

Steps for Approving and Validating Commercial Mycotoxin Test Kits: FGIS/AMS Test Kit Evaluation Program

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FGIS Operates Under Two Federal Statues

- United States Grain Standard Act (USGSA, 1916)

All export corn must be tested for aflatoxin.

≤ 20 ppb total aflatoxins

(Wheat, corn, barley, rye, oat, flaxseed, sorghum, soybeans, triticale, mixed grain, sunflower seed, canola and any other food grains, feed grains, and oil seeds for which standards are established under section 4 of the act)

- Agriculture Marketing Act (AMA, 1946)

(Rice, beans, lentils, and pulses)

FGIS Mission

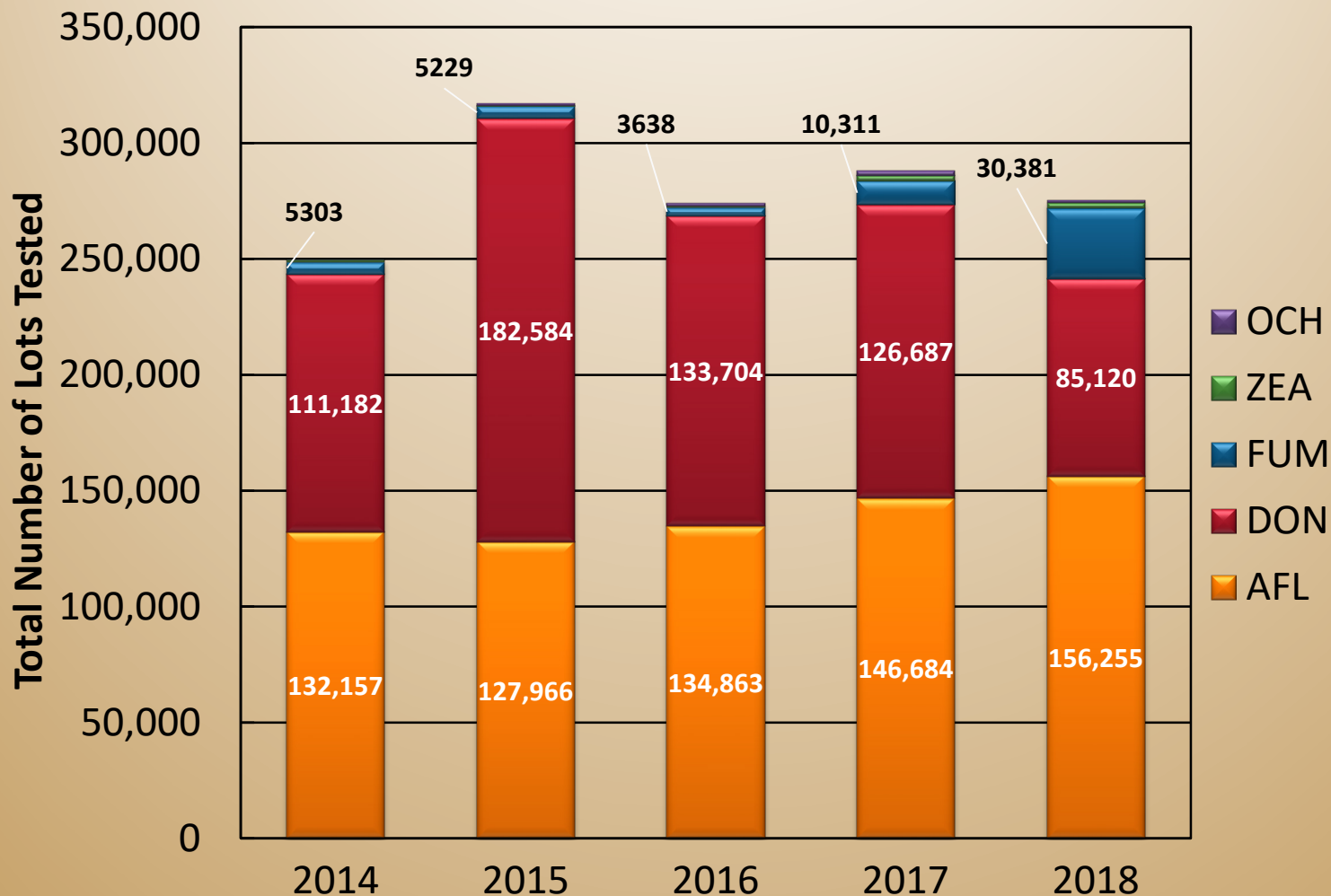
The mission of FGIS is to facilitate the marketing of grain, oilseeds, pulses, rice, and related commodities by:

- Establishing descriptive standards
- Accurately and consistently **certifying quality**
- Providing uniform official inspection and weighing
- Carrying out assigned regulatory & service responsibilities
- Providing the framework for commodity quality improvement incentive to both domestic and foreign buyers

Overall Mycotoxin Testing by Year

Mean number of lots tested: 280809

October 1 – September 30



FGIS Official Mycotoxin Testing Services

- Mycotoxin testing is done at about 100 locations and many of them are elevators & loading facilities (FGIS field offices, delegated and designated agencies)
- Only FGIS-approved commercial test kits are used since they are rapid, simple, and low cost
- Need accurate results to minimize risk to buyer/seller

Steps for Mycotoxin Test Kit Evaluation

- FGIS establishes criteria and performance requirement for rapid test kits (considering the market needs)
- Test kit manufacturers submit data and required documents following the FGIS criteria
- FGIS reviews the submission and if all criteria are fulfilled we accept it for training and evaluation
- Performance evaluation is conducted using FGIS reference material and analysts from both FGIS and manufacturer
 - Pass → Certificate and Test Kit Instructions are issued
 - Fail → Manufacturer can redesign and resubmit

Test Kit Conformance Ranges

- Aflatoxins (total) 5 to 300 ppb
- Deoxynivalenol 0.5 to 30 ppm
- Fumonisin (total) 0.5 to 30 ppm
- Ochratoxin A 5 to 100 ppb
- Zearalenone 100 to 1000 ppb

Mycotoxin Test Kit Performance Criteria

- Analysis time
- Primary grain
- Additional commodities
- Accuracy & precision
- Equipment sensitivity to electromagnetic fields
- Temperature sensitivity
- Reagent stability
- Avoidance of toxic & hazardous substances
- FGIS performance verification

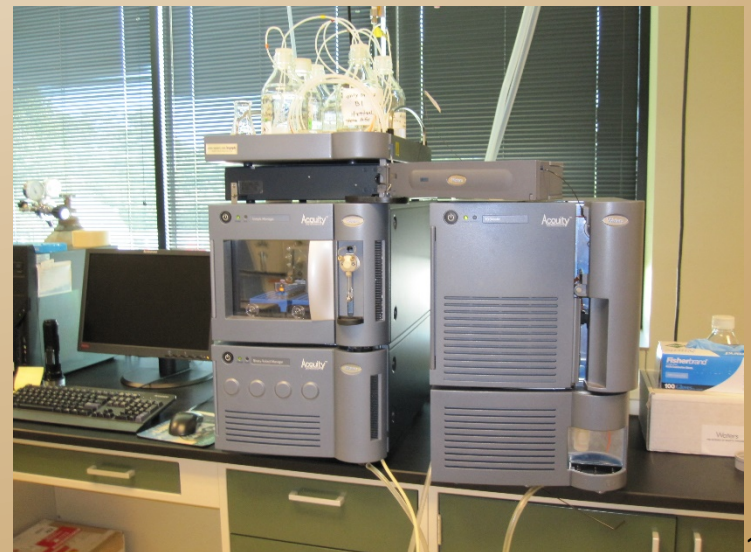
Mycotoxin Test Performance Criteria

Accuracy – Primary Grains

- Primary grain (most frequent testing requests)
 - Naturally contaminated
 - Aflatoxins – corn
 - Deoxynivalenol – wheat and corn
 - Fumonisin – corn
 - Ochratoxin A – wheat
 - Zearalenone – corn
- FGIS certified reference materials are used for evaluation the test kit
- Twenty-one samples at each concentration
 - Three operators / three unique lots
 - Seven samples by each operator / lot at each concentration

FGIS Reference Methods

- Benchmark for accuracy and precision
- Slower, more complex and costly than test kits
- UHPLC/FLD and UHPLC/MS/MS
- Used at National Grain Center only



FGIS Reference Methods

Mycotoxin	Cleanup	Detection Method	LOQ (ppb)	AOAC Method Reference
Aflatoxins	IAC	UHPLC/FLD	1.85 (total)	994.08
Deoxynivalenol	-	UHPLC/MS/MS	250	986.17
Fumonisin	-	UHPLC/MS/MS	400 (total)	995.15
Ochratoxin A	IAC	UHPLC/FLD	1	2000.03
Zearalenone	IAC	UHPLC/FLD	50	FGIS*

IAC – immunoaffinity column

FLD – fluorescence detection

MS/MS – tandem mass spectrometry

UHPLC – ultra high performance liquid chromatography

*Visconti, A *et al.*, *J. of Chrom. A*, 815 (1998) 133-140

Reference Method Quality Control

- Batch Quality Control
 - Calibration standards (r^2 and residuals)
 - Reagent blank
 - Matrix blank
 - Matrix spike (recovery)
 - Naturally contaminated check sample (control charting)
- Proficiency Testing
 - FAPAS
 - AOCS
- Sample exchange with other labs

FAPAS Proficiency Testing Results

Fiscal Year 2017

Mycotoxin	Assigned Value (ppb)	FGIS Result (ppb)	z-score
Aflatoxins	5.12	4.69	-0.4
Deoxynivalenol	1148	1220	0.4
Fumonisin	568	543	-0.3
Ochratoxin A	3.24	2.75	-0.7
Zearalenone	186	165	-0.5

FAPAS Proficiency Testing Results

Fiscal Year 2018

Mycotoxin	Assigned Value (ppb)	FGIS Result (ppb)	z-score
Aflatoxins	4.86	5.16	0.7
Deoxynivalenol	778/743	870/728	0.7/0.1
Fumonisin	485	441	-0.5
Ochratoxin A	4.55	4.46	-0.1
Zearalenone	131	131	0.0

Mycotoxin Test Performance Criteria

Accuracy – Additional Commodities

- Additional Commodities
 - Example: distillers dried grains
 - Fortified samples acceptable
 - Expected to perform with naturally contaminated samples
- Five samples at each concentration
 - One operator / one lot
- All results must be within specified ranges

Quantitative Aflatoxin Criteria

Accuracy and Precision – Updated in 2016

Concentration (ppb)	Maximum RSD %	Standard Deviation	95% Probability Range
5.0	25	1.25	2.5 – 7.5
20	20	4.0	12 – 28
100	16	16	68 – 130
300	16	48	200 – 400
$x > 300$	16	$0.16x$	$x \pm 0.32x$

- Naturally contaminated corn required, except $x > 300$ ppb
- $n = 21$ at each level

Quantitative Deoxynivalenol Criteria

Accuracy and Precision – Updated in 2016

Concentration (ppm)	Maximum RSD %	Standard Deviation	95% Probability Range
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
$x > 30$	10	$0.10x$	$x \pm 0.20x$

- Naturally contaminated wheat and corn required, except $x > 30$ ppm
- $n = 21$ at each level

Summary of Verification Data for Test Kit (20191XX QN)

Deoxynivalenol in Wheat Using XYZ Reader

0.5 ppm Level		2 ppm Level		30 ppm Level	
Analyst	Reading	Analyst	Reading	Analyst	Reading
1	0.46	1	2.2	1	30
1	0.54	1	1.9	1	28
1	0.78	1	2.6	1	31
1	0.60	1	2.1	1	34
1	0.52	1	2.4	1	31
1	0.60	1	1.7	1	33
1	0.61	1	2.4	1	34
2	0.51	2	2.3	2	32
2	0.63	2	2.1	2	39†
2	0.55	2	2.4	2	33
2	0.82†	2	2.3	2	27
2	0.68	2	2.2	2	34
2	0.75	2	2.1	2	33
2	0.52	2	2.3	2	31
3	0.53	3	2.3	3	30
3	0.62	3	2.5	3	36†
3	0.65	3	2.1	3	33
3	0.52	3	2.3	3	34
3	0.77	3	2.4	3	31
3	0.63	3	2.0	3	34
3	0.64	3	2.3	3	33
Total Out-of-Range	1		0		2
Acceptable Ranges	CRV ± 2*0.20*CRV		CRV ± 2*0.12*CRV		CRV ± 2*0.10*CRV
† Out-of-range					
CRV – Certified Reference Value					



Summary of Verification Data for Test Kit (20191XX QN)

Deoxynivalenol in Corn Using XYZ Reader

0.5 ppm Level		2 ppm Level		30 ppm Level	
Analyst	Reading	Analyst	Reading	Analyst	Reading
1	0.46	1	2.3	1	32
1	0.54	1	2.5	1	29
1	0.41	1	1.3†	1	32
1	0.59	1	1.9	1	28
1	0.49	1	2.1	1	29
1	0.42	1	2.2	1	23
1	0.54	1	2.4	1	28
2	0.47	2	2.1	2	26
2	0.49	2	1.9	2	29
2	0.54	2	1.8	2	25
2	0.37	2	2.1	2	30
2	0.54	2	1.9	2	29
2	0.56	2	1.8	2	32
2	0.45	2	2.2	2	23
3	0.59	3	2.4	3	31
3	0.46	3	2.2	3	35†
3	0.63	3	2.2	3	26
3	0.54	3	2.4	3	29
3	0.64	3	2.4	3	32
3	0.62	3	2.1	3	30
3	0.53	3	2.2	3	29
Total Out-of-Range	1		0		1
Acceptable Ranges	CRV ± 2*0.20*CRV		CRV ± 2*0.12*CRV		CRV ± 2*0.10*CRV
† Out-of-range					
CRV – Certified Reference Value					

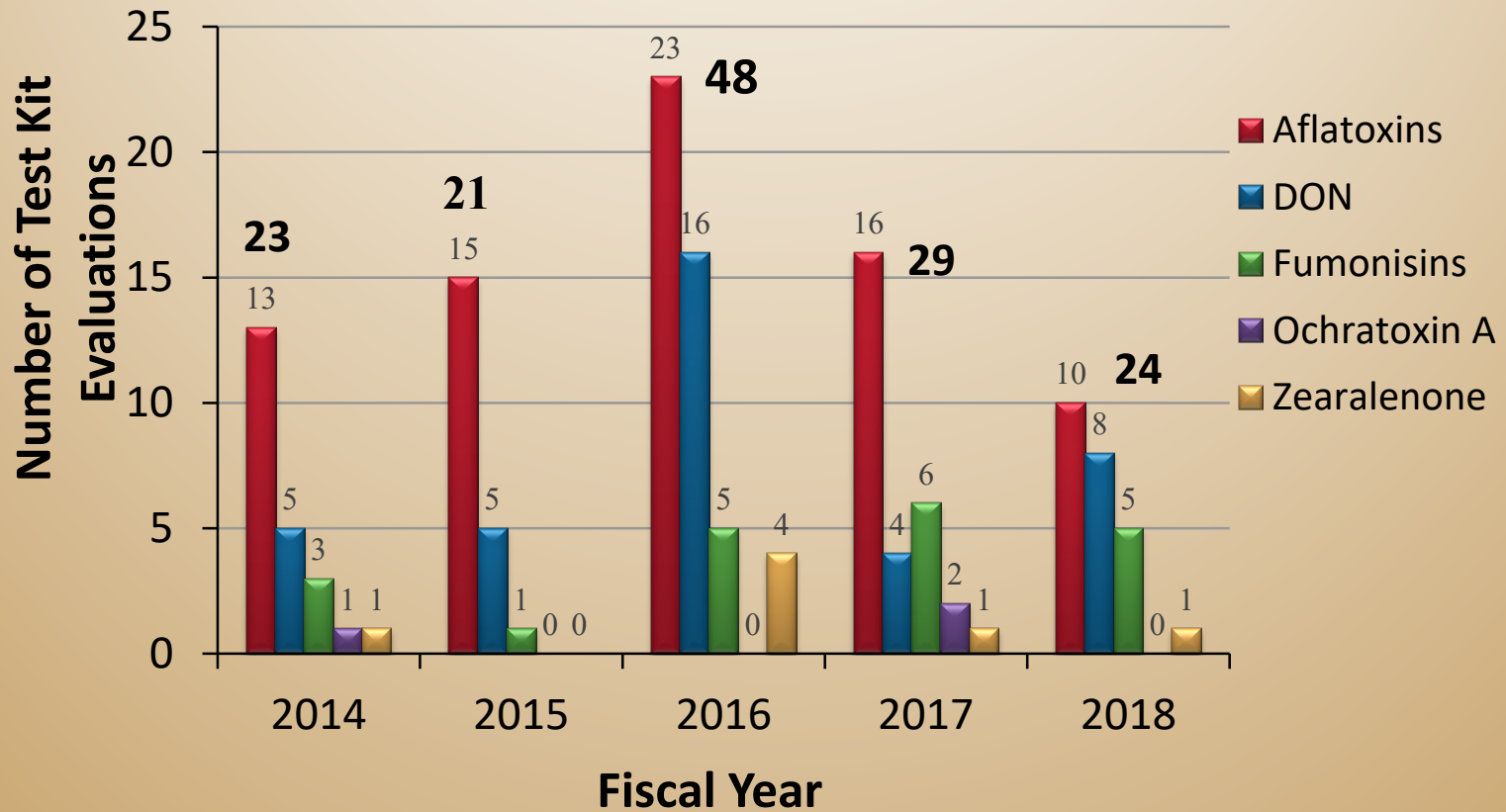
Approved Test Kits and Official Instructions

<https://www.ams.usda.gov/sites/default/files/media/FGISApprovedMycotoxinRapidTestKits.pdf>

FGIS Performance Verified Deoxynivalenol Test Kits – Effective 10/21/2019

Manufacturer	Test Kit	Part Number	FOL Code	Test Type and Test Kit Range	Approved Commodities are Listed Below. Equivalent Commodities can be Found in the Test Kit Design Criteria Appendix C. (page No. 14-15)	Detection Method	Certificate Expiration Date	Official Instructions
Charm Sciences, Inc.	ROSA DONQ2 Quantitative Test	LF-DONQ2	VOMLA	Deoxynivalenol 0.5 – 30 ppm	Water-Based Extraction: corn, wheat, barley, brown rice, buckwheat, corn bran, corn germ meal, corn gluten feed, corn gluten meal, distillers dried grain with solubles (DDGS), hominy, malted barley, milled rice, oats, rice bran, rough rice, rye, sorghum, soybean meal, triticale, wheat bran	EZ-M Reader (LF-ROSA-EZ-M)	11/09/2020	LF-DONQ2 Effective 11-02-2017
EnviroLogix, Inc.	QuickTox Kit for QuickScan DON Flex	AQ 304 BG	VOMNP	Deoxynivalenol 0.5 – 30 ppm	Water-Based Extraction: corn, wheat, distillers dried grains with solubles (DDGS), malted barley, and sorghum	EnviroLogix QuickScan System	10/02/2022	AQ-304-BG Effective 10-02-2019
Neogen Corporation	Reveal Q+ for DON	8385	VOMT	Deoxynivalenol 0.5 – 30 ppm	Water-Based Extraction: corn, corn/soy blend, distillers dried grain with solubles (DDGS), soybeans, malted barley, buckwheat, brown rice, barley (with hull), sorghum, and wheat	AccuScan Gold Reader	12/14/2020	8385-AS-GOLD Effective 03-02-2018
Neogen Corporation	Reveal Q+ for DON	8385	VOMT	Deoxynivalenol 0.5 – 30 ppm	Water-Based Extraction: corn, corn/soy blend, distillers dried grain with solubles (DDGS), soybeans, malted barley, buckwheat, brown rice, barley (with hull), sorghum, and wheat	AccuScan Pro Reader	07/13/2020	8385-AS-PRO Effective 03-02-2018
Neogen Corporation	Veratox DON 2/3	8335	VOMO	Deoxynivalenol 0.5 – 30 ppm	Water-Based Extraction: corn, wheat, corn germ meal, malted barley, oats, rye, wheat bran, sorghum, and corn/soy blend	Stat Fax Reader Model 321 Plus Stat Fax Reader Model 4700	03/08/2021	8335 SFR Effective 03-06-2018
Neogen Corporation	Veratox DON 5/5	8331	VOMC	Deoxynivalenol 0.5 – 30 ppm	Water-Based Extraction: corn, wheat, corn germ meal, malted barley, oats, rye, wheat bran, sorghum, and corn/soy blend	Stat Fax Reader Model 4700	03/08/2021	8331-SF-4700 Effective 03-06-2018
Thermo Fisher Scientific	EZ-Tox DON	600120-DON	VOMTA	Deoxynivalenol 0.5 – 30 ppm	Water-Based Extraction: corn, wheat, barley, and malted barley	BioTek ELX808UI	03/06/2021	600120-DON Effective 02-08-2018
VICAM	DON-V	176004113	VOMVA	Deoxynivalenol 0.5 – 30 ppm	Water-Based Extraction: corn, wheat	Vertu Reader Model Vertu	03/07/2022	176004113 Effective 03-07-2019

Number of Test Kits Evaluated



More Information

- Mycotoxins
 - <https://www.ams.usda.gov/services/fgis/mycotoxins>
- FGIS Rapid Test Evaluation Program
 - <https://www.ams.usda.gov/services/fgis/standardization/tke>
- FGIS Performance Verified Rapid Test Kits
 - <https://www.ams.usda.gov/sites/default/files/media/FGISApprovedMycotoxinRapidTestKits.pdf>
- Contact
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Questions?

