Aflatoxin Proficiency Testing and Control in Africa (APTECA)

Tim Herrman
Professor, State Chemist and Director
Office of the Texas State Chemist
Texas A&M AgriLife Research

Continuous Improvement

You can't improve what you do not control

You can't measure what you do not define

You can't control what you do not measure

PROFICIENCY TESTING

ONE OF THE BIG 3 ALONG WITH UNCERTAINTY AND TRACEABILITY


APTECA Proficiency Testing Program
Corn Meal Sample #4

Proficiency Testing

Interlaboratory comparisons are widely used for a number of purposes

ISO 17043:2010

Purposes of Proficiency Testing

a) Evaluation of the performance of laboratories for specific tests or measurements and monitoring laboratories' continuing performance

b) Identification of problems in laboratories and initiation of actions for improvement which may be related to inadequate test or measurement procedures, effectiveness of staff training and supervision or calibration of equipment

c) Establishment of the effectiveness and comparability of test and measurement methods

ISO 17043:2010

Purposes of Proficiency Testing cont.

d) Provision of additional confidence to laboratory customers

e) Identification of interlaboratory differences

f) Education of participating laboratories based on the outcomes of such comparisons

g) Validation of uncertainty claims

ISO 17043:2010

Proficiency Provider

OFFICE OF THE TEXAS STATE CHEMIST

Texas Feed and Fertilizer Control Service • Agricultural Analytical Service
445 Agronomy Road • College Station, TX 77843-2114

Mailing Address
P. O. Box 3160
College Station, TX 77841-3160

Tel. (979) 845 1121
Fax (979) 845 1389
http://otsc.tamu.edu

Proficiency testing

Proficiency test item

Proficiency testing round

Laboratory Proficiency Program

Aflatoxin in Cornmeal

Round 2

July 2015 APTECA Sample 4

COMESA Laboratories Slurry Results

Participant

Laboratory	Mean Result	Range	Bias	Z value
1	32	NA	+3	0.38
2				
3				
4	91	NA	62	7.84
5	22.6	NA	-6.4	-0.81
6	16.2	NA	-12.8	-1.62
7	35	NA	+6	0.76
8	71	NA	+42	5.31
9	44	NA	+15.1	1.91
10	36	NA	+7	0.88
11	14	NA	-14.9	-1.88
12				
13	36	NA	+7	0.88
14	38.3	NA	+9.3	1.18
15	1.3	NA	-27.7	-3.50

The assigned value was determined by the OTSC reference laboratory using an HPLC method of analysis. The assigned standard deviation was determined using the Horwitz function which in the food sector determines fitness for purpose.

Assigned Value	29
Assigned Standard Dev	7.9

Assigned value

Calculated standard deviation

Twelve aflatoxin slurry results were reported. Two laboratories reported duplicate results from a common extract. Proficiency samples for the slurry method did not contain a sufficient quantity to run a duplicate sample, thus the range calculation was not applicable (NA).

A Dixon outlier test of the means was conducted and showed no outliers.

The mean, bias, and Z value were calculated for each laboratory.

The mean of reported results was 36.5 ppb. The standard deviation of the reported means was 24.5 ppb. The relative standard deviation was 67.1%.

Nine Z values were acceptable (<3) with three being unacceptable >3.

References:

"Processing Data for Outliers": Dixon, W.J. Biometrics March 1953 pg 74-89

"The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories" Pure Appl.Chem. Vol 78 No.1, pp. 145-196, 2006

"Aflatoxins in Corn, Raw Peanuts, and Peanut Butter-Liquid Chromatography with Post-Column Photochemical Derivatization" AOAC Official Method 2005:08

$z=(x-x_a)/\sigma_p$

RSD = 67%

$RSD_R=2^{(1-0.5\log C)}$

Harmonized Protocol for proficiency testing

"It is important to emphasize that the interpretation of z-scores in not generally based on summary statistics that describe the observed participant results." 3.1.2 p 157

A score of zero implies a perfect result
Approximately 95% of z-scores fall between -2 and +2.
A score outside the range from -3 to 3 should be investigated
A score in the ranges -2 to -3 and 2 to 3 would be expected about 1 in 20

Harmonized Protocol for proficiency testing

Assigned Value

- An assigned value and uncertainty may be obtained by a suitably qualified measurement laboratory using a method with sufficiently small uncertainty
- Certified reference material

Consensus - disadvantages

- Not independent of participant results
  - Bias for the population may not be detected
  - Participants whose results are unbiased may unfairly receive extreme z-scores
- Their uncertainty may be too large when the number of labs is small

Assigned Mean – OTSC AAS

APTECA Proficiency #4 (N2013-001095)

	B1	B2	G1	G2	Total
	24.3	2.0	0.0	0.0	26
	26.1	2.3	0.0	0.0	28
	28.8	2.4	0.0	0.0	31
	24.9	2.2	0.0	0.0	27
	24.5	2.7	0.0	0.0	27
	23.8	2.4	4.0	0.0	30
	26.7	2.8	0.0	0.0	30
	27.3	2.7	4.2	0.0	34
	33.9	2.9	0.0	0.0	37
	27.3	2.7	0.0	0.0	30
	22.3	2.3	0.0	0.0	25
	21.8	2.2	0.0	0.0	24
Average	26.0	2.5	0.7	0.0	29
SD	3.3	0.3	1.6	0.0	3.8
RSD (%)	12.6	12.0	233.6		12.9

Horwitzt function to calculation standard deviation

aflatoxin (ppb)	Mass fraction	Log	Expected RSD (%)	Standard Deviation
10	0.00000001	-8.0	32.0	3.2
29	0.000000029	-7.5	27.3	7.9
100	0.0000001	-7.0	22.6	22.6
300	0.0000003	-6.5	19.2	57.5

$RSD_R=2^{(1-0.5\log C)}$

The Horwitz function is often regarded as defining fitness-for-purpose in the food sector Harmonized Protocol for proficiency testing p 163

Reference laboratory standard deviation = 3.8

COMESA laboratories' consensus standard deviation = 24.0

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Laboratory Proficiency Program

Aflatoxin in Cornmeal

Round 2

July 2015, APTECA Sample 4

COMESA Laboratories

Laboratory	Mean Result	Range	Bias	Z value
1	30.4	2	+1.4	0.18
2	1.0	0	-28	-3.54
3	12.5		-16.5	-2.09
4	60.0	4	+31	3.92
5	19.0	0.2	-10	-1.26
6	13.3	0.8	-15.7	-1.98
7	25.0	2	-4.0	-0.51
8	50.5	7	+21.5	2.72
9	33.05	8.9	+4.05	0.51
10	24.0	1.2	-5.0	-0.63
11	23.5	4.3	-5.5	-0.69
12	25.8	8.6	-3.17	-0.40
13	96	0	+67	8.47
14	42.7	9	+13.7	1.73
15	6.05	1.6	-22.95	-2.90

The assigned value was determined by the OTSC reference laboratory using an HPLC method of analysis. The assigned standard deviation was determined using the Horwitz function which in the food sector determines fitness for purpose.

Assigned Value	29
Assigned Standard Dev	7.9
Average Range of duplicates	4.1

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Fifteen proficiency test results were reported. One laboratory reported three sets of results using different testing platforms. The mean, range of duplicates, bias, and Z values were calculated for all 15 results.

A Dixon outlier test of the means was conducted and showed no outliers. The assigned standard deviation was used to calculate the z values.

The mean of reported results was 30.9 ppb. The standard deviation of the means was 24. The relative standard deviation was 78%. The average range of duplicates was 4.1 ppb.

Twelve Z values were acceptable (<3) and three values were unacceptable.

References:

"Processing Data for Outliers": Dixon, W.J. Biometrics March 1953 pg 74-89

"The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories" Pure Appl.Chem. Vol.78 No.1, pp. 145-196, 2006

"Aflatoxins in Corn, Raw Peanuts, and Peanut Butter-Liquid Chromatography with Post-Column Photochemical Derivatization" AOAC Official Method 2005:08

RSD 78%

Kenya Laboratory Proficiency Program

Aflatoxin in Cornmeal

February 2015 Sample

All Laboratories

February report combining industry and government labs

Laboratory	Mean Result	Z value
1	25.25	-0.335
2	26.50	-0.246
3	42.05	0.871
4	32.9	0.214
5	24.85	-0.364
6	29.95	0.002
7	21.50	-0.605
8	3	-1.933
9	36.6	0.480
10	28.05	-0.134
11	56.9	1.937
12	31.75	0.131
13	8.29	-1.553
14	51.3	1.535

Mean	29.92
Standard dev	13.926
Average Range of duplicates	4.43

Consensus mean

Consensus standard deviation

Fourteen laboratories reported results for aflatoxin. Three laboratories reported only one result; they were included in the consensus mean calculation but not in the duplication or range calculation.

A Dixon outlier test was conducted and showed no outliers.

The mean, range of duplicates, and bias was calculated for each laboratory.

The mean of reported result was 29.92. The standard deviation of the reported means was 13.926. While the average range of duplicates was 4.43.

Ten Z values were <1 and four Z values were >1 but <2.

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Kenya Laboratory Proficiency Program

Aflatoxin in Cornmeal

February 2015 Sample

Government Laboratories

Laboratory	Mean Result	Bias	Z value
9	3	-37	3.56
10	36.6	-3.4	0.33
11	28.05	-11.95	1.14
12	56.9	+16.9	1.63
13	31.75	--8.25	0.79
14	8.29	-31.71	3.05
15	51.3	+11.3	1.09
16	2	-38	3.65

OTSC sample N2013-001110 was analyzed at the OTSC laboratory by HPLC (n=12).
The mean value was determined to be 40 ppb. Using the Horwitz formula (R=2C–0.15) to calculate the Predicted Relative Standard Deviation of Reproducibility (PRSD) resulted in a calculated R value of 26%. Therefore, 0.26 x 40 = 10.40.
The following assigned values were then used in evaluating the laboratory data:

Assigned Value	40.00
Assigned Standard Dev	10.40
Average Range of duplicates	3.82

Eight government laboratories reported results for aflatoxin. Two laboratories reported only one result; their results were not used in the range calculation.

One laboratory reported three results; only the first two results were used for calculations.

A Dixon outlier test of the means was conducted and showed no outliers.

The mean, range of duplicates, bias, and Z value were calculated for each laboratory.

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The mean of reported results was 27.24 ppb. The standard deviation of the reported means was 21.22. While the average range of duplicates was only 3.82 the variation between labs was much greater than expected.

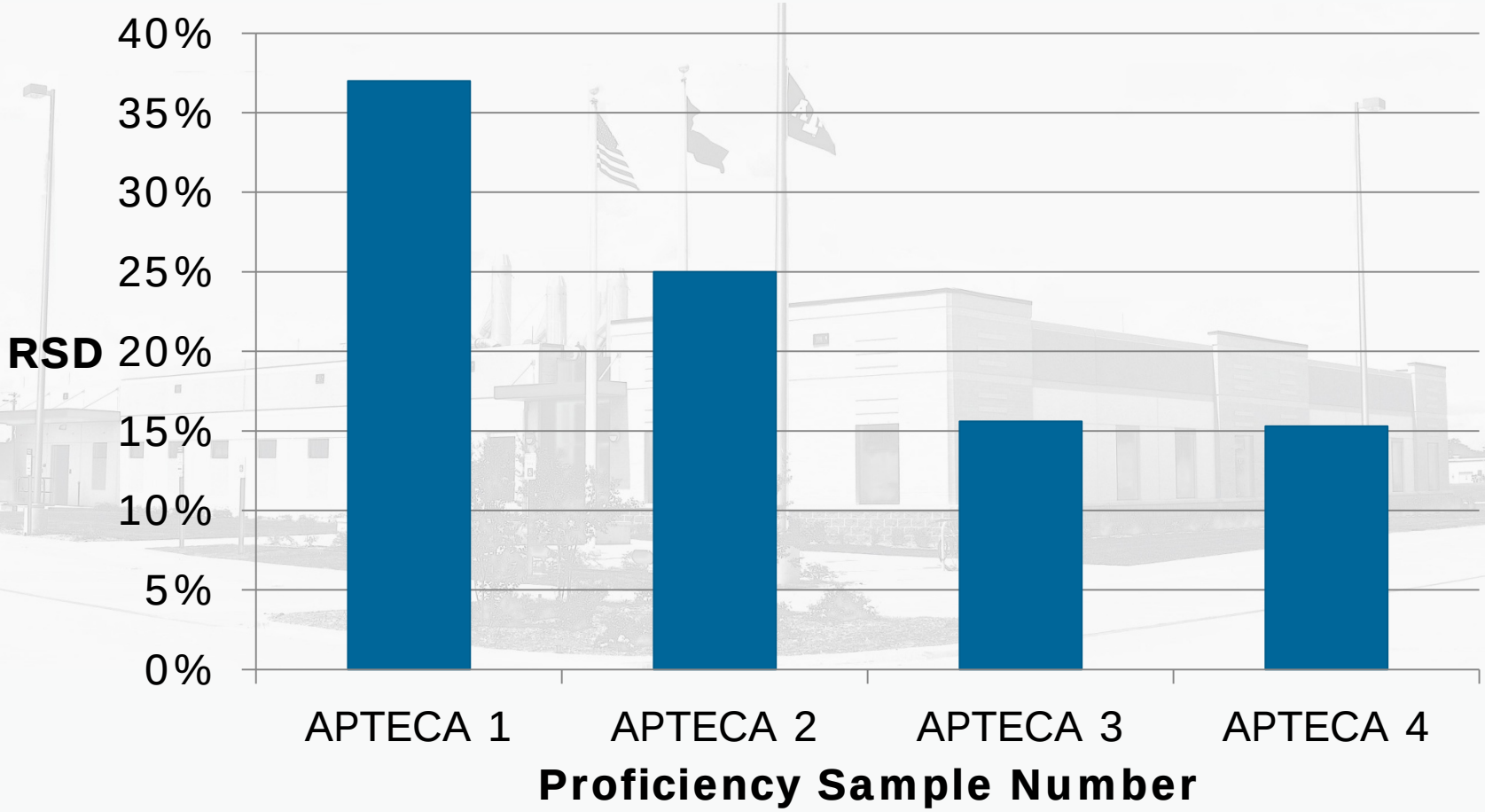
Five Z values were acceptable (<2). Three Z values were >3 and the root cause must be investigated.

RSD = 78%

Horwitz formula to calculate Z value

Assigned value

Kenya Milling Industry Performance



Timeline of OTSC Proficiency Testing Program

- OTSC began an aflatoxin proficiency testing service for the Texas Grain Industry in 2010, also began aftermarket evaluation of USDA approved kits
- Expanded the program to the Kenya maize milling industry in 2014 – began using consensus method
- Collaborated with COMESA and KEBS in 2015 – requested assigned mean and Horwitz function σ
- OTSC has adopted ISO 17043 protocol with APTECA round 4 including use of assigned mean and Horwitz function to calculate the standard deviation and relative standard deviation
- Expand to include FAO in 2016

Summary

- HPLC and TLC results appeared more variable, may be related to calculation or dilution error
- Some test kits displayed a low bias at high levels of aflatoxin and high bias for low levels of toxin
- Use of validate methods and testing platforms is encouraged
- Participation in the proficiency testing has grown to include 13 industry and 15 government labs
- Slurry method didn't improve testing accuracy
- Testing remains a significant source of variability in managing aflatoxin risk
- Problems include timely delivery of kits, proper maintenance of equipment, and quality reagents

Conclusion

SIMPLE IS BETTER