

Mycotoxin Test Kit Program



**GRAIN INSPECTION ADVISORY COMMITTEE MEETING
APRIL 7 – 8, 2015**

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Technology and Science Division**



United States Department of Agriculture
Grain Inspection Advisory Committee Meeting, April 2015

Topics



- Update on water-based test kits
- Accuracy of test kits for distiller dried grains with solubles
- Test kit instructions
- Revision of performance criteria
 - Expansion of concentration ranges in performance criteria
 - Supplemental analysis – results above highest concentration specified in performance criteria (aflatoxins, DON, fumonisins)



Water-Based Test Kits



July 2014 Resolution –

The Advisory Committee supports continued focus on water-based quantitative mycotoxin test kits. Industry efforts to be "green" by reducing use of hazardous chemicals and associated waste are becoming commonplace. Encouraging manufacturers of testing methodology to develop the water-based methods should be continued.



GIPSA-Approved Water-Based Test Kits



- **Aflatoxin – 4 test kits**
 - Charm Sciences (new) – corn and 14 additional commodities
 - EnviroLogix – corn and wheat
 - Neogen – corn and 8 additional commodities
 - Romer Labs (new) – corn and 4 additional commodities
- **Deoxynivalenol – all test kits**
- **Fumonisin – 1 test kit**
 - Charm Sciences – corn and 7 additional commodities
- **Ochratoxin A – 0 test kits**
- **Zearalenone – 0 test kits**



Distillers Dried Grains with Solubles



July 2014 Resolution –

The Advisory Committee recommends continued work in verifying the accuracy of mycotoxin test kits for Distillers Dried Grains with Solubles (DDGS).



GIPSA-Approved Quantitative Test Kits



- GIPSA has performance criteria for DDGS
- Manufacturer required to provide supporting data

Mycotoxin	Number of Test Kits			
	Water-Based Extraction		Organic Solvent Extraction	
	Total	DDGS	Total	DDGS
Aflatoxins	4	2	10	7
DON	10	5	0	0
Fumonisin	1	0	3	0
Ochratoxin A	0	0	2	0
Zearalenone	0	0	2	2

DDGS = Distillers Dried Grains with Solubles



Expansion of Concentration Ranges



July 2014 Resolution –

Whereas GIPSA is exploring the possibility of expanding the concentration ranges in performance criteria for mycotoxin test kits, the Advisory Committee recommends GIPSA consider setting the following ranges for performance criteria:

- Aflatoxin - 5 to 700 ppb
- Vomitoxin - 0.5 to 30 ppm
- Fumonisin - 0.5 to 100 ppm
- Ochratoxin A - No Change
- Zearalenone - No change



Test Kit Instructions, Supplemental Analysis, and Expansion of Concentration Ranges



November 2014 Resolution –

The Advisory Committee recommends FGIS update the current mycotoxin instructions to provide current and clear guidelines for the operation of mycotoxin test kits including supplemental analysis methodology and develop guidelines and testing protocols to enhance mycotoxin rapid test kits to include verification/approval of supplemental analysis ranges and acceptable standard error for each relevant supplemental range consistent with FDA guidelines.



Mycotoxin Test Kit Instructions



November 2014 Resolution –

The Advisory Committee recommends FGIS update the current mycotoxin instructions to provide current and clear guidelines for the operation of mycotoxin test kits including supplemental analysis methodology and develop guidelines and testing protocols to enhance mycotoxin rapid test kits to include verification/approval of supplemental analysis ranges and acceptable standard error for each relevant supplemental range consistent with FDA guidelines.



Mycotoxin Test Kit Instructions



- Previously in mycotoxin handbooks and directives
 - Difficult to update
- Now require final instructions at time of test kit submission
 - Include supplemental analysis
 - Posted upon test kit approval
- Hyperlinks to instructions on GIPSA website
 - July 2014 – 3 test kit instructions posted
 - April 2015 – 26 test kit instructions posted out of 45
 - July 2015 – completion expected



Test Kit Information and Official Instructions

[http://www.gipsa.usda.gov/fgis/metheqp/GIPSA Approved Mycotoxin Rapid Test Kits.pdf](http://www.gipsa.usda.gov/fgis/metheqp/GIPSA%20Approved%20Mycotoxin%20Rapid%20Test%20Kits.pdf)



GIPSA Performance Verified Mycotoxin Test Kits – Effective 3/10/2015

Manufacturer	Test Kit	Part Number	Test Type	Test Kit Range	Approved Commodities		Detection Method	Certificate Expiration Date	Official Instructions
					Water-Based Extraction	Organic Solvent Extraction			
Charm Sciences, Inc.	ROSA FAST5 Zearalenone Quantitative Test	LF-ZEARQ-FAST5	Zearalenone (quantitative)	100 – 1000 ppb		corn, barley, brewer's rice, distillers dried grain with solubles, flaking corn grits, milled rice, oats, rough rice, sorghum, wheat, wheat bran, wheat flour	ROSA-M Reader (LF-ROSAREADER-M-NB) EZ-M Reader (LF-ROSA-EZ-M)	6/23/2017	LF-ZEARQ-FAST5 Revision 2
EnviroLogix, Inc.	QuickTox Kit for Aflatoxin Bulk Grain	10539	Aflatoxin (qualitative)	Detection Threshold, 20 ppb		Corn	Visual	8/26/2016	
EnviroLogix, Inc.	QuickTox Kit for QuickScan Aflatoxin	AQ 109 BG	Aflatoxin (quantitative)	5 – 100 ppb		Corn, wheat, sorghum, and soybean meal.	EnviroLogix QuickScan System	6/27/2016	
EnviroLogix, Inc.	QuickTox Kit for QuickScan Aflatoxin FREE	AQ 209 BG	Aflatoxin (quantitative)	5 – 100 ppb	Corn and wheat	Barley, Corn flour, corn gluten meal, corn gluten feed, corn germ, corn germ meal, delinted cottonseed, distillers dried grains with solubles, rice bran, rough rice, oats, sorghum, soybean meal, and hominy feed	EnviroLogix QuickScan System	9/18/2016	AQ 209BG Revision 1



Test Kit Information and Official Instructions

[http://www.gipsa.usda.gov/fgis/metheqp/GIPSA Approved Mycotoxin Rapid Test Kits.pdf](http://www.gipsa.usda.gov/fgis/metheqp/GIPSA_Approved_Mycotoxin_Rapid_Test_Kits.pdf)



Approved Commodities		Detection Method	Certificate Expiration Date	Official Instructions
Water-Based Extraction	Organic Solvent Extraction			
	corn, barley, brewer's rice, distillers dried grain with solubles, flaking corn grits, milled rice, oats, rough rice, sorghum, wheat, wheat bran, wheat flour	ROSA-M Reader (LF-ROSAREADER-M-NB) EZ-M Reader (LF-ROSA-EZ-M)	6/23/2017	LF-ZEARQ-FAST5 Revision 2
	Corn	Visual	8/26/2016	



Supplemental Analysis and Expansion of Concentration Ranges



November 2014 Resolution –

The Advisory Committee recommends FGIS update the current mycotoxin instructions to provide current and clear guidelines for the operation of mycotoxin test kits including supplemental analysis methodology and **develop guidelines and testing protocols to enhance mycotoxin rapid test kits to include verification/approval of supplemental analysis ranges and acceptable standard error for each relevant supplemental range consistent with FDA guidelines.**



Supplemental Analysis and Expansion of Concentration Ranges



- **Eliminate supplemental analysis?**
 - Expansion of concentration ranges
 - Feedback – high values needed
 - Result – continue to allow supplemental analysis
- **Expand concentration ranges**
 - Use FDA maximum levels
 - Establish accuracy requirements at high levels



Outreach to Stakeholders



- Letter to test kit manufacturers – October 2014
- Increase highest concentration level in performance criteria
- Establish %RSDs to determine acceptable ranges
- Feedback on –
 - Impact with additional analyses
 - Impact with NO additional analyses
 - Additional challenges for water-based extraction?

Mycotoxin	Current Highest Level	Proposed New Highest Level	Proposed New %RSD	Proposed New Acceptable Range
Aflatoxins	100 ppb	300 ppb	14	216 – 384 ppb
Deoxynivalenol	5 ppm	30 ppm	6.0	26.4 – 33.6 ppm
Fumonisin	5 ppm	100 ppm	8.0	84 – 116 ppm



Feedback from Test Kit Manufacturers



- Received feedback from 5 of 9 manufacturers
- Common feedback
 - Additional analyses needed to cover expanded ranges
 - Proposed range requirement at highest level too narrow
 - Challenges for water-based extraction
 - ✦ Mixed feedback
 - Naturally-contaminated materials difficult to find
 - ✦ 30 ppm DON
 - ✦ 100 ppm fumonisins



Current Proposal – Expand Concentration Ranges

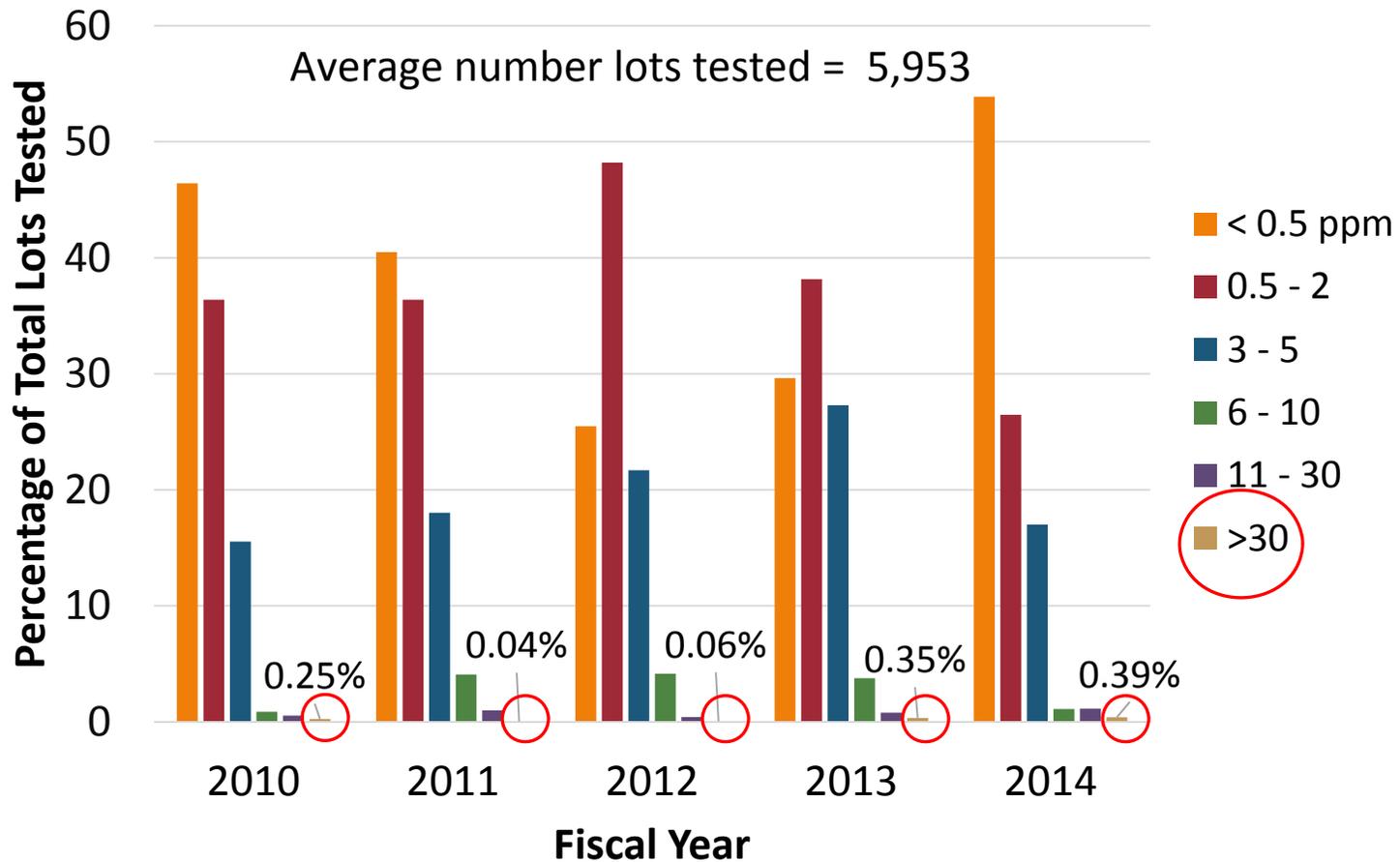


- **Aflatoxins and DON – FDA Action and Advisory Levels**
 - Aflatoxin 300 ppb
 - DON 30 ppm
- **Fumonisin – set to 30 ppm instead of 100 ppm**
 - Difficulty obtaining naturally-contaminated reference materials at 100 ppm
 - Very few detections above 30 ppm



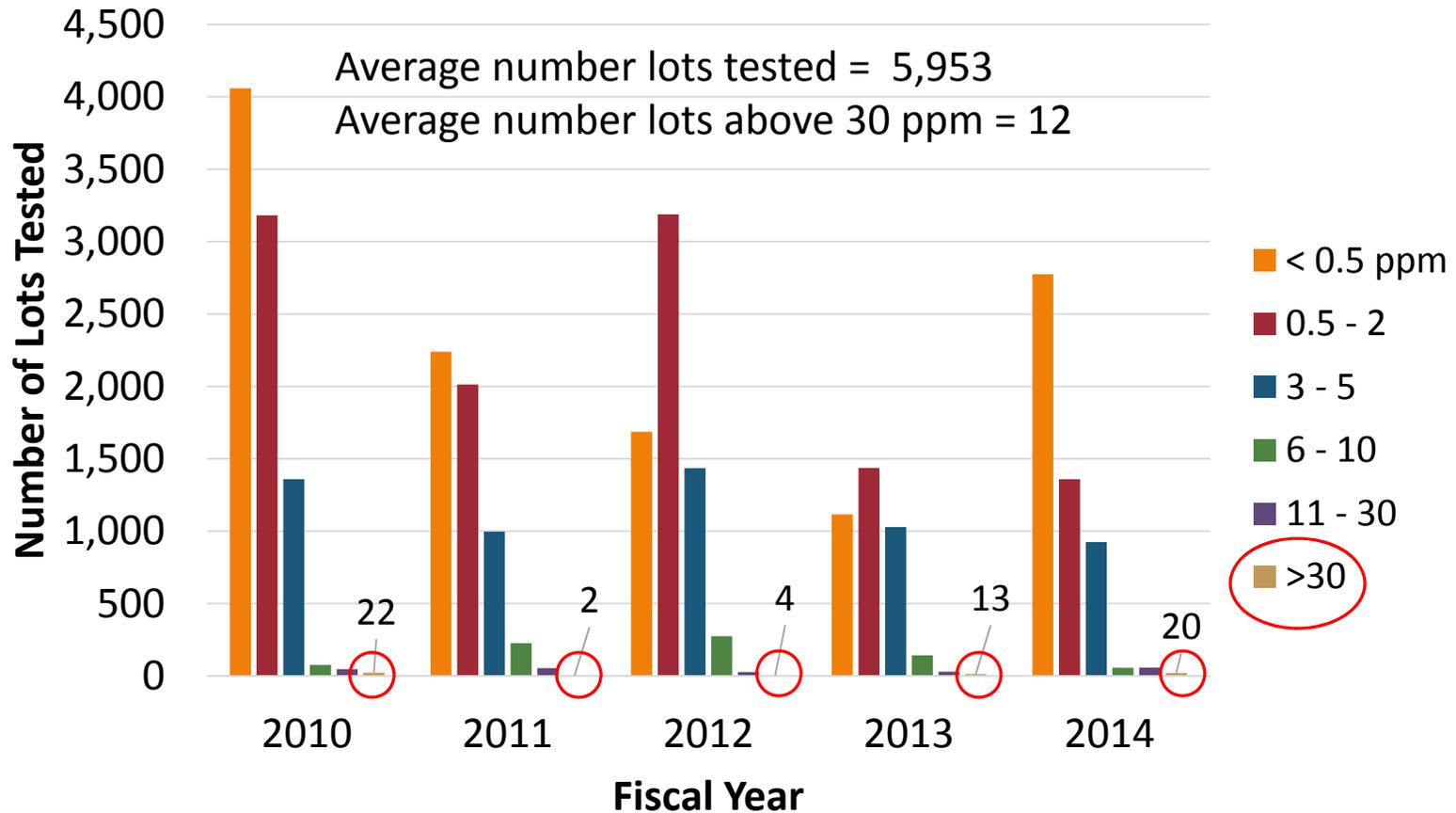
Fumonisin Testing Results by Fiscal Year

October 1 – September 30



Fumonisin Testing Results by Fiscal Year

October 1 – September 30



Recommended GIPSA Performance Criteria



	Current		Recommended		
Mycotoxin	Highest Level	% RSD	Highest Level	% RSD	Acceptable Range
Aflatoxins	100 ppb	16	300 ppb	16	200 – 400 ppb
Deoxynivalenol	5 ppm	10	30 ppm	10	24 – 36 ppm
Fumonisin	5 ppm	13	30 ppm	13	22 – 38 ppm



Recommended GIPSA Performance Criteria



- Supplemental Analysis
 - > 300 ppb aflatoxin
 - > 30 ppm DON
 - > 30 ppm fumonisins
- Recommended by GIPSA but not required of test kits
- Accuracy requirement same as that for new highest concentration
- Manufacturer must –
 - Provide specific procedure
 - Specify upper limit
 - Provide supporting data at upper limit
 - Standard solution-fortified samples accepted



Future Work



- Complete test kit instruction posting – July 2015
- Revise Performance Criteria Documents – FY 2015
 - Expand concentration ranges (aflatoxins, DON, fumonisins)
 - ✦ Aflatoxins 5 – 300 ppb
 - ✦ DON 0.5 – 30 ppm
 - ✦ Fumonisins 0.5 – 30 ppm
 - Establish supplemental analysis performance criteria



Implementation Plan



- Revised performance criteria effective one year from issuance
- Existing certificates not affected
- Transition year – submission under old or new criteria
 - Old criteria – certificate expires after one year
 - New criteria – certificate expires after three years



Questions?

