

Test Kit Instruction

May 14, 2020

Charm Sciences, Inc. **ROSA WET-S5 Zearalenone Quantitative Test**

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 FGIS 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 of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

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1. GENERAL INFORMATION

ROSA WET-S5 Zearalenone Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology and Water Extraction Technology (WET) that eliminates the use of organic solvents (e.g., methanol and ethanol). WET uses a non-hazardous extraction powder added to the sample followed by water to extract zearalenone into the aqueous solvent. Zearalenone interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the Charm EZ-M reader and interpreted as parts per billion (ppb) zearalenone.

Approved Test Kit Information

Test Kit Vendor:	Charm Sciences, Inc. 978-687-9200
Test Kit Name:	ROSA WET-S5 Zearalenone Quantitative Test
Product Number:	LF-ZEARQ-WETS5
Effective Date of Instructions:	05/14/2020
Conformance Range:	100 – 1000 ppb
Number of Analyses to Cover Conformance Range:	1
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), barley (with hull, including malting barley), distillers dried grains with solubles (DDGS), sorghum, and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings)
Extraction Method:	Shake vigorously 50-gram sample and contents of one packet of WET-S Extraction Powder with 150 mL deionized or distilled water for 1.5 minutes. For barley and distillers dried grain with solubles (DDGS), use two packets of WET-S Extraction Powder and 250 mL of deionized or distilled water.
Test Format:	Lateral flow test strip
Detection Method:	Reflectance
Reader:	Charm EZ-M reader, Model LF-ROSA-EZ-M; REQUIRES CALIBRATION UPDATE FOR USE

2. PREPARATION OF TESTING MATERIALS

a. Test Strips

Remove from the container only the number of test strips to be used in 1 day. Keep these test strips at room temperature (18 °C to 30 °C) during daily use for up to 12 hours; discard the unused test strips after the 12-hour period.

b. ZEARQ-WETS5 Dilution Buffer

(1) Dispense buffer into a clean micro-centrifuge tube and label for each sample to be tested.

(2) Use pre-dispensed buffer tubes and buffer solution at room temperature.

c. EP Control

(1) Prepare EP Control by dissolving one (1) packet of WET-S Extraction Powder in 150 milliliters (mL) deionized or distilled water (using 250 mL graduated cylinder) by gently swirling until extraction powder is dissolved.

(2) Use to prepare Positive Control.

d. Negative Control

(1) Use ZEARQ-WETS5 Dilution Buffer as Negative Control.

(2) Use Negative Control in TEST PROCEDURES section.

e. Positive Control

(1) Reconstitute the dry Positive Control (provided with test kit) by adding 300 microliters (µL) EP Control (using 100-1000 µL or 300 µL pipet) followed by 2.7 mL ZEARQ-WETS5 Dilution Buffer (using 100-1000 µL pipet as three 900 µL additions). Cap the bottle, shake well, and allow to stand for 10 minutes at room temperature; mix before use.

(2) Use reconstituted Positive Control to prepare Positive Control Extract.

f. Positive Control Extract

(1) Add 900 µL ZEARQ-WETS5 Dilution Buffer (using 100-1000 µL pipet) into a clean micro-centrifuge tube. Add 300 µL reconstituted Positive Control (using 100-1000 µL or 300 µL pipet) to buffer in the micro-centrifuge tube. Cap the micro-centrifuge tube, mix (shake vigorously for 5 seconds), and label.

(2) Use Positive Control Extract (within 4 hours of preparation) in TEST PROCEDURES section.

g Reader and Calibration Strip Performance Testing

- (1) Enter performance mode in Charm EZ-M reader by selecting Perf. Mon. from the Main Menu, followed by Perf. Test.
 - (a) Follow the system prompts to test calibration strips (LO CAL and HI CAL).
 - (b) Follow the system prompts to test controls (NEG CTRL and POS CTRL); select **ZEARQ-WETS5** from the TESTS list if prompted.
- (2) Test calibration strips daily to verify Charm EZ-M reader performance. Calibration strips must test/perform in the specified ranges; only use calibration strips that match the serial number of the Charm EZ-M reader.
- (3) Test Negative Control and Positive Control Extract weekly to verify test strip performance. Valid control ranges are:
 - (a) Negative Control: less than 15 ppb
 - (b) Positive Control Extract: 100 ppb to 300 ppb

If calibration strips or controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance. Notify your monitoring field office or TSD with any documented information for quality control purposes.

h ROSA Incubator

- (1) ROSA Incubator must be clean and level.
- (2) The ROSA Incubator temperature must be at $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (the temperature indicator should match the incubator temperature).

3. EXTRACTION PROCEDURES

- a **3:1 Extraction Procedure for corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), sorghum, and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings):**
- (1) Weigh 50.0 grams \pm 0.2 grams ground sample into a clean extraction container.
 - (2) Add contents of one (1) packet of WET-S Extraction Powder.
 - (3) Add 150 mL deionized or distilled water (using 250 mL graduated cylinder).
 - (4) Extract for 1.5 minutes by shaking vigorously by hand or mechanical shaker.

- (5) Transfer 1 mL to 1.5 mL extract (using transfer pipet) into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (centrifuge within 30 minutes of extraction and use within 2 hours).

For sorghum only, filter centrifuged extract by drawing into syringe and passing through RC15 filter (purchased separately). Collect filtered extract in a clean micro-centrifuge tube and label.

- (6) Repeat steps for additional samples.

b. **5:1 Extraction Procedure for barley (with hull, including malting barley) and distillers dried grain with solubles (DDGS):**

- (1) Weigh 50.0 grams \pm 0.2 grams ground sample into a clean extraction container.
- (2) Add contents of two (2) packets of WET-S Extraction Powder.
- (3) Add 250 mL deionized or distilled water (using 250 mL graduated cylinder).
- (4) Extract for 1.5 minutes by shaking vigorously by hand or mechanical shaker.
- (5) Transfer 1 mL to 1.5 mL extract (using transfer pipet) into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (centrifuge within 30 minutes of extraction and use within 2 hours).
- (6) Repeat steps for additional samples.

4. SAMPLE PREPARATION FOR QUANTITATION

a. Prepare **Diluted Extract** for corn, sorghum, and wheat for 100 ppb to 1000 ppb quantitation.

- (1) Add 900 μ L ZEARQ-WETS5 Dilution Buffer (using 100-1000 μ L pipet) into a clean micro-centrifuge tube.
- (2) Add 100 μ L centrifuged or filtered extract (using 100 μ L pipet) to the micro-centrifuge tube containing 900 μ L ZEARQ-WETS5 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label as **Diluted Extract**.
- (3) Repeat steps for additional samples.
- (4) Use **Diluted Extract** (within 6 hours of preparation) as your test sample in Sample Analysis found in TEST PROCEDURES section.

b. Prepare **Diluted Extract** for barley and DDGS for 100 ppb to 1000 ppb quantitation.

- (1) Add 1000 μ L ZEARQ-WETS5 Dilution Buffer (using 100-1000 μ L pipet) into a clean micro-centrifuge tube.

- (2) Add 200 µL centrifuged extract (using 200 µL or 100-1000 µL pipet) to micro-centrifuge tube containing 1000 µL ZEARQ-WETS5 Dilution Buffer, cap, mix (shake vigorously for 5 seconds), and label as **Diluted Extract**.
- (3) Repeat steps for additional samples.
- (4) Use **Diluted Extract** (within 6 hours of preparation) as your test sample in Sample Analysis found in TEST PROCEDURES section.

5. TEST PROCEDURES

a Sample Analysis.

- (1) Check that the ROSA Incubator temperature is $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
- (2) Label test strip(s) to identify sample.
- (3) Place test strip in the ROSA Incubator with the flat side facing upward.
- (4) Hold the test strip flat in the ROSA Incubator and use tab to expose sample compartment by peeling tape back to “Peel to Here” line.

Avoid lifting the test strip and sponge under tape and bending back the white wick and sponge under the tape.

- (5) Hold the pipet vertically and slowly add 300 µL test sample (**Diluted Extract** or control using 300 µL pipet) into the sample compartment at the ROSA Incubator line.
- (6) Reseal the tape over the sample pad compartment.

NOTE: Incubate no more than two test strips in a single ROSA Incubator at a time:

- (a) Peel, pipet, and reseal before starting next test strip.
 - (b) Complete procedure for both test strips within 1 minute.
- (7) Close lid on the ROSA Incubator.
 - (8) Incubate test strip(s) for 5 minutes.
 - (9) Remove test strip from the ROSA Incubator.

Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted.

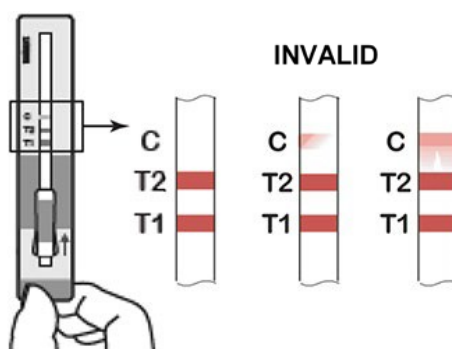
NOTE: When running multiple test strips in ROSA Incubator, remove and interpret one test strip at a time.

- (a) Wipe foreign matter (dust, etc.) from the test strip.

- (b) Inspect and read test strips within 1 minute of incubation completion.
- (c) Lower ROSA Incubator lid; do not re-latch.

b. Visual Inspection.

- (1) The test strip is INVALID if any of the following are observed:
 - (a) C (Control) line is missing.
 - (b) T1 or T2 (Test) lines or C line is smeared or uneven.
 - (c) T1, T2, or C line is obscured by diluted extract or control.
 - (d) Beads do not flow past T1, T2, or C lines.



- (2) Do not put INVALID test strips in the Charm EZ-M reader.
- (3) If test strip is INVALID, re-test the diluted extract or control.

c. Interpretation.

- (1) Insert a clean and valid test strip into the Charm EZ-M reader. Slide the test strip into the slot with the sample compartment in the down position until it stops.

NOTE: Ensure that test strip is fully inserted until it stops in the slot of the Charm EZ-M reader. Failure to insert properly can result in misinterpretation.

- (2) Read results on **ZEARQ-WETS5** from the TESTS list with COMMODITY and DILUTION (DE for Diluted Extract) selected for sample. If desired, enter OPERATOR ID, SAMPLE ID, and/or LOT NUMBER. Close door to read.

NOTE: For controls, see Reader and Test Strip Performance Testing in PREPARATION OF TESTING MATERIALS AND EQUIPMENT section.

- (3) **READING:** The number displayed is the concentration of zearalenone (ppb) in the sample.

A Diluted Extract READING greater than 1000 ppb indicates that the sample concentration is greater than the sensitivity range of the sample dilution; report test result as greater than 1000 ppb on the work record and certify as “Zearalenone exceeds 1000 ppb”.

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 strips refrigerated (0 °C to 7 °C) in tightly closed supplied container.
- (2) Store dilution buffer bottle and pre-dispensed micro-centrifuge tubes refrigerated.
- (3) Store WET-S Extraction Powder at room temperature in supplied packet.
- (4) Store EP Control refrigerated for up to 1 week or aliquot (at least 1.5 mL) to clean micro-centrifuge tubes, label, and freeze (-15 °C or below) within 6 hours of reconstitution for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed EP Control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.
- (5) Store dry Zearalenone Positive Control refrigerated.
- (6) Store reconstituted Positive Control refrigerated for up to 1 week or aliquot (at least 0.5 mL) to clean micro-centrifuge tubes, label, and freeze within 6 hours of reconstitution for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed Positive Control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.

b Precautions.

- (1) Test Strips.
 - (a) To open test strip canister, remove and save plastic lid with foil-lined foam insert to reseal container, lift foil tab, and peel foil seal off container. Discard foil seal.
 - (b) In high humidity, limit condensation by opening container after it has warmed to room temperature.

- (c) Inspect/verify desiccant indicator. Beads inside desiccant packets should be blue. Discard test strips if the blue beads have turned purple or pink.
- (d) Re-shape dented sample compartments to fit into ROSA Incubator.
- (2) Use ZEARQ-WETS5 Dilution Buffer supplied with each test kit only at room temperature. Keep buffer at room temperature during daily use for up to 12 hours; place back in refrigerator after daily use.
- (3) WET-S Extraction Powder is non-hazardous and may be disposed as normal waste. Do not open WET-S Extraction Powder until ready to use.
- (4) Do not use the test kits beyond the noted expiration date.
- (5) Debris on test strips may alter the reader optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into reader.
- (6) ROSA Incubator must be clean and level. ROSA Incubator temperature must be $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The temperature indicator should match the ROSA Incubator temperature. A daily thermometer check is recommended. Keep ROSA Incubator lid lowered, but not latched, unless performing test procedure. ROSA Incubator may take 10 minutes to reach proper temperature depending on ambient temperature.
- (7) Charm EZ-M reader must be clean and level. Keep reader lid closed unless performing procedure.

8. EQUIPMENT AND SUPPLIES

a Test Strips.

- (1) LF-ZEARQ-WETS5-20K/-20ESK
 - (a) One container of 20 ZEARQ-WETS5 test strips
 - (b) Zearalenone Positive Control(s)
 - 1 One control in LF-ZEARQ-WETS5-20K
 - 2 Two controls in LF-ZEARQ-WETS5-20ESK
 - (c) One ZEARQ-WETS5 Dilution Buffer
- (2) LF- ZEARQ-WETS5-100K/-100ESK
 - (a) One container of 100 ZEARQ-WETS5 test strips
 - (b) Zearalenone Positive Control(s)
 - 1 One control in LF-ZEARQ-WETS5-100K

- 2 Five controls in LF-ZEARQ-WETS5-100ESK
 - (c) Two ZEARQ-WETS5 Dilution Buffers
 - (3) LF- ZEARQ-WETS5-500K/-500ESK
 - (a) Five containers of 100 ZEARQ-WETS5 test strips
 - (b) Zearalenone Positive Controls
 - 1 Five controls in LF-ZEARQ-WETS5-500K
 - 2 Twenty-five controls in LF-ZEARQ-WETS5-500ESK
 - (c) Ten ZEARQ-WETS5 Dilution Buffers
- b. WET-S Extraction Powder
 - (1) LF-WET-EXTS50G-20: WET-S Extraction Powder for 50 gram sample (20/pack)
 - (2) LF-WET-EXTS50G-100: WET-S Extraction Powder for 50 gram sample (100/pack)
- c. Materials required but not provided
 - (1) 100 µL pipet and pipet tips (Charm order code: PIP-100UL-1STOP and 100-ULT-X1 (rack of 96 tips))
 - (2) 200 µL pipet and pipet tips (Charm order code: PIP-200UL and 100-ULT-X1 (rack of 96 tips))
 - (3) 300 µL pipet and pipet tips (Charm order code: PIP-300UL-1STOP-M and 1-MLT-96 (rack of 96 tips))
 - (4) 100-1000 µL variable volume pipet and pipet tips (Charm order code: PIP-100-1000UL-1STOP and 1-MLT-96 (rack of 96 tips))
 - (5) 250 mL graduated cylinder (Charm order code: GRAD-CYL-250ML)
 - (6) FGIS-approved scale (balance) with minimum division of 0.1 g
 - (7) Charm EZ-M reader (Charm order code: LF-ROSA-EZ-M)
 - (8) Deionized or distilled water
 - (9) Extraction containers or Whirl-Pak bags (Charm order code: WHIRLPK-50 (50/PK))
 - (10) Mechanical shaker (Charm order code: SHAKER-110V)
 - (11) Micro-centrifuge tubes (Charm order code: CEN-2-0ML-TUBES-100 (100/PK))

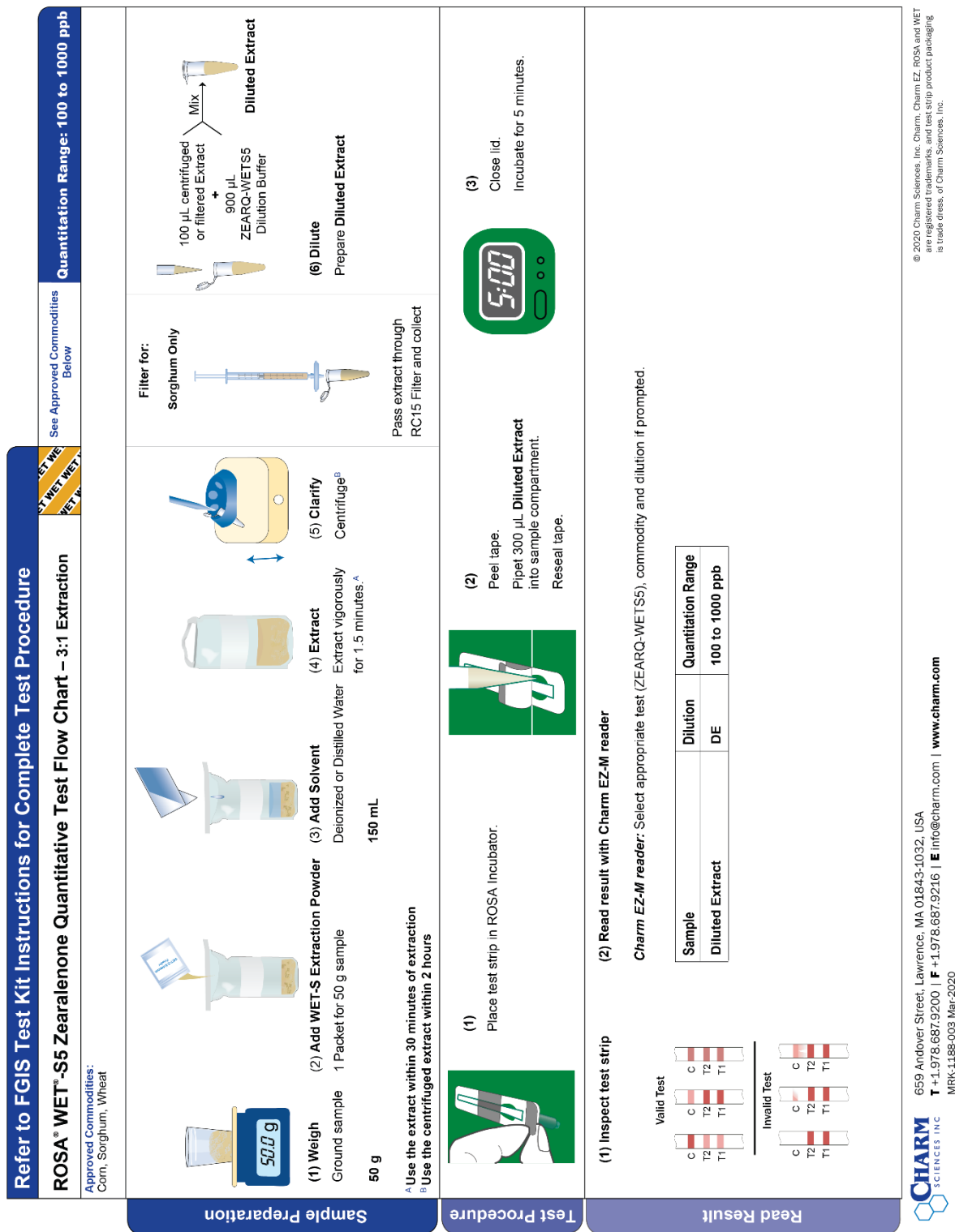
- (12) Mini-centrifuge (Charm order code: MINICEN-110V)
- (13) Printer for Charm EZ-M reader (Charm order code: PRN-THERM-CITIZEN)
- (14) ROSA Incubator (Charm order code: LF-INC4-5-45D or LF-INC2-5-45)
- (15) Sample grinder (see FGIS Mycotoxin Handbook)
- (16) Transfer pipets (Charm order code: PIP-3ML-100K (100/PK))

9. REVISION HISTORY

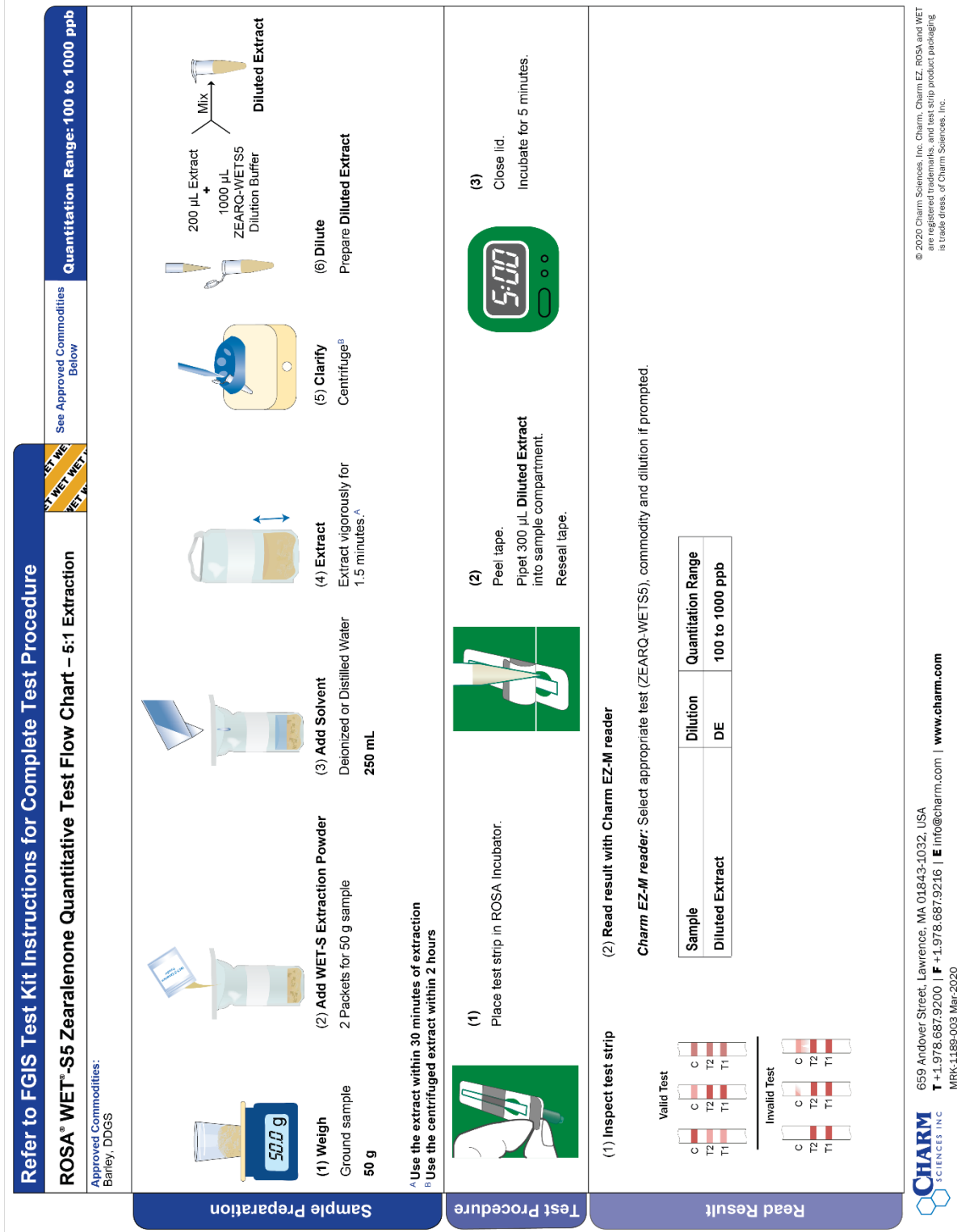
Effective 05/14/2020

10. FLOW CHARTS

a Flow chart for 3:1 extraction procedure and assay for corn, sorghum, and wheat:



b. Flow chart for 5:1 extraction procedure and assay for barley and DDGS:



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