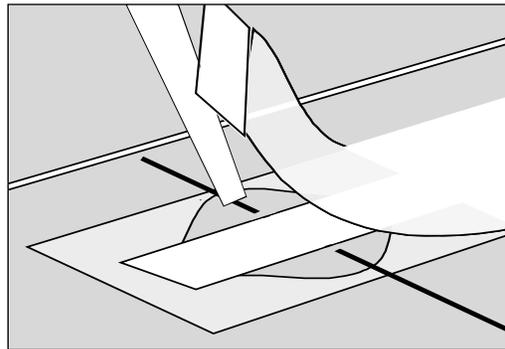
- (1) Check to ensure the ROSA Incubator temperature is 45 +/- 1° C.
- (2) Label the test strip to identify sample(s).
- (3) Open the incubator lid and place test strip in the ROSA-M Incubator with the flat side facing upward.
- (4) While holding the strip flat on the incubator, use tab to peel tape back to the indicated line exposing the sample pad. Avoid bending back the white wick and sponge under the tape.



i. Sample Analysis.

- (1) Pipet 300 µl of diluted extract or control into the side of the strip sample compartment at the position indicated by the black line on the incubator.

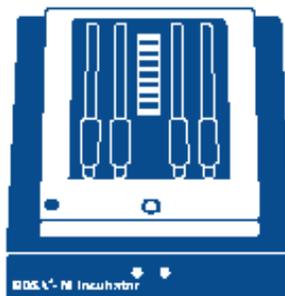
**NOTE: Pipet very slowly.**



- (2) Reseal the tape over the sample pad compartment. When testing multiple samples, complete the peel, pipet, and reseal steps on each strip before going to the next strip.

**NOTE: Add diluted extract to all strips within 1 minute. If a quad incubator is used, four samples can be incubated simultaneously.**

- (3) Close lid on the incubator and tighten the latch. The solid red timer light will automatically start when the lid is closed.



**LF-INC4-3-45D: Quad incubator**

- (4) Incubate for 3 minutes. After the incubation step is complete, a 2 minute beeper will sound and the yellow “test complete” light will begin to flash.
- (5) Remove strips and interpret the results. **Strips must be removed from the incubator and read within 2 minutes of incubation completion.** After strip removal, lower but do not latch the incubator lid.

j. Visually Interpreting the Lateral Flow Test Strip.

Development of a Control Line indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded. A second preparation of the diluted extract (using a fresh dilution) should be made and tested using another test strip.

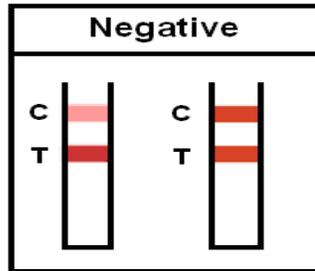
**Note: The examples shown below depicting invalid, negative, and positive results are for illustration purposes only. Do not use these color bars as actual intensity measurement for determining if the sample is positive or negative.**

- (a) Invalid Result.

A test is invalid if a Control Line is missing, smeared, or uneven, or if the Test Line is uneven. It is invalid if the diluted extract is obscuring either the Control (C) or Test Line (T).

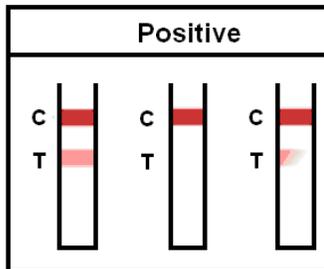
(b) Negative Result.

A sample containing DON residues less than or equal screening level will develop a Test Line that is darker or equal in intensity to the Control Line in the test area.



(c) Positive Result.

A sample containing DON residues greater than the screening level will develop a Test Line that is lighter in intensity than the Control Line.



(4) Interpreting the Lateral Flow Test Strip using the ROSA-M Reader.

The ROSA-M Reader interprets the test strip and displays either **NEGATIVE** or **POSITIVE**.

- (a) A **NEGATIVE** sample contains DON less than or equal to the screening level.
- (b) A **POSITIVE** sample contains DON greater than the screening level.

**NOTE: Periodically enter Performance Mode in reader by selecting DON channel and pressing ESC and then 5. Follow reader prompts. Run each of the calibration strips to verify reader performance. The strips should read +/- 2 ppb from the average written on the strips themselves.**



**LF-ROSA READER-M:** ROSA-M Reader supplied with calibrators.

- (c) Read result on **DON** Channel (2-Line Mode) using the appropriate Matrix. The appropriate Matrix numbers are as follows:

|                  |  |
|------------------|--|
| <b>MATRIX 00</b> | <b>Used to screen for 0.5 ppm DON (Diluted Extract #1)</b> |
| <b>MATRIX 01</b> | <b>Used to screen for 1.0 ppm DON (Diluted Extract #2)</b> |
| <b>MATRIX 02</b> | <b>Used to screen for 2.0 ppm DON (Diluted Extract #3)</b> |
| <b>MATRIX 03</b> | <b>Used to screen for 5.0 ppm DON (Diluted Extract #4)</b> |
|                  |  |

If desired enter **Sample** and/or **Operator**. Press **ENTER** to read.

**Note: It is very important to select the correct matrix according to the screening level threshold (diluted extract) tested.**

k. Reporting and Certifying Test Results.

- (1) Report Negative results on the pan ticket and inspection log as being equal to or less than the request screening (0.5, 1, 2, or 5 ppm) threshold level tested ( $\leq 0.5$ ,  $\leq 1$ ,  $\leq 2$ , or  $\leq 5$  ppm), as applicable.

Report Positive results on the pan ticket and inspection log as being greater than the requested screening (0.5, 1, 2, or 5 ppm) threshold level tested ( $> 0.5$ ,  $> 1$ ,  $> 2$ , or  $> 5$  ppm), as applicable.

- (2) Certify Negative results as being equal to or less than the request screening (0.5, 1, 2, or 5 ppm) threshold level tested ( $\leq 0.5$ ,  $\leq 1$ ,  $\leq 2$ , or  $\leq 5$  ppm), as applicable.
- (3) Certify Positive results as being greater than the request screening (0.5, 1, 2, or 5 ppm) threshold level tested ( $> 0.5$ ,  $> 1$ ,  $> 2$ , or  $> 5$  ppm), as applicable.

Refer to the Certification Chapter of the DON Handbook for more detailed certification procedures.

l. Cleaning Labware.

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

m. Waste Disposal.

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

n. Equipment and Supplies.

(1) Materials Supplied in Test Kits.

Kits can be purchased that contain 20, 100, or 500 strips and include Control and DON Dilution Buffer.

(a) LF-DONPN-20-K:

- 1 1 package containing 20 ROSA® DON P/N strips packed in a moisture resistant container.
- 2 1 - 0.5 ppm Positive Control.
- 3 1 DON Dilution Buffer.

(b) LF-DONPN-100 K:

- 1 1 package containing 100 ROSA® DON P/N strips packed in a moisture resistant container.
- 2 1 - 0.5 ppm Positive Control.
- 3 1 DON Dilution Buffer.

- (c) LF- DONPN-500-K:
  - 1 5 packages each containing 100 ROSA® DON P/N strips packed in a moisture resistant container.
  - 2 5 - 0.5 ppm Positive Controls.
  - 3 5 DON Dilution Buffers.

(2) Materials Required, but not Provided.

- (a) Sample grinder.
- (b) Balance.
- (c) Deionized or Distilled water.
- (d) Sample extraction containers.
- (e) 1.0 ml pipettor and pipette tips.
- (f) 300 µl pipettor and pipette tips.
- (g) 100 µl pipettor and pipette tips.
- (h) 50 µl pipettor and pipette tips.
- (i) 25 ml graduated cylinder.
- (j) LF-INC4-3-45D ROSA Quad Incubator (45° C and 3 minute timer).

(3) Optional Equipment and Supplies.

- (a) Mini-centrifuge.
- (b) 1.5 micro-centrifuge tubes.
- (c) Filter funnels.
- (d) Whatman 2V filter paper or equivalent.
- (e) LF-ROSAREADER-M-NB ROSA-M Reader.

o. Storage Conditions.

Test kits should be refrigerated between 32°- 45° F.

p. Precautions.

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

**4. FILING**

Retain a copy of this program notice with the DON Handbook until the handbook is revised to include the test method revisions stated herein.

**5. QUESTIONS**

Direct any questions concerning this program notice to Carl Jackson, Policies, Procedures, and Market Analysis Branch (PPMAB), at (202) 720-8286, or email at [carl.jackson@usda.gov](mailto:carl.jackson@usda.gov), or Patrick McCluskey, PPMAB, at (816) 659-8403, or email at [patrick.j.mccluskey@usda.gov](mailto:patrick.j.mccluskey@usda.gov).

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