NEOGEN REVEAL Q+ MAX FOR AFLATOXIN USING ACCUSCAN GOLD AND ACCUSCAN PRO READERS

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GENERAL INFORMATION

The REVEAL Q+ MAX FOR AFLATOXIN test method provided by the Neogen Corporation is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The test provides quantitative analysis for the presence of Aflatoxins, using distilled or deionized water and a MAX 1 extraction packet along with an Aflatoxin-antibody particle complex coated test strip. The test strips are analyzed on the Neogen AccuScan Gold and AccuScan Pro readers.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the Mycotoxin Handbook for information on use of this test kit in the official inspections including sampling, general sample preparation, reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

Approved Test Kit Information

Test Kit Vendor:	*Neogen Corporation 800/234-5333*
Test Kit Name:	REVEAL Q+ MAX FOR AFLATOXIN
Product Number:	8088
Effective Date of Instructions:	07/25/2016
Instructions Revision Number	0
Conformance Range:	5 – 300 ppb
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Approved Commodities:	corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), lightly pearled barley, popcorn, brown rice, rice, sorghum, and wheat
Extraction method:	Vigorously shake 50g sample and one packet of MAX-1 G50 extraction powder in 250 mL of distilled or deionized water for 3 minutes.
Test Format:	Lateral Flow Strip
Detection Method:	AccuScan Gold Reader, Model #9595; AccuScan Pro Reader, Model #9565

PREPARATION OF TESTING MATERIALS

AccuScan Pro Reader Set-up:

The system provides an easy method to objectively read, store, and analyze results from Neogen's line of lateral flow strips.

Note: Please keep and store all packaging materials included in the kit for future storage.

(1) Set-up: Press the Home icon at any time to return to the main screen. 

a. Plug the outlet of the cord into the power source. Plug the small end of the power cord into the reader.

b. This will turn the reader on. To turn the reader off, unplug the reader.

c. Plug the USB key into the USB port on the side of the reader.

USB, Ethernet, and power cord ports

Start up screen

(2) Settings: 

Press the designated icon to set time, date, volume, remote connection, language setting, and allows the user to review current information.

(3) QR Codes: 

The reader is capable of reading information from predefined QR codes. Disposable QR codes containing lot specific information are included in each Reveal Q+ kit.

a. Whenever a new lot is obtained, and before you run a test, you must verify the QR codes. Simply place the QR code card into the QR code cartridge. From the **Home screen**, select the QR code icon and place the QR code cartridge containing the card in the reader. The specific information automatically will be downloaded into the reader.

b. The specific QR code cartridges are entered into the reader to identify unique users with a five digit user ID code.

Note: Technicians must update or verify reader information (lot number of test strips in use) before official testing. If needed refer to the AccuScan Pro manual for more detailed instructions.

(4) Run test protocol: 

a. Touch the **Run test** icon, which looks like a test strip, on the **Home screen**.

b. The test category will display in the first box and the test type will display in the second box. The reader will default to the last test analyzed.

c. Select the appropriate test category by touching the desired option (e.g., Mycotoxin Q+ MAX).

d. Touch the green check mark to confirm the selection.

e. Touch the **Test type** icon. Select the desired test by touching the name of the test (e.g., Afla Q**+** MAX). To scroll through the test options, use the white up and down arrows. Touch the green check mark to confirm the selection.

f. Insert the test strip to begin interpreting the test or enter the sample ID by touching the **Sample ID** button. **NOTE**: Strips may be tested without a sample ID. To do this, insert the test strip cartridge containing the test strip into the reader square end first. The reader automatically will begin analyzing the strip.

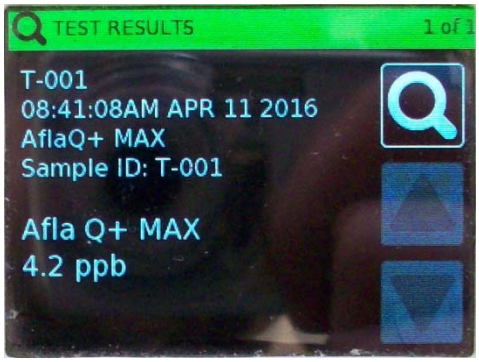
g. If entering a sample ID, type the ID in and press the green check mark to continue.

h. Insert the test strip into the appropriate strip cartridge. Insert the cartridge containing the strip into the slot on the lower left portion of the reader. The reader automatically will begin analyzing the strip.

(5) Test Results:

a. For a quantitative test, a numerical value will be displayed.

b. Touch the screen to view additional test information such as test line and control line ratios.



c. To print, select the **Print** icon. A report will automatically be sent to the attached printer.

d. To begin/run a new test, select the "**New test"** icon. This will return the user to the test setup screen.

Reader Notes and Cautions:

- Ensure device is fully inserted into cartridge.
- Removing the cartridge prior to completion can result in invalid readings.
- Reading should be made as soon as development is done.

AccuScan Gold Reader Set-up:

(1) Enter the lot-specific QR code by selecting Scan QR code from the main screen.

(2) Place the QR code into the white cartridge adapter labeled Cal/QR and insert the cartridge into the reader.

(3) The valid code will be scanned by the reader and provide information on the lot number and expiry date. Verify this information is correct and then add the lot ID to the reader by pressing Add Lot ID.

(4) Return to the home screen and select the test strip icon.

(5) Select Mycotoxin Q+ MAX for the Category

(6) Select the **Q+ M Afla** test type. See Analysis Procedure to determine which one to use.

SAMPLE PREPARATION AND EXTRACTION PROCEDURES

Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), lightly pearled barley, popcorn, brown rice, rice, sorghum, and wheat

The sample to be tested should be collected and prepared according to accepted sampling techniques (see **Mycotoxin Handbook**):

Sample extraction (for quantitation range 5-50 ppb)

(1) Weigh 50 ± 0.2 grams of ground sample into a whirl-pak bag.

(2) Add one packet of MAX 1-G50 Aqueous Extraction Powder to the bag.

(**CAUTION**: Do not inhale the powder. Perform this step in a vent hood.)

(3) For all commodities except rice, add 250 mL of distilled or deionized water. For rice samples, use 350 mL of distilled or deionized water.

(4) Shake vigorously by mechanical shaker (250 rpm) for 3 minutes.

(5) Allow the sample to settle for 5 minutes.

(6) Filter about 3 mL of the extract through a Neogen syringe filter. The filtered sample extract should be free of particulates.

The **filtered sample extract** is now ready for testing or dilution, as applicable.

Diluted sample extract (for quantitation range 50-300 ppb)

For samples with levels known to be between 50 and 300 ppb (or have tested above 50 ppb), a dilution needs to be made. Determine the appropriate dilution factor for the sample based on testing of the undiluted extract or on expected concentration.

(1) For a quantitation range of **50-300 ppb**, a ten-fold dilution is required. Dilute the samples as follows:

a. Pipette 100 µL of filtered extract into a suitable tube or other container.

b. Add 900 µL of distilled or deionized water, and vortex to mix well.

The **diluted sample extract** is now ready for testing.

TEST PROCEDURES

Analysis of extracts

(1) Set up one red sample dilution cup and one clear sample cup for each extract to be tested. Place the cups in a holder or rack, labeled as applicable. Follow the same procedure for each extract.

(2) Add 100 µL of **sample diluent** to the red sample dilution cup.

(3) Add 100 µL of **filtered sample extract** (or 100 µL of **diluted sample extract for 50 to 300 ppb quantitation**) to the red cup containing the diluent.

(4) Mix by pipetting the mixture in the cup up and down at least 5 times.

(5) Pipette 100 µL of the contents of the red cup (diluent + extract) into a new clear cup.

(6) Place a new Reveal Q+ MAX for Aflatoxin test strip into the clear cup, with the arrow end down, and let it develop for exactly 6 minutes.

(7) Remove strip from the sample cup as soon as the 6-minute development is complete, and read the results immediately.

(8) Fully insert the Reveal Q+ test strip into the black cartridge adapter, sample end first and results facing out.

(9) Insert the cartridge with test strip side up into the AccuScan reader. The reader will automatically read the strip and display the result.

a. If the filtered sample extract was undiluted, the result displayed on the reader is the final result for the sample.

b. If the filtered sample extract was diluted, the displayed result must be multiplied by the dilution factor to obtain the final result for the sample.

c. If the result (diluted or undiluted) is above 50 ppb, the sample must be re-analyzed after appropriate dilution to bring it down to a level between 5 and 50 ppb.

Notes about reading the results

(1) The strips must be read immediately after development using either Neogen's AccuScan Gold or AccuScan Pro Reader. Test results will be displayed and stored in the reader.

(2) Readings should be made between 6 and 7 minutes. Reading results after 7 minutes may be inaccurate due to over-development of the test strip, and should not be reported.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

Store kit components at room temperature (18-30ºC, 64-86ºF) to ensure full shelf life. Test strips should remain capped in their original tubes until used to ensure optimal performance.

b. Precautions

(1) Do not use test kit components beyond the expiration date.

(2) Test strip development times, other than those specified in Test Procedures section, may give inaccurate results.

(3) The test strips must remain inside the stay-dry tube before use.

(4) Treat all used liquids, including sample extract, and labware as if contaminated with Aflatoxin, gloves and other protective apparel should be worn at all times.

(5) To avoid cross-contamination, use new tips for each measurements.

(6) Ensure the device, lot number and curve details match the lot ID number selected on the reader. Failure to update the lot-specific QR code within the AccuScan Gold or AccuScan Pro reader will cause inaccurate results.

(7) Commodity extracts should have a pH of 6 – 8 before testing. Excessively acidic or alkaline samples should be adjusted. For instructions on adjusting pH contact a Neogen representative or Technical Services.

EQUIPMENT AND SUPPLIES

a. Materials provided in test kits.

(1) 25 Reveal Q+ for Aflatoxin test strips

(2) 25 red sample dilution cups

(3) 25 clear sample cups

(4) 1 bottle of sample diluent

(5) 25 MAX Aqueous Extraction packets

(6) Instructions for use

b. Materials required but not provided.

(1) Extraction Materials
   a. Sample collection cups with lids 125 mL (Neogen item #9428, #9428B)
   b. Sample collection tubes with caps (Neogen item #9421, 9421B)
   c. Neogen filter syringe, Whatman #1 filter paper, or equivalent (Neogen item #9420, 9519, 9429)
   d. Centrifuge, mini (Neogen item #9330)
   e. Micro centrifuge tubes (Neogen item #9372)
   f. Dispensing pump or graduated cylinder (Neogen item #9448, #9447)

(2) Timer (Neogen item #9426)

(3) 100 µL pipettor (Neogen item #9272, #9278)

(4) 100 µL pipette tips (Neogen item #9407, #9410, #9417)

(5) 500 µL pipettor (Neogen item #9291, #9336)

(6) 200-1000 µL pipette tips (Neogen item #9464, #9487, #9292, #9293)

(7) Reveal sample rack (Neogen item #9475)

(8) Reveal AccuScan Gold Reader (Neogen item #9595)

(9) Reveal AccuScan Pro Reader (Neogen item #9565)

(10) Disposable polyethylene transfer pipettes

(11) Agri-Grind grinder or equivalent (Neogen item #9427)

(12) Scale capable of weighing 5 – 50 grams (Neogen item #9427)

(13) Distilled or deionized water

(14) MAX-1 G50 Aqueous Extraction packets (Neogen item #8089G)

REVISION HISTORY

- Revision 0 (07/25/2016)