Sampling variation in the quantification of fumonisins in maize samples

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Fumonisins produced by F. verticillioides and F. proliferatum cause mycotoxicoses in horses, swine and rats and have been associated with oesophageal cancer in humans. Accurate measurement of mycotoxins is essential for determining the safety of grain and their products for consumption. Four sources of variation were studied, namely sub-sample size, variation within a single maize sub-sample, number of replicates and toxin detection techniques used by independent laboratories. Variation in detected fumonisin levels within a single maize sample was high using the 25 g sub-samples proposed in the Neogen Veratox protocols. A 250 g sub-sample significantly reduced variation in fumonisin levels of samples. An incremental increase in sample size also improved the number of positive samples recorded. Increasing the number of replicates using the recommended sub-sample size (25 g) did notreduce variation except when the sample had high fumonisin levels. Improved accuracy was recorded when a 250 g sub-sample was used in conjunction with increased replicates. Data from laboratory analyses indicated that ELISA reactions (Agricultural Research Council – Grain Crops Institute) correlated significantly with HPLC results of the Medical Research Council (MRC), but neither of these correlated with results from an independent laboratory. Concentrations determined using ELISA were consistently higher than those from the HPLC (MRC) technique. Quantification technique, sample size, replicate number and laboratory where analyses are conducted, appear to be important sources of variation for quantification of fumonisins.

Keywords: ELISA, Fusarium, HPLC, replications, sub-sample size

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Introduction

Maize (Zea mays L.) is an important nutrient source for humans and livestock worldwide and the screening of meal for mycotoxins, and in particular, fumonisins caused by Fusarium verticillioides (Sacc.) Nirenberg and F. proliferatum (Matsushma) Nirenberg is important to ensure food safety. Identifying potentially dangerous grain or products and removing them from the food chain could reduce the impact of mycotoxin-infected grain on society. To date, substantial mycotoxin research has been carried out but high variation in toxin test results and inaccurate species identification (Janse van Rensburg et al. 2008) has confounded statistical analysis of data. The grain sampling technique used to ensure accurate estimates of mycotoxin contamination is critical. Since mycotoxin production is not homogenous, poor grain sampling procedures may result in highly infected grains escaping detection (Whitaker et al. 2001). Alternately, coincidental sampling of contaminated grain in what otherwise is a relatively healthy grain sample can result in grain being unnecessarily discarded, with considerable economic implications (Whitaker, 2003). Whitaker (2006) states that sampling is usually the largest source of variability associated with the mycotoxin test procedure. Sampling together with preparation and analytical methods contribute to the total variation associated with any test procedure (Whitaker, 2006).

A variety of fumonisin detection methods have been proposed but results are often variable. Methods of fumonisin detection include capillary gas chromatography, thin layer chromatography (TLC), Neogen Veratox, which uses direct competitive, enzyme-linked, immunosorbent assay (ELISA), capillary electrophoresis with mass spectrometry and high-performance liquid chromatography (HPLC) (Dilkin et al., 2001; Betancourt et al., 2006; Krska & Welzig, 2006). ELISA, TLC and HPLC have different levels of sensitivity and also differ in cost and time required for analysis. Subsequent to 1990 the Association of Official Analytical Chemists (AOAC) officially-approved method of testing for mycotoxins was the TLC technique, which was recently replaced by the HPLC technique (Sydenham et al., 1996).

The ARC-Grain Crops Institute (ARC-GCI) uses the ELISA-Veratox protocol because it allows for rapid screening of a large number of samples and is a cost-effective technique. However, ELISA fumonisin test results vary considerably, even within single samples. Reduced variation in the overall fumonisin testing procedure could ensure that the mycotoxin concentration of the primary sample is estimated with more confidence and accuracy (Whitaker et al., 1998) and reduce the risk of false positives or negatives, with the concomitant financial implications for producers and processors. The aim of this study, therefore, was to address variation associated with sampling of maize grain by identifying sources of variation and improving sampling methods. Four sources of variation were studied, namely sub-sample size, variation within a single sub-sample, number of replicates and toxin detection techniques used by independent laboratories.

Material and methods

In April 2004, nineteen, random-aggregate maize samples of 50 kg were collected from South African silos at sixteen localities in the dry western area (western Free State and

North-West provinces). A further seven samples were collected at six localities in the wetter eastern maize production area (eastern Free State, Gauteng, Mpumalanga and KwaZulu Natal). Sampling was conducted by removing approximately 1 kg samples from a moving conveyer belt over a period of approximately two hours. Maize samples ranged from grade 1 to 3, based on the general maize grading system used locally (Anonymous, 2008). Samples included yellow and white maize, depending on availability at the time of collection. Samples were placed in a polyethylene bag and stored at 5ºC for approximately one month before fumonisin testing.

Samples for further study were selected based on initial fumonisin levels as determined at the ARC-GCI, using the ELISA – Neogen Veratox quantitative fumonisin 5/10 test kit (Neogen Corp, Lansing, MI, USA) according to the manufacturer's instructions. Selection was based on 15 random samples of 25 g each, which were drawn from each silo-sample and evaluated according to the standard protocol.

Increased sub-sample size

Five of the initial 50 kg silo samples with low to high fumonisin concentrations were selected for this study, based on the above initial analyses. A series of grain sub-samples (25, 50, 75, 100, 125, 150, 200, 250, 500, 750, 1000 g) were randomly drawn from each of the 50 kg samples. Sub-sampling from each respective mass class was replicated five times. Each sub-sample was individually milled in a Cyclotech 1093 sample mill (with a 1 mm mesh sieve). The mill was thoroughly cleaned with high-pressure air between each sub-sample to prevent cross contamination of mycotoxins from the different sub-samples. A final 5 g aliquot was independently drawn from each of the milled sub-samples as specified in the ELISA-Veratox protocol (Anonymous, 2006) for fumonisin extraction and quantification.

Variation within a single maize sub-sample

Maize grain visibly infected with Fusarium spp. was collected from a farm in Vredefort (Free State Province). The visibly infected kernels were separated by hand while the apparently healthy grains were discarded. Five random sub-samples of 250 g each were taken from the visibly infected kernel sample. Each sample was milled and evaluated for fumonisin concentration using the ELISA-Veratox protocol (Anonymous, 2006). The sample had a mean fumonisin concentration of 5.3 ppm. Grain from this sample was subsequently used to spike a grain silo sample from Leeudoringstad, which had a low fumonisin level (0-1 ppm) as determined at the initiation of the study.Visibly infected grains were added at rates of 75%, 50% and 25% by weight to 5 kg of Leeudoringstad grain and thoroughly mixed to ensure homogeneity. A visibly infected sample served as a control. Variation in fumonisin quantification within each spiked maize sample and the control was then determined. Twenty-one replications of 25 g samples from each of the 5 kg spiked sub-samples and the control were separately milled as previously described. A 5 g aliquot from each 25 g milled sub-sample was analysed as described by the ELISA-Veratox protocol (Anonymous, 2006).

The entire procedure was repeated using 250 g sub-samples, which were milled before 5 g aliquots were drawn for fumonisin testing.

Number of replicates

Data from the initial fumonisin tests were used to select grain from five localities with fumonisin levels varying from 0 - 7.5 ppm, i.e. Bronkhorstspruit (0 ppm), Hartebeesfontein (1.7 ppm), Ventersdorp (2.3 ppm), Sannieshof (2.4 ppm) and Derby (7.5 ppm). Five random 25 g sub-samples were drawn from each of the initial 50 kg silo samples. Sub-sampling was repeated five times to obtain a total of 25 independent replicates. Each sub-sample was milled as described above and a final 5 g aliquot was taken for fumonisin quantification according to the ELISA-Veratox protocol. This procedure was repeated by drawing 25 replicates of 250 g each, from which a final 5 g aliquot was taken for fumonisin quantification.

This study was repeated with fumonisin-contaminated grain, which was added at rates of 100%, 75%, 50% and 25% weight to 5 kg Leeudoringstad grain batches as described above. Twenty-five independent grain sub-samples of 25 g each were drawn, separately milled and a final 5 g aliquot was taken for fumonisin determination. This procedure was repeated by drawing 25 replicates of 250 g each, from which a final 5 g aliquot was taken for fumonisin quantification.

Consistency between laboratories

Two replications of 500 g milled maize of each of the five samples selected above were sent to the Medical Research Council - PROMEC (MRC) in Cape Town and an independent, certified private laboratory for fumonisin analysis. MRC and the independent laboratory analysed the samples using the HPLC technique (Shephard, 2000). Two replicates of the same samples were evaluated for fumonisin concentration using the ELISA-Veratox protocol (Anonymous, 2006) at the ARC-GCI.

Data analysis

All data were analysed using Statgraphics Version 5.0. Non-linear regression analysis ($Y=aX^b$) was used to quantify the relationship between maize sub-sample sizes, mean number of positive sub-samples (> 1ppm) and detected fumonisin concentrations. Fumonisin data for the 25 g and 250 g samples were analysed by calculating the standard deviation and coefficient of variation (CV) for each spiked sample (100%, 75%, 50% and 25%). CV's were plotted against percentage spiked samples using linear regression analysis. Data from the respective laboratories were compared using correlation and regression analysis to quantify the relationship between the two techniques and repeatability between laboratories.

Results

Sub-sample sizes

Analysis of variance indicated significant differences in fumonisin concentrations (P0.05) associated with the various samples sizes. No locality x sample-size interaction was recorded. Tukey-Kramer's means separation indicated that variation in fumonisin concentration was high between 25 g and 200 g samples (Table 1) and there appeared to be no consistent separation of means within this range. At sub-sample

sizes 250 g a more consistent quantification of fumonisins was recorded and no significant increase in detection efficiency was observed with sub-sample sizes > 250 g (Table 1). Non-linear regression ($Y=aX^b$) with a $R^2=0.64$ ($P<0.05$) indicated a significant relationship between the detected fumonisin concentration using the ELISA method and size of the grain sub-sample tested (Figure 1). A similar relationship ($R^2=0.81$, $P=0.001$) was recorded between sample size and the recorded number of positive sub-samples. Use of 25 g sub-samples resulted in 23 of the 25 sub-samples being tested negative for fumonisins. In contrast, 250 g sub-samples resulted in 23 of the 25 sub-samples testing positive for fumonisins, with concentrations ranging from 1 - 17 ppm.

Table 1 Fumonisin concentrations (ppm) determined using varying sub-samples (21) sizes prior to the application of the ELISA-Veratox protocol

Locality	25 g	50 g	75 g	100 g	125 g	150 g	200 g	250 g	500 g	750 g	1000 g	Mean
Hartebeesfontein	0.00	0.00	0.26	2.91	1.41	0.41	1.50	1.28	3.27	1.30	2.63	1.36a
Derby	2.24	5.24	12.15	9.21	5.62	3.96	4.09	8.30	5.62	5.62	6.69	6.25b
Sannieshof	0.00	0.00	0.35	1.13	2.32	0.49	1.90	4.44	4.10	3.71	3.49	1.99a
Ventersdorp	0.00	0.23	0.40	2.20	1.33	1.18	2.95	4.07	2.42	2.41	2.87	1.82a
Bronkhorstspruit	0.00	2.54	0.83	0.21	1.25	1.83	2.26	1.20	1.56	1.64	1.61	1.36a
Mean	0.45a	1.60a	2.80b	3.13b	2.39a	1.57a	2.54a	3.88b	3.39b	2.94b	3.46b	2.56

Mean values with the same letter are not statistically significant at P=0.05

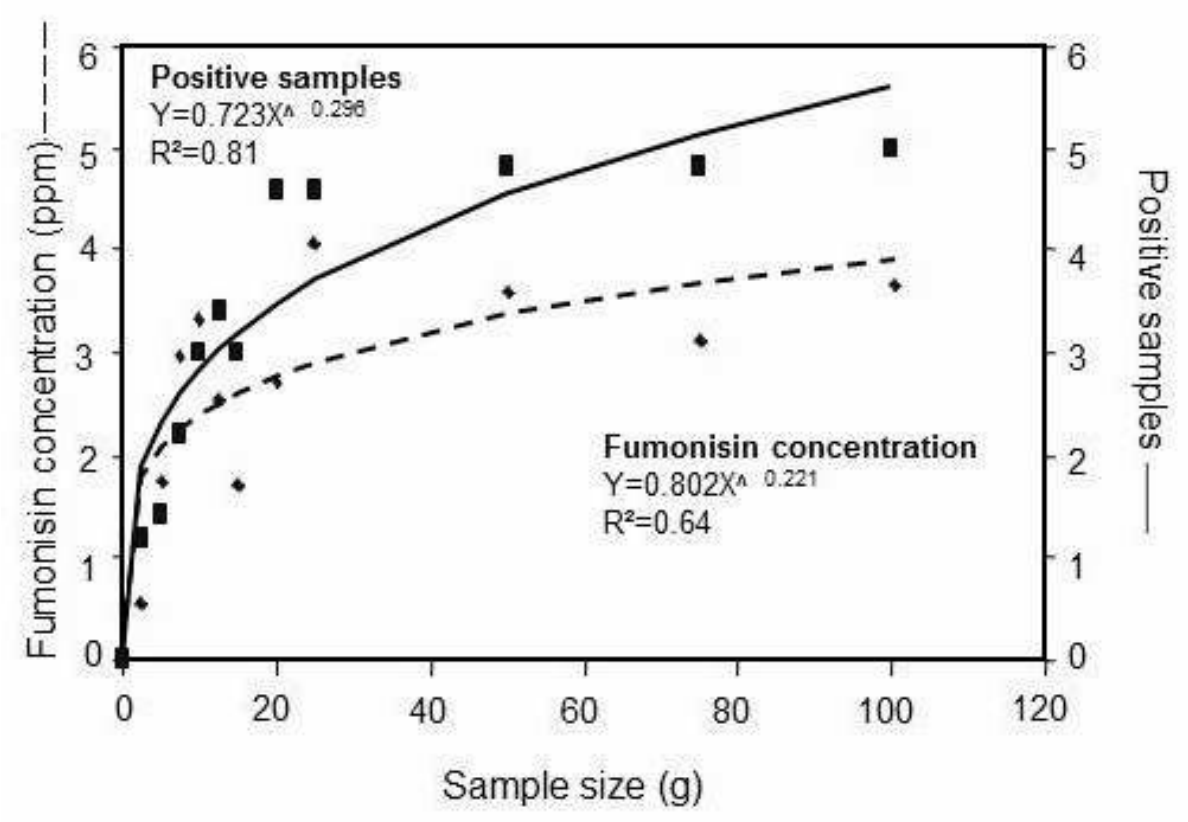

Figure 1 Relationship between increasing sub-sample size, number of positive hits and detected fumonisin concentration.

Variation within a single maize sample

High CV's within the 25 g sub-samples from the spiked Leeudoringstad grain were recorded (Figure 2). Although a linear decline in CV was recorded as the level of contaminated grain increased, this relationship was not significant ($R^2=0.65$, $P=0.14$). The CV for the 250 g sub-samples decreased from 52.4% in the 25% spiked sub-sample to 30.5% in the 100% spiked sub-sample (Figure 2) and a significant linear relationship between level of contamination and CV was recorded ($R^2=0.83$; $P=0.05$). Results indicate that the use of 250 g sub-samples reduced variation substantially when compared with

results from the 25 g sub-samples. The prescribed ELISA-Veratox protocol of 25 g sub-samples yielded 6, 15 and 12 positive sub-samples in the 25, 50 and 75% spiked sub-samples, respectively'compared to the 250 g sub-samples, which had only one false negative in the 25% spiked sub-sample (Figure 2).

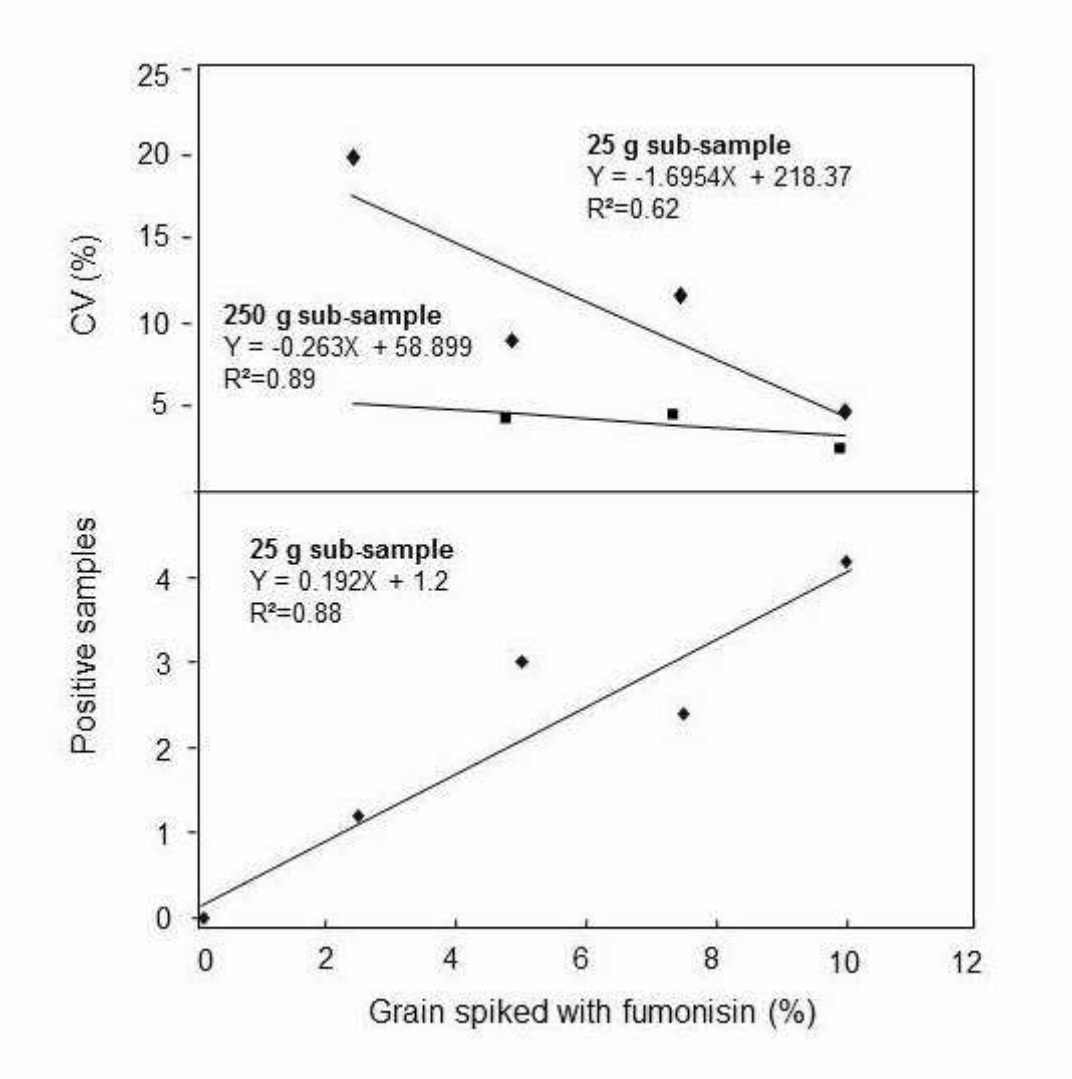

Figure 2 The relationship between coefficient of variation, number of positive samples and spiked fumonisin concentrations for 25 g and 250 g sub-samples.

Number of replications

No significant reduction in standard deviation or CV was observed when using up to 25 replications of 25 g sub-samples. Variation indicators increased with each increase in replication due to the high number of negative sub-samples and zerovalues included in the analyses. The number-of-replication study using the 25 g sub-sample size showed a slight improvement in standard deviation for the 50% and 100% spiked samples with increased replication. A reduction in the CV from 85.5 to 67.11 occurred after 10 replications in the 50% spiked samples. The 75% spiked samples showed a reduction in CV from 157.93 to 118.20 after 15 replications, and from 61.23 to 46.92 after 10 replications in the 100% spiked samples. The standard deviation was lower with high fumonisin concentrations compared to low fumonisin concentrations in the 25 g samples.

Where larger (250 g) samples were used in the spiked-sample study no consistent reduction in CV or standard deviation was observed. Tendencies indicate that at lower fumonisin concentrations more replications are needed, while at higher fumonisin concentrations fewer replications would suffice.

Consistency between laboratories

ELISA-Veratox fumonisin levels correlated significantly (r=0.91; P=0.032) with HPLC fumonisin levels obtained at the MRC, although fumonisin concentrations using the ELISA technique were consistently higher (by approximately 33%) than fumonisin concentrations using the HPLC technique (Figure 3). Fumonisin levels from the independent laboratory obtained using HPLC level did not correlate with the the ELISA-Veratox technique used at the ARC-GCI (R^2=0.534; P=0.354) or with HPLC results from the MRC (R^2=0.166; P=0.798) indicating that both technique and laboratory may be sources of variation.

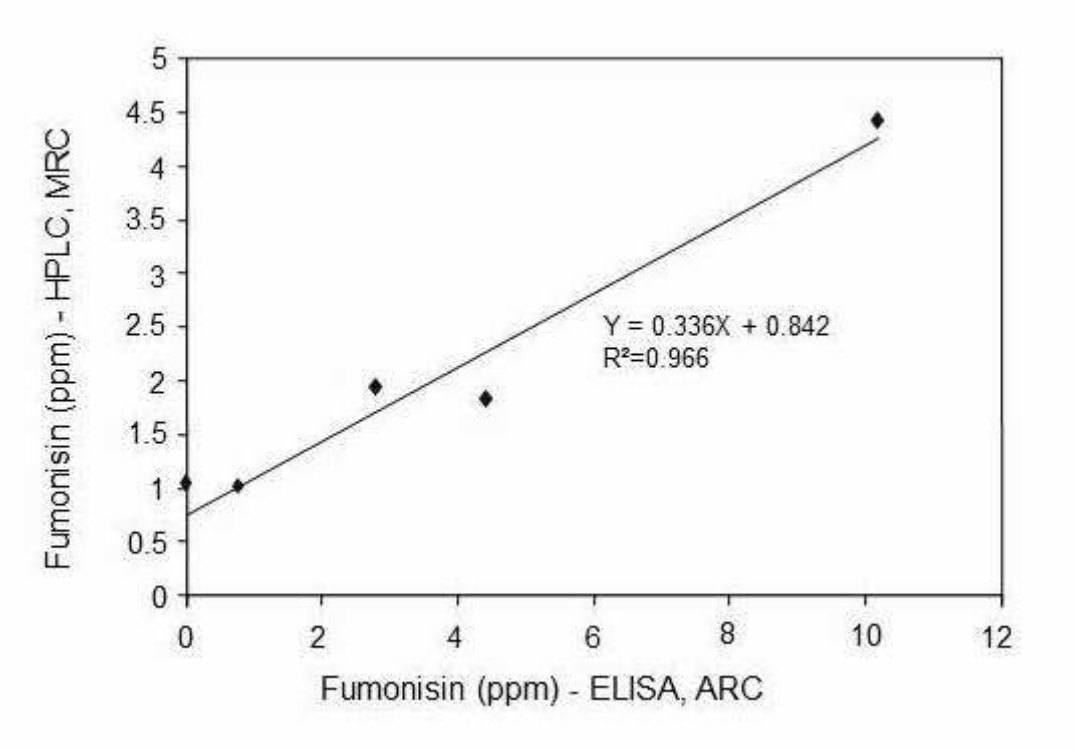

Figure 3 Fumonisin concentrations detected by ELISA compared to HPLC fumonisin concentrations.

Discussion

The ELISA-Veratox protocol requires that a 25 g sub-sample be drawn from a larger sample for milling. A 5 g aliquot is subsequently used for fumonisin testing. Results obtained in the present study showed variation to be high in small sub-samples (25 g), especially those with a low fumonisin content. Variation was lower in samples with a high fumonisin content (50%, 75% and 100% spiked sub-samples) and notably so when using larger sub-samples (250 g). Whitaker and Johansson (2005) explain that sub-samples with high fumonisin content have a more homogeneous spread of the toxins thus contributing to lower variation in their detection. Variation increases in small samples with low fumonisin concentrations because of decreasing homogeneity of the mycotoxin. This increases the possibility that the whole sample will be discarded because the sub-sample was not representative of that sample. An increase in replications using the 25 g samples had little effect on reducing standard deviation and CV percentages remained high.

This study showed that substituting the protocol 25 g sub-sample with a modified 250 g sub-sample before drawing a final 5 g aliquot from the 250 g sub-sample significantly reduced variation because the larger sample was more representative of the total sample. At lower sub-sample sizes the variation was more extreme and as sub-samples increased, variation decreased. It was shown that using 25 g sub-samples resulted in 23 out of 25 false negatives. The implication of this finding is that fumonisin levels in a small sub-sample can be easily underestimated or worse, not detected (Whitaker & Johansson, 2005). This implies that maize samples with a fumonisin level higher than the legal limit could be accepted and these fumonisin-contaminated samples may be consumed by humans and animals, resulting in health-related problems. By using the modified 250 g sub-sample, increased accuracy and precision of fumonisin levels were obtained, thereby reducing the risk of incorrectly classifying maize samples.

In the 25 g sub-sample study only the 75% and 100% spiked samples produced a slight reduction in the CV and standard deviation compared with lower percentages (Table 2). Whitaker and Johansson (2005) explained that random variation among fumonisin levels within the same maize sample may occur. When the sample fumonisin level increases, the variation between fumonisin levels will also increase but the CV will decrease. Mean fumonisin levels in the 25 g sub-samples were 1 ppm (25% spiked sub-sample), 2.9 ppm (50% spiked sub-sample), 3.4 ppm (75% spiked sub-sample) and 5 ppm (100% spiked sub-sample). Fumonisin levels ranged from 0 - 8.1 ppm (25% spiked sub-sample), 0 - 7.1 ppm (50% spiked sub-sample), 0 - 9 ppm (75% spiked sub-sample) and 1.9 - 10.1 ppm (100% spiked sample). Although variation in fumonisin levels was high for all the sub-samples, only the 75% and 100% spiked sub-samples produced a slight reduction in CV and standard deviation, indicating that the high fumonisin levels in spiked sub-samples slightly reduced the variation of fumonisin levels within these sub-samples.

The 250 g sub-samples had a reduction in the CV from 25% - 100% spiked sub-samples (Table 2). The 250 g sub-samples were more representative than the 25 g sub-samples, which yielded 30 false negatives. Whitaker and Johansson (2005) found that when a specific sample is tested there is a greater than 50% chance that the sample fumonisin level will test lower than the true fumonisin level. The skewness was

greater for small sub-sample sizes and the distribution became more symmetrical as the sample size increased. Increasing the sub-sample size to 250 g significantly reduced variation of fumonisin levels in samples containing high and low fumonisin levels.

Table 2 Correlation between fumonisin spiked 25g and 250g sub-sample effects and coefficient of variation

	Fumonisins (ppm)							
Replication	25 g samples				250 g samples			
	25% spiked	50% spiked	75% spiked	100% spiked	25% spiked	50% spiked	75% spiked	100% spiked
1	0.0	4.0	9.0	10.1	3.1	8.4	4.7	5.9
2	0.0	7.1	0.0	2.4	0.0	3.6	7.1	7.0
3	2.2	10.0	0.0	7.3	2.8	5.6	5.3	5.6
4	0.0	2.1	0.0	1.9	1.7	2.9	4.6	5.1
5	0.0	0.0	3.5	8.1	1.2	2.4	5.7	2.9
6	0.0	3.2	3.7	5.2	1.2	4.1	5.3	7.0
7	0.0	3.4	12.0	3.3	1.0	4.6	2.1	5.1
8	0.0	4.0	0.0	5.2	1.6	7.2	5.4	5.6
9	4.1	2.9	3.2	7.0	1.1	4.8	9.3	5.7
10	0.0	4.1	2.1	5.4	0.4	2.8	5.2	5.8
11	0.0	5.2	0.0	2.4	1.6	4.9	5.2	5.8
12	3.4	2.7	0.0	4.3	2.0	8.3	2.9	7.0
13	0.0	0.0	1.2	2.9	1.3	3.3	3.5	5.9
14	0.0	0.0	8.2	8.6	1.0	5.3	5.3	7.0
15	0.0	4.5	7.1	6.3	0.1	1.9	5.9	7.0
16	0.0	0.0	7.1	4.2	2.2	3.9	4.1	3.2
17	8.1	1.2	9.3	7.3	2.1	2.2	5.8	5.3
18	1.2	0.0	4.0	2.3	1.7	4.3	2.1	1.2
19	0.0	3.8	0.0	3.9	1.4	3.7	1.8	2.3
20	0.0	3.2	0.0	2.5	1.8	2.3	1.8	5.8
21	3.3	0.0	0.0	4.4	1.7	3.1	1.6	5.4
Mean	1.0	2.9	3.4	5.0	1.5	4.3	4.5	5.3
CV	197.59	88.99	115.63	47.43	52.38	43.53	43.45	30.49

Whitaker and Johansson (2005) reported that 10 replicated aflatoxin test results from the same sample yielded CV's that ranged from 31.6% to 196.3%. As the aflatoxin concentration in the sample increased, the variance among aflatoxin level results increased but the CV decreased. Even though a small sub-sample (25 g) was used in this study, the high percentage of fumonisin levels in the 75% and 100% spiked samples with increasing replicates ensured more accurate fumonisin quantification, explaining the slight reduction in CV and standard deviation (Whitaker & Johansson, 2005). It appears that high fumonisin levels, rather than replications reduced the CV and standard deviation of small sub-samples.

Increased replication had a greater effect on reducing variation in the modified 250 g sub-samples. This could be due to the 250 g sub-samples that were more representative of the original sample, thereby reducing variation in fumonisin levels in each sub-sample (Whitaker& Johansson, 2005). The number of replications needed to improve accuracy will depend on the level of fumonisins in a sample. Samples with high fumonisin levels require less replications than moderately or low fumonisin contaminated samples (Whitaker & Johansson, 2005).

This study emphasised the importance of using more replications together with a 250 g sub-sample to reduce variation in detected fumonisin levels. The use of small sub-sample sizes (25 g), with increased replications will not reduce variation except when the sample has high fumonisin concentrations.

There was a significant correlation between ELISA fumonisin levels detected by the ARC-GCI and the HPLC fumonisin levels carried out at the MRC. Fumonisin levels obtained using the ELISA technique were consistently 33% higher in this study than fumonisin levels obtained using the HPLC, which was in agreement with Shelby et al. (1994) and Desjardins (2006). These authors suggested that ELISA reacted to antibodies of all known members of the fumonisin class of mycotoxins (B_1, B_2 and B_3). These antibodies also detected additional fumonisin-like molecular structures and the result was inflated when compared to other methods of analysis such as HPLC (Shelby et al., 1994; Desjardins, 2006). The same HPLC technique used by both MRC and the independent laboratory correlated poorly. This is an indication that the laboratory where analyses are carried out is an important additional source of variation, even though the same quantification technique is used.

A reduction in variation of fumonisin levels using the

ELISA-Veratox protocol will result in increased confidence and accuracy of fumonisin quantification by addressing the problems highlighted in this study. The higher percentage fumonisin levels of ELISA can be reduced by 33% to obtain fumonisin levels in accordance with HPLC fumonisin levels. To reduce variation associated between laboratories it is advisable to make use of one reputable laboratory for fumonisin quantifications.

References

ANONYMOUS, 2006. Veratox quantitative fumonisin test instructional pamphlet. Neogen Corporation, 620 Lesher Place, Lansing, MI 48912.

ANONYMOUS, 2008. Agricultural products standards act of 1990. Government Gazette, 25 January 2008, No 30681.

DESJARDINS, A.E., 2006. Fusarium mycotoxins. Chemistry, Genetics and Biology. St Paul, The American Phytopathological Society. 260p.

DILKIN, P., MALLMANN, C.A., DE ALMEIDA, C.A.A. & CORRÊA, B., 2001. Robotic animated clean-up for detection of fumonisins B_1 and B_2 in corn and corn-based feed by high performance liquid chromatography. J. of Chrom. 925, 151-157.

JANSE VAN RENSBURG, B., FLETT, B.C., MCLAREN, N.W. & MCDONALD. A.H., 2008. Variation in the identification of Fusarium spp. in maize samples due to enumerator and growth medium. S. Afr. J. Plant Soil 25, 105-110.

KRSKA, R. & WELZIG, E., 2006. Mycotoxin analysis: An overview of classical, rapid and emerging techniques. p.225-247. In: The mycotoxin fact book. D. Barug, D. Bhatnagar, H.P. van Egmond, J.W. van der Kamp, W.A.Osenbruggen & A. Visconti (eds.). The Netherlands, Wageningen Academic Publishers.

PETA BETANCOURT, S.D., FLORES, G.H., ROSAS, S.J. & GONZLEZ, L.A., 2006. Detection of fumonisins in maize (Zea mays L.) by three analytical techniques (HPLC, TLC and ELISA). p. 101-106. In: Mycotoxins and Phycotoxins. H. Njapau, S. Trujillo, H.P. van Egmond & D.L. Park (eds.). The Netherlands, Wageningen Academic Publishers.

SHELBY, R.A., WHITE, D.G. & BAUSKE, E.M., 1994. Differential fumonisin production in maize hybrids. Plant Dis. 78, 582-584.

SHEPHARD, G.S., 2000. Liquid chromatographic method for fumonisins in corn. Meth. Mol. Biol.157, 147-157.

SYDENHAM, E.W., SHEPHARD, G.S., THIEL, P.G., STOCKENSTRÖM, S., SNIJMAN, P.W. & VAN SCHALKWYK, D.J., 1996. Liquid chromatographic determination of fumonisins B^1, B^2 and B^3 in corn. J. Assoc. Off. Anal. Chem. 79, 688-696.

WHITAKER, T.B., TRUCKSESS, M.W., JOHANSSON, A.S., GIESBRECHT, F.G., HAGLER, W.M.& BOWMAN, D.T., 1998. Variability associated with testing shelled corn for fumonisin. J. Assoc. Off. Anal. Chem. 81, 1162-1168.

WHITAKER, T.B., HAGLER JR, W.M., JOHANSSON, A.S., GIESBRECHT, F.G. & TRUCKSESS, M.W., 2001. Distribution among sample test results when testing shelled corn lots from fumonisins. J. Assoc. Off. Anal. Chem.84, 770-776.

WHITAKER, T.B., 2003. Standardisation of mycotoxin sampling procedures: an urgent necessity. Food Control14, 233-237.

WHITAKER, T.B. & JOHANSSON, A.S., 2005. Sampling uncertainties for the detection of chemical agents in complex food matrixes. J. Food. Prot.68, 1306-1313.

WHITAKER, T.B., 2006. Sampling foods for mycotoxins. Food. Addit. Contam. 23, 50-61.