

Criteria & Specifications

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Design Criteria and Test Performance Specifications for Quantitative Deoxynivalenol Test Kits

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1. PURPOSE AND SCOPE

The United States Department of Agriculture's Federal Grain Inspection Service (FGIS) provides official mycotoxin testing services throughout the United States for domestic and export grains, oilseeds, and processed-grain commodities. Official testing services are available for aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, and zearalenone. Testing at field locations requires rapid, simple, inexpensive, and accurate methods to effectively assess the quality of U.S. grain. An important part of quality assurance for official mycotoxin testing is the Mycotoxin Test Kit Evaluation (MTKE) Program, through which FGIS evaluates and certifies the conformance of quantitative rapid mycotoxin test methods to specific criteria. Only rapid test kits having FGIS certification are approved for official mycotoxin testing. Updated test kit design criteria and performance specifications for quantitative deoxynivalenol test kits are covered herein. These requirements are effective March 1, 2016.

NOTE: Until March 1, 2017, applicants may submit test kits for evaluation under the previous requirements issued in September 2010. Certificates issued under the September 2010 requirements will expire one year from the date of issuance. For a copy of the previous requirements, contact the MTKE Program Leader at 816-891-0401 or [MTKE.Program.Leader](#).

2. SUMMARY OF TEST KIT EVALUATION PROCESS

FGIS establishes design criteria and performance specifications that mycotoxin test kits must meet to be considered for use in official inspection. Test kit manufacturers may submit a test kit for evaluation by FGIS staff. Submission packets are reviewed by FGIS staff in order of receipt. The submission packet must include all documentation needed to demonstrate that the test kit meets the established FGIS design criteria and performance specifications. Incomplete submissions, submission not conforming to FGIS requirements, and submissions containing excessive errors will be rejected. If the submission is accepted, arrangements for analyst training and FGIS performance verification will be made with the applicant. The FGIS analysts that conduct performance verification testing will be trained in the operation of the test kit by the applicant.

If the test kit meets all design and performance requirements, FGIS issues a Certificate of Conformance (COC) stating that the test kit has met the criteria. Upon issuance of the official test kit instructions, the test kit may be used for official inspection. The COC will be valid for three years from its date of issuance. Renewal of a COC requires a full submission and evaluation.

If the test kit fails to meet all of the criteria specified herein, the test kit can be resubmitted after a three-month waiting period. When the test kit is resubmitted, the applicant must state the corrective action that was taken to bring the test kit into conformance with FGIS requirements.

3. PROGRAM CONTACT INFORMATION

Send submission packets via email to [MTKE.Program.Leader](#). For questions and comments regarding mycotoxin test kit evaluation, contact the Mycotoxin Test Kit Evaluation Program Leader at 816-891-0401 or [MTKE.Program.Leader](#).

4. FEE FOR TEST KIT EVALUATION

FGIS will assess a fee for evaluating a test kit, including all documentation reviews. Payment is expected within 30 days of the invoice date.

5. DEFINITIONS

Deoxynivalenol (DON). 3, 7, 15-trihydroxy-12,13-epoxytrichothec-9-en-8-one, also known as vomitoxin.

Applicant. The manufacturer of the test kit under evaluation.

Fortified Samples. Samples to which a known amount of DON was previously added using a standard solution.

Lower Conformance Limit. The lowest concentration at which the test kit has demonstrated acceptable quantitation of DON according to FGIS requirements. Submitted test kits must have a lower conformance limit (LCL) of 0.50 ppm.

Upper Conformance Limit. The highest concentration at which the test kit has demonstrated acceptable quantitation of DON according to FGIS requirements. Submitted test kits must have an upper conformance limit (UCL) of at least 30 ppm DON. Submissions with a desired UCL of greater than 30 ppm must be accompanied by additional performance data from the applicant at this level, and the test kit must pass a FGIS performance verification study at the proposed limit.

Range of Conformance. The concentration range for which a test kit conforms to FGIS accuracy requirements. This range is bracketed by the upper and lower conformance limits. The minimum range of conformance required by FGIS for quantitative DON test kits is 0.50 – 30 ppm.

Range of Quantitation. The concentration range associated with a single analysis. A test kit may have more than one range of quantitation if additional dilutions, reader calibrations, and analyses are needed to cover the range of conformance.

Test Kit. A commercially packaged system of the principal or key components of an analytical method used to determine the presence of DON in specified matrices. Test kits include directions for their use and are self-contained, complete analytical systems; however, they may require supporting supplies and equipment. The key components frequently represent proprietary items that may be available only from the manufacturer of the test kit.

6. DESIGN AND PERFORMANCE REQUIREMENTS

a. Grinding and Homogenizing Sample Materials

All commodities used in the performance studies must be ground so that at least 95% passes through a U.S. Standard No. 20 sieve and mixed thoroughly to ensure homogeneity prior to removing samples for testing.

b. Sample Storage

Sample materials must be stored in sealed containers at ≤ 8 °C, and refrigerated samples must be allowed to reach room temperature before use.

c. Preparation of Reference Materials

A validated analytical reference method, preferably the FGIS method, must be used for determining the DON concentration in all naturally contaminated materials and materials used to prepare fortified samples that are used in the performance studies. The applicant must state what analytical method was used to certify the reference materials. If a method other than the current FGIS reference method is used, a copy of the method instructions and validation report or literature reference must be supplied by the applicant. This method must have performance characteristics (accuracy and precision) that are equivalent to the FGIS reference method. Determination of method equivalence resides with the applicant. The FGIS reference method is available upon request.

The DON concentration in each naturally contaminated reference material must be determined by analyzing the minimum of twenty-one samples (25.0 ± 0.1 g each) across three batches (seven samples in each batch) using the FGIS reference method or equivalent method. For use of a material in the test kit trials, the mean DON concentration must be within $\pm 15\%$ of the target concentration.

DON-contaminated materials produced through fungal inoculation and growth under laboratory conditions may be used in place of naturally contaminated materials. A relevant fungal species and strain isolated from the Continental United States must be used for producing the contaminated commodity. Although inoculation is allowed for generating contaminated samples, the test kit will be expected to meet FGIS performance criteria when determining DON on any naturally contaminated grain or processed-grain commodity sample originating in the United States.

d. Preparation of Standard Solutions

The purity of the neat DON standard must be $\geq 98\%$. A certificate of analysis from the supplier of each neat standard must be provided in the submission packet. The concentration of the DON in standard solutions must be determined by UV spectrophotometry as specified in the FGIS reference method.

e. Conditions of Analysis

All analyses must be conducted at room temperature (i.e., 20 – 24 °C) unless specified otherwise.

When generating test kit data for submission, all samples must be labeled with a code and fully randomized so that the analyst conducting the test cannot know the level of analyte. Furthermore, the same person performing the analyses must not prepare the test samples.

f. Written Instructions for Test Kit Use

The applicant must provide complete, written instructions for use of the test kit that accurately reflect the procedures followed to generate the data in the submission. These instructions must be formatted following the guidelines in Appendix B. FGIS will follow these written instructions during training and when verifying test kit performance.

g. Multiple Procedures

A single, well-defined, analytical method will be evaluated for each submission. Multiple procedures, such as options for the use of blending vs. shaking for sample extraction or filtration vs. centrifugation for sample clarification, require a full data submission for each procedure; each procedure will be evaluated by FGIS. Other instances of multiple procedures may require a full data submission packet and evaluation for each procedure depending on the significance of the variation. FGIS will determine the need of a separate evaluation on a case-by-case basis taking into consideration the possible effect of procedural variation and its benefit to the official inspection system. Prior to test kit submission, the MTKE Program Leader should be contacted for further guidance on data submission and evaluation requirements in such cases.

h. Time for Completion of Analysis

For a pre-ground sample, the test kit procedure must be capable of generating an accurate result across the minimum required range of 0.50 – 30 ppm DON in 30 minutes or less (i.e., time from extraction to final result). If the test kit has a limited quantitative range and the need for additional dilution and analysis to cover the minimum range of conformance, the 30-minute limit includes all steps needed to generate the final result.

i. Significant Figures and Unit of Measurement

The test kit reader (or detector) must display results using at least two significant figures in parts per million (ppm). Test kit results will be evaluated using two significant figures. When rounding to two significant figures, the number will be rounded up when the third digit is 5 or more. For example, 5.25 ppm would be rounded to 5.3 ppm, and 5.24 ppm would be rounded to 5.2 ppm. To avoid rounding error, at least three significant figures should be used when performing calculations, and only the final calculated result should be rounded to two significant figures.

j. Accuracy

(1) Minimum Required Range of Conformance

The applicant must provide data demonstrating accuracy of the test kit using naturally contaminated corn and wheat at the targeted concentrations of 0.50, 2.0, 5.0, and 30 ppm DON.

Three analysts must each extract seven separate samples (50 ± 0.2 g each) at each concentration according to the test kit instructions. Each analyst must use a separate test kit manufactured lot. All samples must be analyzed as if the concentrations were unknown.

At least 95% of the results (20 out of 21) for each concentration level must be within the acceptable ranges specified in Table 1. The acceptable range for each material will be adjusted using the mean concentration derived from the reference method analyses and the corresponding Maximum Relative Standard Deviation (RSD) from Table 1.

Table 1. Acceptable Limits			
DON* (ppm)	Maximum RSD (%)	Standard Deviation (ppm)	Acceptable Range (ppm)
0.50	20	0.10	0.30 – 0.70
2.0	12	0.24	1.5 – 2.5
5.0	10	0.50	4.0 – 6.0
30	10	3.0	24 – 36
* $\pm 15\%$			

(2) Extended Range of Conformance

For an UCL above 30 ppm DON, the applicant is required to provide data demonstrating the test kit's capability for meeting the performance requirements at the proposed UCL. The applicant must also provide the analytical procedure used to generate the supporting data. The use of naturally contaminated materials to generate supporting data is preferred; however, fortified samples are acceptable. The FGIS reference method or equivalent must be used to characterize the naturally contaminated material as described previously. The standard solution used for fortification of additional commodity samples must be prepared according to Section 6.d. After fortification, samples must be left open in a fume hood for approximately 30 minutes to allow the solvent to evaporate prior to extraction.

Three analysts must each extract seven separate samples (50.0 ± 0.2 g each) at the UCL according to the test kit instructions. Each analyst must use a different test kit manufactured lot. All samples must be analyzed as if the

concentrations were unknown. At least 95% of the results (20 out of 21) must be within the range specified in Table 2.

Table 2. Acceptable Limits for Extended UCL			
DON (ppm)	Maximum RSD (%)	Standard Deviation (ppm)	Acceptable Range (ppm)
UCL > 30	10	0.10·UCL	UCL ± 0.20·UCL

(3) Multiple Ranges of Quantitation

Analyses associated with additional dilutions and reader calibrations may be needed to cover the entire range of conformance. A range of quantitation is associated with each one of these analyses. The test kit must conform to FGIS accuracy requirements across each range of quantitation. If multiple analyses are needed to cover the range of conformance, the applicant must provide data demonstrating conformance to FGIS accuracy requirements at the upper and lower limits of each range of quantitation. As a result, additional data to that specified in Section 6.j.(1) and 6.j.(2) may be required depending on the individual ranges of quantitation.

To determine the Maximum RSD for concentrations not covered in Table 1, use the following equation and round to the nearest whole percentage:

Maximum RSD (%) = $15.848 \cdot C^{-0.308}$; where C is the DON concentration in ppm. For concentrations 5.0 ppm and above, the Maximum RSD is 10%.

(4) Additional Commodities

Application for approval of additional commodities can be made at the time of original submission or after the test kit has been approved. The list of acceptable commodities is found in Appendix C. Contact the MTKE Program Leader for possible incorporation of additional commodities not included in this list. The applicant must provide the additional commodity name and definition.

Applicants must provide data in accordance with the following guidelines for each additional commodity and the specific procedures followed for analysis. Fortified samples can be used to generate the supporting data; however, the test kit will also be expected to adhere to the accuracy requirements for naturally contaminated samples. The standard solution used for fortification of additional commodity samples must be prepared according to Section 6.d. After fortification, samples must be left open in a fume hood for approximately 30 minutes to allow the solvent to evaporate prior to extraction. GIPSA may verify performance of the test kit using fortified or naturally contaminated samples.

Uncontaminated (blank) samples of each additional commodity must be ground with a mill so that at least 95% of the material passes through a U.S. Standard No. 20 sieve. Ground portions must be mixed to ensure homogeneity and divided in a manner to maintain sample representativeness. A description of the specific equipment and procedures used in grinding, mixing, sieving processes, and dividing must be provided.

For each additional commodity, five fortified samples (50 ± 0.2 g each) must be tested at each targeted level of 0.50, 2.0, 5.0, and 30 ppm DON. For an UCL limit above 30 ppm, five samples at the proposed UCL must also be included in the study.

One analyst must extract all samples according to the test kit instructions. For each additional commodity, the analyst must analyze one aliquot of each extract.

All of the results must be within the ranges specified for the fortification levels according to Table 1 and Table 2.

k. Hazardous Materials and Waste

Use of the test kit must not expose employees, without access to fume hood or respirator, to toxic or hazardous substances beyond OSHA Standards specified in 29 CFR. The test kit must not require special waste disposal (e.g., special waste disposal includes all radioactive material, P-listed hazardous waste, or hazardous waste listed for its toxicity characteristic as defined in 40 CFR). The applicant must attest that the test kit meets these requirements. Safety Data Sheets (SDS) must be provided for all materials used in the test kit

l. Sensitivity to Electromagnetic Fields

Electronic equipment used with the test kit must not be sensitive to electromagnetic fields spanning a frequency of 800 MHz to 6 GHz and an intensity of 1.0 volts/meter to 5.0 volts/meter. Electric fields must not cause the equipment display to be corrupted or otherwise affect readings during the test. The applicant must provide a statement of certification from an accredited laboratory verifying the equipment meets these requirements. Laboratories conducting the electromagnetic study must be accredited under the National Volunteer Laboratory Accreditation Program operated by the National Institute of Standards and Technology, Gaithersburg, Maryland. Tests must be performed according to the guidelines in (EMC)-IEC 61000-4-3 (International Electrotechnical Commission, Geneva, Switzerland).

m. Temperature Sensitivity

Wheat that is naturally contaminated with DON at 2.0 ppm must be used in the temperature sensitivity study. Seven samples (50.0 ± 0.2 g each) must be extracted, and one analysis of each extract must be performed at each of three temperature conditions (i.e., 18 °C, 24 °C, and 30°C) to yield seven analyses at each temperature. Extracted samples must be protected from light during this study. All test materials (i.e., extracts and test kit components) must be allowed to equilibrate to the targeted temperature for one hour using an environmental chamber prior to analysis. At least 95% of the results (20 out of 21) must be within the range as specified in Table 1.

n. Shelf Life

The applicant must provide a shelf life label statement and supporting data under the recommended storage conditions for all materials and reagents used in their tests. In addition, the applicant must provide the procedure followed for generating and analyzing the stability data. Real-time data is preferred for supporting stability claims; however, accelerated aging based on the Arrhenius equation may be used. The stability data must demonstrate conformance to all applicable criteria in this document.

o. FGIS Performance Verification

After acceptance of a submission, the applicant will be contacted to arrange for training of three analysts who will conduct the FGIS performance verification. The training allows the applicant to provide hands-on training to the FGIS analysts for the sample preparation and equipment operation procedures. The analysts will receive up to one day of training in the use of each test kit by the applicant or designee. The applicant is required to provide all equipment and supplies needed to complete the training and FGIS performance verification. Any naturally contaminated materials used for the performance verification will be characterized as specified in Section 6.c using the current FGIS reference method.

(1) Minimum Required Range of Conformance

FGIS will verify the test kit's performance following the procedure as described in Section 6.j.(1) at the targeted levels of 0.50, 2.0, and 30 ppm DON in corn and wheat.

(2) Extended Range of Conformance

For an UCL greater than 30 ppm, FGIS will verify the test kit's performance following the procedure as described in Section 6.j.(2).

7. MANUFACTURER'S NOTIFICATION RESPONSIBILITIES

Manufacturers holding a COC must notify the MTKE Program Leader in writing when any changes or alterations are made to the test kit, including reagents, shelf life of test kit materials, equipment used in the test kit, software or calibration revisions, or any part of the analytical method. Alterations to a test kit will be evaluated by FGIS for significance and may require another full FGIS performance verification. Failure to notify FGIS of such changes will serve as grounds for immediate cancellation of the COC. Changes to the kit packaging, brochure, or other marketing information are exempt from this requirement.

Appendix A: Protocol & Notification Agreement Statement

This is to certify that I am an official representative of _____, that I fully understand the conditions that FGIS will use to determine if our quantitative deoxynivalenol test kit marketed under the trade name

will be given a Certificate of Conformance for use in the official inspection system. If issued, the Certificate of Conformance will be valid for three years from the date. **FGIS monitors the performance of all approved test kits and reserves the right to verify the performance of the test kit at any time.** I understand that if the kit fails to meet the criteria set forth in the performance specification document, the Certificate of Conformance will be revoked immediately. I further understand that the Certificate of Conformance will expire after three years and the kit must be completely re-evaluated to renew certificated status. I accept these conditions and agree to abide by the Manufacturer's Notification Responsibilities provided in this document.

Name

Date

Title

Appendix B: Guidelines for Test Kit Instructions

The following section headings and outline format must be used for official test kit instructions. In addition to the instructions, FGIS recommends the use of a flow chart to summarize the test procedure. The flow chart may be submitted as an appendix. An example of a completed set of test kit instructions is available from FGIS upon request. Pictures should be included in the written instructions only if necessary.

Section Headings

GENERAL INFORMATION
PREPARATION OF TESTING MATERIALS AND EQUIPMENT
EXTRACTION PROCEDURES
SAMPLE PREPARATION FOR QUANTIFICATION
TEST PROCEDURES
SUPPLEMENTAL ANALYSIS
REPORTING AND CERTIFYING TEST RESULTS
STORAGE CONDITIONS AND PRECAUTIONS
EQUIPMENT AND SUPPLIES
REVISION HISTORY
FLOW CHARTS

Outline Format

1. XXXXX

Text aligns under the indent level

a. XXX
b. XXX

(1) XXX
(2) XXX

(a) XXX
(b) XXX

1 XXX
2 XXX

a XXX
b XXX

Appendix C. Commodity List

Commodity	
1.	Amaranth
2.	Barley (with hull, including Malting Barley)
3.	Malted Barley (including Malted Barley Flour)
4.	Hulled Barley (including Hulless Barley)
5.	Pearl or Pearled Barley (including Quick Pearl Barley)
6.	Barley Grits
7.	Barley Flakes
8.	Buckwheat
9.	Corn (including Dent or Field Corn, Corn Meal, Corn Flour, Cracked Corn, Corn Grits or Polenta, and Corn screenings)
10.	Corn Bran
11.	Corn Germ
12.	Corn Germ Meal
13.	Corn Gluten Feed (including Corn Gluten Feed Meal)
14.	Corn Gluten Meal
15.	Flaking Corn Grits
16.	Corn Starch
17.	Corn/Soy Blend
18.	Corn/Soy Blend Plus
19.	Corn/Soy/Whey Blend
20.	Popcorn
21.	Super Cereal Plus
22.	Sweet Corn
23.	Condensed Distillers Solubles
24.	Cottonseed Meal
25.	Cottonseed Hulls
26.	Whole Cottonseed (including Linted Cottonseed and Delinted Cottonseed)
27.	Distillers Dried Grain

Appendix C. Commodity List (continued)

Commodity	
28.	Distillers Dried Grain w/Solubles
29.	Hominy (including Hominy Grits)
30.	Hominy Feed
31.	Millet
32.	Oats (Whole Oats with Hull)
33.	Oat Groats
34.	Oat Bran
35.	Rapeseed
36.	Rapeseed Meal
37.	Brown Rice
38.	Milled Rice (including Brewer's Rice and Glutinous Rice)
39.	Rough Rice
40.	Rice Bran
41.	Wild Rice
42.	Rye (or Rye Berries, including Whole Grain Rye Flour, Rye Meal, Cracked Rye, and Rye Chops)
43.	White Rye Flour
44.	Rye Flakes
45.	Wheat (including Whole Grain Wheat Flour, Wheat Middlings, Wheat Red Dog, Wheat Flour 2nd Clear, and Wheat Screenings)
46.	White Wheat Flour
47.	Wheat Bran (including Wheat Bran Aleruone)
48.	Wheat Gluten
49.	Sorghum
50.	Soybean (including Whole Soybean and Full-Fat Soy Flour)
51.	Defatted Soy Flour
52.	Soybean Meal
53.	Soybean Hulls
54.	Triticale

Appendix D: Test Kit Evaluation Submission Form

The applicant must fill out the submission form and provide it along with the submission packet. The information provided in the submission form will be used in creating the certificate if the test kit meets all FGIS requirements; therefore, it is imperative that the form be filled out correctly. A fillable version of this form is available on the AMS Website.

Contact Information	
Applicant (i.e., test kit manufacturer)	
Street Address	
City, State, Zip Code	
Contact Person	
Phone Number	
Email Address	
Billing Address (if different than manufacturer's address)	
City, State, Zip Code	

Test Kit Information	
Test Kit Name	
Product Identification Number	
Test Method Format	
Reader Name	
Reader Model or Identification Number	
Detection Method	
Upper Conformance Limit	
Additional Commodities (list in alphabetical order)	

Appendix D: Test Kit Evaluation Submission Form (continued)

Design Criteria and Performance Specification Check List	
The test kit meets the performance requirements and submission packet components are included.	Initials
Time for completion of analysis ≤ 30 minutes	
FGIS reference method or equivalent used for reference analyses (validation report or literature reference provided if FGIS reference method not used)	
Accuracy study for naturally contaminated corn and wheat from 0.50 – 30 ppm DON	
Additional accuracy data for optional extension of upper limit of conformance above 30 ppm DON in corn and wheat	
Data supporting accuracy of multiple ranges of quantitation	
Additional commodities – accuracy data; sample preparation and analytical procedure is described for each additional commodity (including grinding procedure); type of reference material is given (naturally-contaminated or standard-solution fortified)	
Use of the test kit does not expose the employees to toxic or hazardous substances higher than OSHA Standards per 29 CFR when used without a fume hood or respirator.	
Use of the test kit does not generate radioactive waste, P-listed hazardous waste, or hazardous waste listed for its toxicity characteristic as defined in 40 CFR	
Safety Data Sheets (SDS)	
Reader electromagnetic field study certification statement (renewals exempt if same reader)	
Temperature sensitivity study	
Shelf life study	
Certificates of analysis for reference materials	
Written test kit instructions (provided as Microsoft Word document)	
Method flow chart is included (optional)	
Protocol & Notification Agreement Statement signed by applicant	

Comments

8. REVISION HISTORY

Revision 1 (June 1, 2018)

Added Corn Gluten Meal and Wheat Gluten to Appendix C: Commodity List.

Revision 0 (March 1, 2016)

Original Release