

Test Kit Instruction

July 05, 2017

ROMER LABS **AGRASTRIP TOTAL FUMONISIN QUANTITATIVE TEST WATEX**

FORWARD

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 official inspections including sampling, general sample preparation, grinding and dividing, reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

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1. GENERAL INFORMATION

The AgraStrip Total Fumonisin (FUM) Quantitative Test WATEX is a one-step lateral flow immunochromatographic assay for the quantitative screening of Total Fumonisin in samples. The test is based on a competition immunoassay format. Antibody-colloidal gold complex (conjugate) lyophilized in a microwell is mixed with sample extract. A Total Fumonisin strip is placed into the microwell. The mixed content is then wicked onto a membrane of the strip, which contains a test zone and a control zone. The test zone captures free antibody-particle complex (conjugate), allowing color particles to concentrate and form a visible line. The color intensity of the line is inversely proportional to the concentration of Total Fumonisin in the sample. The line is always visible in the control zone regardless of the presence of Total Fumonisin. The strips are measured using an AgraVision Reader and the results are determined.

Approved Test Kit Information

Test Kit Vendor:	Romer Labs, Inc. (636) 583-8600
Test Kit Name:	AgraStrip Total Fumonisin Quantitative Test WATEX
Product Number:	COKAS3000W
Effective Date of Instruction:	07/05/2017
Instruction Revision Number:	0
Conformance Range:	0.5 ppm – 30 ppm
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).
Extraction method:	Shake vigorously by hand a 50-gram sample with 150 milliliters (mL) of water and one buffer packet for 2 min. A mechanical shaker with similar shaking action (as hand shaking) may be substituted.
Test Format:	Lateral Flow Strip
Detection Method:	AgraVision Reader, Model No. EQASR1000

2. PREPARATION OF TESTING MATERIALS AND EQUIPMENT

All reagents and kit components must be at room temperature 20-24°C (68-75°F) before use.

a. AgraStrip Incubator

The temperature of AgraStrip Incubator is set at 45°C.

It is recommended to switch on the incubator in the morning and to keep it on throughout the whole day. The incubator must be switched on at least 15 minutes before use.

b. AgraVision Reader

- (1) Use the AgraVision Reader to read the strip and interpret the result.

Note: follow the instructions of AgraVision Reader to read the strips. Strips should not be read more than 1 minute after completion of the run.

- (2) Turn on the AgraVision reader using the power button located on the back.
- (3) Use the arrow keys on the keypad to highlight "TEST". Select it using the checkmark key.
- (4) Use the arrow keys on the keypad to highlight "Mycotoxin". Select it using the checkmark key.
- (5) The barcode scanner will turn on. Scan the barcode included in the test kit on the certificate of analysis.

Note: Separate bar codes for the separate quantitation ranges are provided in the test kit.

- (6) Create a sample ID by using the alphanumeric keys on the keypad. Use the checkmark key to enter.
- (7) Since only one strip is being read; use the pound key to bypass to the next screen.
- (8) Enter the operator ID. Press the checkmark key to enter, and press it a second time to move to the next screen.
- (9) The reader is ready to read and will display "start measurement".

3. SAMPLE PREPARATION AND EXTRACTION PROCEDURES

a. Extraction Procedure for Corn (including Dent or Field Corn, Corn Meal, Corn Flour, Cracked Corn, Corn Grits or Polenta, and Corn screenings):

- (1) Weigh out 50.0 ± 0.2 grams ground sample into one side of a filtering Whirl-Pak bag.
- (2) Place one soluble buffer bag onto the ground sample in the Whirl-Pak bag.
- (3) Add 150 mL of distilled or deionized water and securely close Whirl-Pak bag using a 250 mL graduated cylinder.
- (4) Shake closed Whirl-Pak bag vigorously by hand for 2 min or shake with similar shaking action (as hand shaking) for 2 minutes using a mechanical shaker.
- (5) Allow sample to settle for 2 minutes to get filtered extract (extract can only be used for next 5 minutes).

b. Dilution Procedure:

- (1) Dilute the filtered extract 1:21 with dilution buffer. Use a 1000 μ L pipette to add 1000 μ L of dilution buffer to a dilution tube. Then use a 50 μ L pipette or 10-100 μ L pipette to add 50 μ L of extract into the dilution tube.
- (2) Close tube, mix well by inverting a few times and the sample is ready for assay (this diluted sample can only be used for next one hour).

Note: Extract should be pipetted from the side of filter bag that is opposite of the ground sample. If the sample has a large foam head, tilt the bag for easier access to the supernatant.

4. TEST PROCEDURES

a. Analysis Procedure for Quantitation Ranges 0.5 to 6 ppm

- (1) Place the cover of the heat block on the top of the heat block. Break off the appropriate number of conjugate coated microwells for the samples to be run. Remove sealing caps from conjugate microwells, and place the conjugate microwells inside the heat block. Ensure that the wells are fully seated in the heat block. **Only one sample should be run at one time.**
- (2) Using a 100 microliter (μL) pipet, add 100 μL of **diluted sample** (from step “b” under Dilution Procedure) to each conjugate microwell.
- (3) Mix the content in each microwell by **pipetting it up and down 10 times**.
- (4) Put one test strip into one microwell. Place the cover back into the heat block to cover the microwells and test strips.
- (5) Allow the test strip to develop color for 3 minutes. Lift the heat block cover and place it on the top of the heat block.
- (6) Wipe the end of the strip test onto an absorbent paper and insert the strip into the strip holder/tray for reading.

b. Reading the Results

- (1) Use the AgraVision Reader to read the strip and interpret the result. Strips should not be read more than 1 minute after completion of the run.
- (2) Insert the strips into the tray, and insert the tray into the reader. The strips should go in the tray with the white end facing into the reader. Press the checkmark key to read.
- (3) After completion of reading, press the checkmark key to save the result in the AgraVision Reader’s memory or the pound key to print the result.

Note: If the reader gives a concentration greater than 6 ppm the previously diluted sample extract needs to be further diluted.

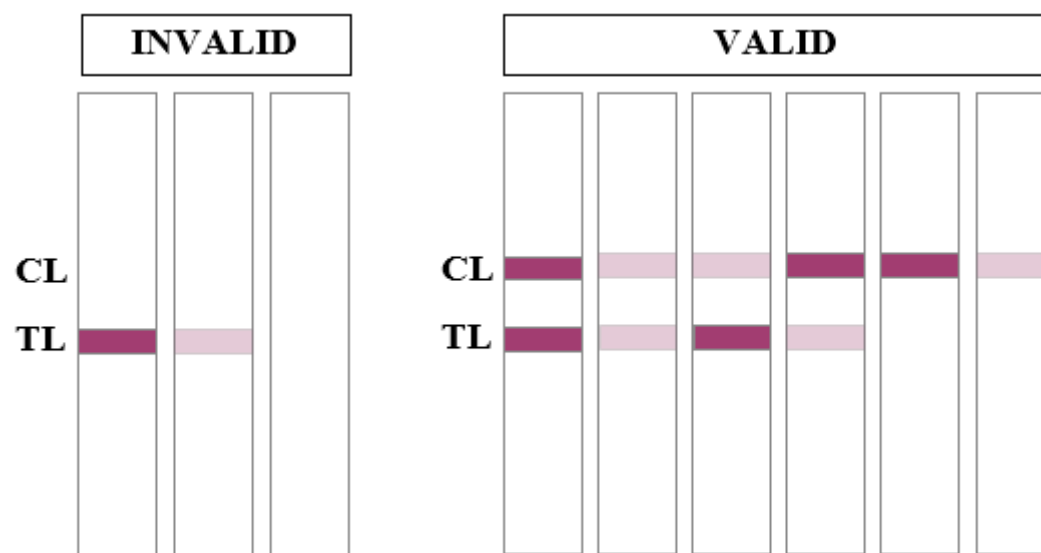
c. **Analysis Procedure for Quantitation Ranges 5 to 30 ppm**

- (1) Using a 100-1000 microliter (μL) pipet, add 900 μL dilution buffer to a clean dilution tube. Using a 100 μL pipet, add 100 μL **diluted sample** (from step “b” under **Dilution Procedure**) to the dilution tube containing 900 μL dilution buffer (Dilution factor is 10). Close tube, mix well by inverting a few times and the sample is ready for assay.
- (2) Perform an additional measurement by repeating steps (1) – (6) of the Analysis Procedure for Quantitation Ranges 0.5 to 6 ppm.

Note: To interpret results of samples for the second quantitation range a different bar code for reading the results using the AgraVision Reader has to be scanned prior to repetition of the analysis.

d. **Interpretation of Results:**

- (1) A color line always appears in the upper section of the test strip to indicate that the test strip is working properly. This line is the Control Line (C). A line in the lower section of the test strip indicates the test result. This line is the Test Line (T).
- (2) **Invalid results:** If there is no control line in the control zone, the test is invalid and the sample should be re-tested by using a new test strip. The AgraVision Reader will also indicate “invalid” if the strip is invalid.



- (3) **Valid results:** 2 lines are visible, or CL only. The intensity of the line in the test zone is dependent on Total Fumonisin concentration in the sample and must be measured with an AgraVision Reader.

5. 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).

6. STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

- (1) Store test kits at 4-8°C (40-47°F) when not in use, and do not use beyond the expiration date. Do not freeze. Do not leave it in direct sunlight.
- (2) Test strips must be kept inside their original tubes.
- (3) Conjugate microwells must be kept inside their original tubes.

b. Precautions

- (1) All reagents must be at room temperature before the assay is run.
- (2) Adhere to the GIPSA-issued test kit instructions for test procedures.
- (3) Do not re-use test strips and conjugate wells.
- (4) Consider all materials, containers and devices that are exposed to the sample to be contaminated with toxin. Wear protective gloves and safety glasses when using the kit.
- (5) The components in this test kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

7. EQUIPMENT AND SUPPLIES

a. **Materials Supplied with the Kit:**

- (1) 1 tube containing 24 AgraStrip Total Fumonisin test strips
- (2) 1 tube containing 24 AgraStrip Total Fumonisin WATEX Conjugate wells with lyophilized antibody particle complex (conjugate)
- (3) 1 bag containing 24 AgraStrip WATEX Extraction Buffer Bags.
- (4) 1 bottle of 30 mL AgraStrip Total Fumonisin WATEX Dilution Buffer
- (5) 1 bag of 48 yellow or white pipette tips.
- (6) 1 bag of blue pipette tips.
- (7) 1 bag of 24 micro centrifuge tubes (dilution tubes).
- (8) 24 Filter Whirl-Pak bags

b. **Materials Required but not Provided with Kit**

- (1) Romer Series II Mill or equivalent
- (2) EQOLE1010: Balance, 400 grams
- (3) EQOLE1050: 250 mL Graduated cylinder
- (4) Distilled or deionized water

c. **Assay Procedure**

- (1) Single channel pipette capable of pipetting up to 100 µL.
- (2) Single channel pipette capable of pipetting up to 1000 µL.
- (3) EQOLE1300: Timer
- (4) EQASR1003: AgraVision Reader without printer or EQASR1000: AgraVision Reader with printer
- (5) EQOEV2060: AgraStrip Incubator with timer and metal tweezers or EQASR1005: AgraStrip heat block with cover.





8. REVISION HISTORY

Revision 0 (07/05/2017)

9. FLOW CHART

AgraStrip Mycotoxin WATEX Quick Guide

Read the GLPSA issued instructions completely before performing any test.
Procedure of AgraStrip Fumonisin Quantitative Test WATEX.

 <p>1</p> <p>AgraStrip Incubator set to 45°C (pre-heat about 30 min)</p>	 <p>2</p> <p>50 g ground sample + 1 buffer bag</p>	 <p>3</p> <p>Add 150 mL distilled water</p>	 <p>4</p> <p>Shake vigorously for 2 min open bag and settle for 2 min</p>	<p>Result Interpretation A color line always appears in the upper section of the test strip to indicate that the test strip is working properly. This line is the control line.</p> <p>Invalid results If there is no control line in the control zone, the test is invalid and the sample should be re-tested by using a valid test strip.</p> <p>Valid results 2 lines are visible. The intensity of the line in the test zone is indirectly proportional to the mycotoxin concentration and has to be measured with the AgraVision Reader</p>
 <p>5</p> <p>1000 µL Fumonisin Dilution Buffer + 50 µL sample extract</p>	 <p>6</p> <p>Put lyophilized gold wells into heatblock add 100 µL diluted sample</p>	 <p>7</p> <p>Insert strip close heat block lid incubate for 3 min</p>	 <p>8</p> <p>Wipe strip onto an absorbent paper and read results within 1 min</p>	