

Test Kit Instructions

September 9, 2020

ROMER LABS

AGRASTRIP TOTAL AFLATOXIN QUANTITATIVE 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, reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

The U.S. Department of Agriculture (USDA) prohibits discrimination in its programs on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, and marital or familial status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternate means for communication of program information (Braille, large print, audiotope, etc.) should contact USDA's TARGET Center at (202) 720-2600 (voice and TDD).

To file a complaint, write to the USDA, Office of Civil Rights, Room 326-W, 1400 Independence Avenue, SW, Washington, DC 20250-9410, or call (202) 720-5964 (voice and TDD). USDA is an equal employment opportunity employer.

TABLE OF CONTENTS

1.	GENERAL INFORMATION	3
2.	PREPARATION OF TESTING MATERIALS AND EQUIPMENT	4
3.	EXTRACTION PROCEDURES	5
4.	SAMPLE PREPARATION FOR QUANTITATION	5
5.	TEST PROCEDURES	6
6.	REPORTING AND CERTIFYING TEST RESULTS	8
7.	STORAGE CONDITIONS AND PRECAUTIONS	8
8.	EQUIPMENT AND SUPPLIES	9
9.	REVISION HISTORY	10
10.	FLOW CHARTS	11

1. GENERAL INFORMATION

The AgraStrip Total Aflatoxin Quantitative WATEX test is a one-step lateral flow immunochromatographic assay for the quantitative screening of Aflatoxins 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. An AgraStrip Aflatoxin WATEX strip is placed into the microwell. The mixed content is then wicked onto the 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 Aflatoxins in the sample. The line is always visible in the control zone regardless of the presence of Aflatoxins. The strips are analyzed using an AgraVision Reader and the results are determined.

Approved Test Kit Information	
Test Kit Vendor:	Romer Labs, Inc. 1- 636 - 583-8600
Test Kit Name:	AgraStrip Total Aflatoxin Quantitative WATEX
Product Number:	10002138
Effective Date of Instructions:	9/9/2020
Conformance Range:	5.0 – 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)
Extraction Method:	Shake vigorously 50-gram sample with 150 milliliters (mL) of deionized or distilled water and one buffer packet by hand for 2 min or shake with similar shaking action (as hand shaking) for 2 minutes using a mechanical shaker.
Test Format:	Lateral Flow Strip
Detection Method:	AgraVision Reader (Product Model No.10002418)

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) Turn on the AgraVision reader using the power button located on the back.
- (2) Use the arrow keys on the keypad to highlight "TEST". Select it using the checkmark key.
- (3) Use the arrow keys on the keypad to highlight "Mycotoxin". Select it using the checkmark key.
- (4) 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.
- (5) Create a sample ID by using the alphanumeric keys on the keypad. Use the checkmark key to enter.
- (6) Since only one strip is being read; use the pound key to bypass to the next screen.
- (7) Enter the operator ID. Press the checkmark key to enter, and press it a second time to move to the next screen.
- (8) The reader is ready to read and will display "start measurement".

Note: Strips should not be read more than 1 minute after completion of the run.

Allow all samples, water used for extraction, and test kit materials to equilibrate to room temperature before use.

3. EXTRACTION PROCEDURE

- 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 on top of the ground sample in the Whirl-Pak bag.
 - (3) Using a 250 mL graduated cylinder, add 150 mL of distilled or deionized water and securely close Whirl-Pak bag.
 - (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 obtain the filtered extract (take extract from the opposite side of the filter from the sample).
This extract can only be used for the next 5 minutes.

4. SAMPLE PREPARATION FOR QUANTITATION

- a. Dilution procedure for corn: range 5 to 100 ppb
 - (1) Dilute the settled and filtered extract 1:21 with Dilution Buffer. Using a 1000 μ L pipette and a blue pipette tip, add 1000 μ L of Dilution Buffer to a dilution tube. Then, using a 100 μ L pipette and a yellow or white pipette tip, add 50 μ L of filtered extract into the dilution tube.
 - (2) Close tube, mix well by inverting a few times and the sample is ready for assay. This **diluted extract** 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.

5. TEST PROCEDURES

a. Analysis procedure for Quantitation Ranges 5 to 100 ppb and 100 to 300 ppb

- (1) Place the cover of the heat block on the top of the heat block. Break off one conjugate coated microwell for the sample to be run. Remove the sealing cap from conjugate microwell, and place the conjugate microwell inside the heat block. Ensure that the well is fully seated in the heat block. **Only one sample should be run at a time.**
- (2) Using a 100 µL pipette, add 100 µL of **diluted extract** to the conjugate microwell and mix the contents by pipetting it up and down 4 times.
- (3) Put one test strip into the microwell. Place the cover back into the heat block to cover the microwell and test strip.
- (4) Start the timer and develop the test strip for 3 minutes. Remove the test strip immediately once the 3 minutes are complete.
- (5) Wipe the end of the test strip onto an absorbent paper and insert the strip into the strip holder/tray for reading.

Note: After the test, the used microwell can be removed with tweezers.

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.

- (4) If the reader gives a value greater than 100 ppb, the previously diluted sample extract needs to be further diluted and run the test following “Quantitation Range 101 to 300 ppb” below.

c. Analysis procedure for Quantitation Range 101 to 300 ppb

If the reader gives a concentration greater than 100 ppb, the **diluted extract** needs to be further diluted and retested.

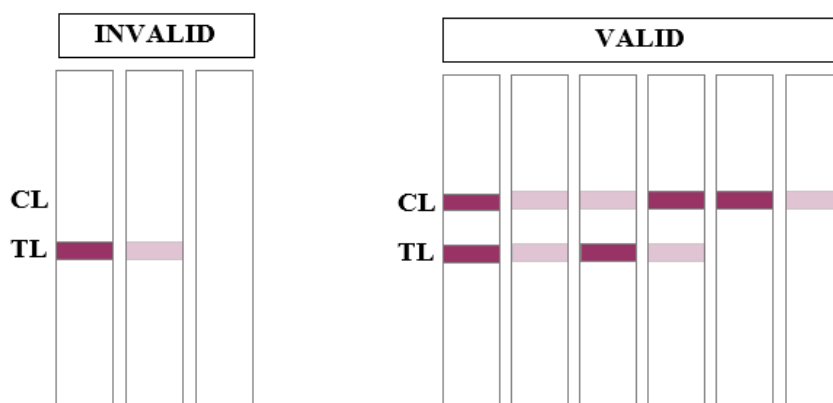
- (1) Dilute the **diluted extract** in a ratio of 1:10, i.e. using an adjustable 1000 µL pipette, add 900 µL of Dilution Buffer to a dilution tube.
- (2) Using a 100 µL pipette, add 100 µL of **diluted extract** to the Dilution Buffer. Close tube, mix well by inverting a few times and the sample is ready for assay
- (3) Perform the test by repeating steps (1) – (5) of section 5 “Test procedures” sections a. “Analysis Procedure for Quantitation Ranges 5 to 100 ppb and 100 to 300 ppb” followed by section b. “Reading the results.”
- (4) To interpret the results for the second quantitation range, a different bar code must be scanned using the AgraVision Reader prior to repetition of the analysis.

A final result less than 53 ppb (using 101 – 300 ppb quantitation range) is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the analysis using 5.0 – 100 ppb quantitation range and only perform the analysis using 101 – 300 ppb quantitation range again if the value is greater than 100 ppb.

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 (CL). A line in the lower section of the test strip indicates the test result. This line is the Test Line (TL).
- (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 or only CL are visible. The intensity of the line in the test zone is dependent on the Aflatoxin concentration in the sample.



6. 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@usda.gov).

7. 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) Conjugate microwells and the Test strips must be kept inside their original tubes.

b. Precautions and Testing Notes:

- (1) All reagents must be at room temperature before the assay is running.

- (2) Adhere to the FGIS issued instructions of 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.

8. EQUIPMENT AND SUPPLIES

a. Materials supplied with the kit

- (1) 1 tube containing 24 AgraStrip Aflatoxin WATEX test strips
- (2) 1 tube containing 24 AgraStrip Aflatoxin 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 Aflatoxin WATEX Dilution Buffer
- (5) 1 bag of 48 yellow or white pipette tips, 1 bag of 24 micro centrifuge tubes (dilution tubes)
- (6) 24 Filter Whirl-Pak bags

b. Materials Required but not Provided with the Kit

- (1) Romer #10002523: Romer Series II Mill or equivalent
- (2) Romer #10002581: Balance, 400 grams
- (3) Romer #10002612: 250 mL Graduated cylinder
- (4) Distilled or deionized water
- (5) Romer #10002573: Tweezers to easily remove microwells from incubator
- (6) Optional: Any shaker that is capable of 250 rpm

- (7) Romer #10002634: Single channel pipette capable of pipetting up to 100 µL
- (8) Romer #10002630: Single channel pipette capable of pipetting up to 1000 µL
- (9) Romer #10002421: AgraVision Reader without printer or 10002418: AgraVision Reader with printer
- (10) Romer #10002566: AgraStrip Incubator with timer and 10002423: AgraStrip heat block with cover


9. REVISION HISTORY

Effective 9/9/2020: Test kit was approved

10. FLOW CHART

AgraStrip Mycotoxin WATEX Quick Guide

Read the FGIS issued instruction completely before performing any test.
Procedure of AgraStrip Total Aflatoxin Quantitative Test WATEX



1

AgraStrip Incubator
set to **45°C**
(pre-heat about 15 min)



2

50 g ground sample
+ **1 buffer bag**



3

Add **150 mL** distilled or
deionized water



4

Shake vigorously for **2 min.**
open bag, settle for 2 min



5

1000 µL Fumonisin
Dilution Buffer +
50 µL sample extract



6

Put lyophilized gold wells
into heatblock, add **100 µL**
diluted sample, mix by
pipette up and down **4x**



7

Insert strip
close heat block lid
incubate for **3 min**



8

Wipe strip onto an
absorbent paper and read
results **within 1 min**

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.

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.

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