



**The Southern African Grain Laboratory NPC**

We measure so that you know



# *Evaluation of commercial screening kits for the determination of Mycotoxins in maize in non-laboratory environment*

*AGBIZ SYMPOSIUM*

15 AUGUST 2017



## INTRODUCTION

There is a need in the maize industry to measure the Mycotoxin contamination in all stages of maize production, storage and processing, by using reliable screening kits in non-laboratory environments.

In this project rapid test kits for the quantitative analyses of:

- aflatoxins (Afla),
- fumonisins (FUM) and
- deoxynivalenol (DON) in maize samples in a non-laboratory environment, such as at silos was evaluated.

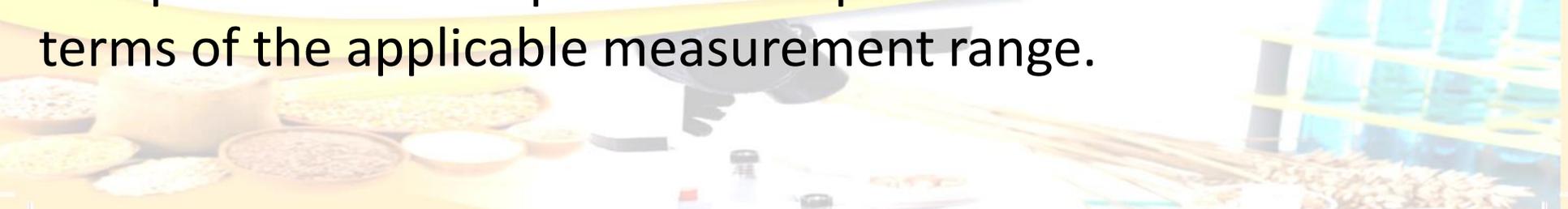
There are a wide variety of commercially available rapid test kits for different mycotoxins and a variety of testing matrices.

The challenge for commercial mycotoxin test kits to accurately determine the mycotoxin concentration is huge. Both qualitative and quantitative screening kits are available on the market;

The qualitative testing kits provide rapid results confirming the presence of the target mycotoxins

and

the quantitative kits provide the quantitative measurements in terms of the applicable measurement range.



## The Project focused on:

- The collection of maize samples with the mycotoxins at different concentration levels.
- The analysis of the samples with the accredited SAGL LCMSMS multi-mycotoxin test method.
- The selection and verification of quantitative screening kits available in South Africa according to the GIPSA (Grain Inspection, Packers and Stockyards Administration) criteria.

## COLLECTION OF MAIZE SAMPLES

We had to collect enough maize samples with aflatoxin, fumonisin and deoxynivalenol at different concentration levels.

The SAGL sourced enough naturally contaminated maize samples to continue with the verification of the rapid kits.

The samples were analysed with the accredited SAGL LCMSMS multi-mycotoxin test method.

# PREPARATION OF MAIZE SAMPLES

**Milling to particle size < 1mm**



**Mixing of milled sample for 90 min to ensure homogeneity of the sample**

## SELECTION OF TEST KITS

The United States Department of Agriculture, Grain Inspection, Packers and Stockyards Administration (GIPSA) has an official test program to evaluate test kits according to design criteria and test performance specifications for quantitative test kits to measure the most important mycotoxins, aflatoxins, fumonisins or deoxynivalenol, in various grain commodities.

Test kits for quantitative analysis were selected from the list GIPSA performance verified mycotoxin test kits

Only kits that use a water-based extraction can be used in a non-laboratory environment,

therefore all kits that use an organic solvent extraction and additional clean-up with Solid Phase Extraction (SPE) cartridges were not selected for the verification.

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Manufacturer	SA Supplier and contact person	Mycotoxin and concentration range	Test kit	Detection method
Charm Sciences	Anatech Instruments	Aflatoxin 5 – 100 ppb	ROSA WET-S5 Aflatoxin Quantitative test	ROSA-M Reader
		Deoxynivalenol 0.5 – 5 ppm	ROSA DON WET-S5 Quantitative test	
		Fumonisin 0.5 – 5 ppm	ROSA WET-S5 Fumonisin Quantitative test	
Envirologix	Stargate Scientific	Aflatoxin 5 – 100 ppb	Quick Tox Kit Afla Free	Quick Scan
		Deoxynivalenol 0.5 – 5 ppm	Quick Tox Kit DON3	
		Fumonisin 0.5 – 5 ppm	Quick Tox Kit Fumonisin Flex	
Neogen Corporation	Analytical & Diagnostic Products CC	Aflatoxin 5 – 100 ppb	Reveal Q+ Max for Aflatoxin	AccuScan Gold Reader
		Deoxynivalenol 0.5 – 5 ppm	Reveal Q+ for DON	
		Fumonisin	-	
Romer Labs	Biomin South Africa	Aflatoxin 5 – 100 ppb	Agrastrip Total Aflatoxin WATEX	AgraVision Reader
		Deoxynivalenol 0.25 – 6 ppm	Agrastrip Deoxynivalenol WATEX	
		Fumonisin Total (B <sub>1</sub> , B <sub>2</sub> and B <sub>3</sub> ) 0.5 – 5 ppm	Agrastrip Total Fumonisin WATEX	

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Weigh 50 g sample



Add extraction powder



Add 150 mL water



Sample extraction



Settling time



Transfer 1 mL sample to tube



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Centrifuge for 10 sec



Extract after centrifuge



Pipette 950  $\mu$ L dilution buffer



Add 50  $\mu$ L of clear extract into  
950  $\mu$ L buffer



Mix the buffer and extract well



Inspect the strip before use



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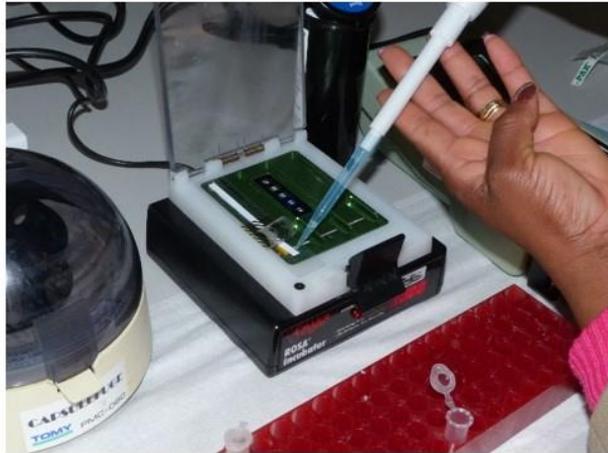
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Load extract on the strip



Incubate for 5 min



Reader performance check



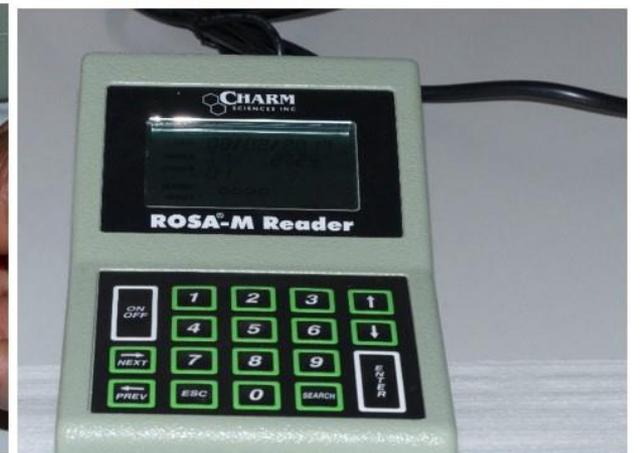
After incubation



Read the strip



Get results with correct  
calibration



## VERIFICATION PARAMETERS

The verification parameters were **specificity**, **accuracy**, **precision** and the speed and ease to conduct the tests.

- **Specificity** over the concentration range was evaluated with samples containing the different combinations of Afla B<sub>1</sub>, Afla B<sub>2</sub> and Afla G<sub>1</sub>, FUM B<sub>1</sub>, FUM B<sub>2</sub>, FUM B<sub>3</sub> and / or deoxynivalenol as well as related mycotoxins e.g. 15-ADON.

- **Accuracy** was determined by:
  - comparing the test kit results with the LCMSMS results obtained with the SANAS accredited SAGL In-House method.
- **Precision:**
  - Replicate samples were tested to determine the repeatability and reproducibility.
  - Five replicate analyses at three different concentration levels  
and
  - five replicate analysis of blank maize with no mycotoxins were conducted for each of the three mycotoxins



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Sample Info		LCMSMS Aflatoxin					KIT aflatoxin results			
							ROSA	Enviroligix	Neogen	Romer
		B <sub>1</sub>	B <sub>2</sub>	G <sub>1</sub>	G <sub>2</sub>	Total	Afla B <sub>1</sub>	Total	Total	Total
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND	ND	ND	ND	ND
MSK 6	Mean (n=5)	59.9 µg/kg	4.79 µg/kg	ND	ND	64.7 µg/kg	46 µg/kg	51 µg/kg	43.6 µg/kg	66.4 µg/kg
	RSD	16%	12%	-	-	15%	7%	6.7%	9.4%	6.8%
	% Recovery	-	-	-	-	-	77%	85%	73%	103%

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Sample Info		LCMSMS Aflatoxin					KIT aflatoxin results			
							ROSA	Envirologix	Neogen	Romer
		B <sub>1</sub>	B <sub>2</sub>	G <sub>1</sub>	G <sub>2</sub>	Total	Afla B <sub>1</sub>	Total	Total	Total
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND	ND	ND	ND	ND
MSK 6	Mean (n=5)	59.9 µg/kg	4.79 µg/kg	ND	ND	64.7 µg/kg	46 µg/kg	51 µg/kg	43.6 µg/kg	66.4 µg/kg
	RSD	16%	12%	-	-	15%	7%	6.7%	9.4%	6.8%
	% Recovery	-	-	-	-	-	77%	85%	73%	103%
MSK 7	Mean (n=5)	42.8 µg/kg	<LOQ	13.7 µg/kg	ND	56.4 µg/kg	41 µg/kg	52 µg/kg	48.5 µg/kg	82.5 µg/kg
	RSD	7%	-	18%	-	6%	11%	7.7%	4.7%	4.7%
	% Recovery	-	-	-	-	-	96%	122%	113%	146%

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Sample Info		LCMSMS Aflatoxin					KIT aflatoxin results			
							ROSA	Envirologix	Neogen	Romer
		B <sub>1</sub>	B <sub>2</sub>	G <sub>1</sub>	G <sub>2</sub>	Total	Afla B <sub>1</sub>	Total	Total	Total
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND	ND	ND	ND	ND
MSK 6	Mean (n=5)	59.9 µg/kg	4.79 µg/kg	ND	ND	64.7 µg/kg	46 µg/kg	51 µg/kg	43.6 µg/kg	66.4 µg/kg
	RSD	16%	12%	-	-	15%	7%	6.7%	9.4%	6.8%
	% Recovery	-	-	-	-	-	77%	85%	73%	103%
MSK 7	Mean (n=5)	42.8 µg/kg	<LOQ	13.7 µg/kg	ND	56.4 µg/kg	41 µg/kg	52 µg/kg	48.5 µg/kg	82.5 µg/kg
	RSD	7%	-	18%	-	6%	11%	7.7%	4.7%	4.7%
	% Recovery	-	-	-	-	-	96%	122%	113%	146%
MSK 13	Mean (n=5)	11.7 µg/kg	ND	ND	ND	11.7 µg/kg	16 µg/kg	15 µg/kg	13.5 µg/kg	11.6 µg/kg
	RSD	17%	-	-	-	17%	2.5%	24%	5.2%	19%
	% Recovery	-	-	-	-	-	137%	128%	115%	99%

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Sample info		LCMSMS Fumonisin			Total	KIT Fumonisin results			
		B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>		ROSA	Envirologix	Neogen	Romer
						Total			
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND	-	ND	

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Sample info		LCMSMS Fumonisin			Total	KIT Fumonisin results			
		B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>		ROSA	Enviroligix	Neogen	Romer
						Total			
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND	-	ND	
MSK 5	Mean (n=5)	3.62 mg/kg	1.01 mg/kg	2.17 mg/kg	4.93 mg/kg	3.9 mg/kg	5.3 mg/kg	-	3.8 mg/kg
	RSD	2%	2%	2%	1%	10.3%	11.3%	-	10.5%
	% Recovery	-	-	-	-	79%	107%	-	77%

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Sample info		LCMSMS Fumonisin			Total	KIT Fumonisin results			
		B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>		ROSA	Enviroligix	Neogen	Romer
						Total			
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND	-	ND	
MSK 5	Mean (n=5)	3.62 mg/kg	1.01 mg/kg	2.17 mg/kg	4.93 mg/kg	3.9 mg/kg	5.3 mg/kg	-	3.8 mg/kg
	RSD	2%	2%	2%	1%	10.3%	11.3%	-	10.5%
	% Recovery	-	-	-	-	79%	107%	-	77%
MSK 10	Mean (n=5)	1.73 mg/kg	3.86 mg/kg	1.14 mg/kg	2.23 mg/kg	2.1 mg/kg	2.4 mg/kg	-	2.7 mg/kg
	RSD	5%	4%	5%	4%	14.3%	8.3%	-	11.1%
	% Recovery	-	-	-	-	94%	108%	-	121%

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Sample info		LCMSMS Fumonisin			Total	KIT Fumonisin results			
		B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>		ROSA	Enviroligix	Neogen	Romer
						Total			
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND	ND	-	ND
MSK 5	Mean (n=5)	3.62 mg/kg	1.01 mg/kg	2.17 mg/kg	4.93 mg/kg	3.9 mg/kg	5.3 mg/kg	-	3.8 mg/kg
	RSD	2%	2%	2%	1%	10.3%	11.3%	-	10.5%
	% Recovery	-	-	-	-	79%	107%	-	77%
MSK 10	Mean (n=5)	1.73 mg/kg	3.86 mg/kg	1.14 mg/kg	2.23 mg/kg	2.1 mg/kg	2.4 mg/kg	-	2.7 mg/kg
	RSD	5%	4%	5%	4%	14.3%	8.3%	-	11.1%
	% Recovery	-	-	-	-	94%	108%	-	121%
MSK 11	Mean (n=5)	0.803 mg/kg	0.197 mg/kg	0.040 mg/kg	1.04 mg/kg	1.0 mg/kg	1.2 mg/kg	-	1.3 mg/kg
	RSD	5%	2%	6%	4%	10%	8.3%	-	15.4%
	% Recovery	-	-	-	-	96%	115%	-	120%

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Sample Info		LCMSMS DON	KIT Deoxynivalenol results			
			ROSA	Envirologix	Neogen	Romer
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND

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Sample Info		LCMSMS DON	KIT Deoxynivalenol results			
			ROSA	Envirologix	Neogen	Romer
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND
MSK 4	Mean (n=5)	1.48 mg/kg	2.4 mg/kg	2.6 mg/kg	1.7 mg/kg	2.0 mg/kg
	RSD	7%	24%	3.8%	23.5%	20%
	% Recovery	-	169%	176%	115%	136%

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Sample Info		LCMSMS DON	KIT Deoxynivalenol results			
			ROSA	Envirologix	Neogen	Romer
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND
MSK 4	Mean (n=5)	1.48 mg/kg	2.4 mg/kg	2.6 mg/kg	1.7 mg/kg	2.0 mg/kg
	RSD	7%	24%	3.8%	23.5%	20%
	% Recovery	-	169%	176%	115%	136%
MSK 8	Mean (n=5)	0.74 mg/kg	0.8 mg/kg	1.2 mg/kg	0.9 mg/kg	0.8 mg/kg
	RSD	8%	13.8%	8.3%	11.1%	25%
	% Recovery	-	108%	162%	122%	108%

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Sample Info		LCMSMS DON	KIT Deoxynivalenol results			
			ROSA	Envirologix	Neogen	Romer
MSK 17	Mean (n=5)	ND	ND	ND	ND	ND
MSK 4	Mean (n=5)	1.48 mg/kg	2.4 mg/kg	2.6 mg/kg	1.7 mg/kg	2.0 mg/kg
	RSD	7%	24%	3.8%	23.5%	20%
	% Recovery	-	169%	176%	115%	136%
MSK 8	Mean (n=5)	0.74 mg/kg	0.8 mg/kg	1.2 mg/kg	0.9 mg/kg	0.8 mg/kg
	RSD	8%	13.8%	8.3%	11.1%	25%
	% Recovery	-	108%	162%	122%	108%
MSK 14	Mean (n=5)	4.0 mg/kg	4.9 mg/kg	6.9 mg/kg	4.2 mg/kg	5.0 mg/kg
	RSD	7%	6.1%	4.3%	4.8%	22.6%
	% Recovery	-	122%	172%	105%	125%

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## CONCLUSIONS

Rapid results are obtained with the different test kits, but the time to correctly mill and properly mix the samples is not included in the kit information.

Operating procedures are supplied but not all the procedures have enough detail to conduct the tests without any training.

In all the tests, micro hand pipettes are used to pipette the required volumes of the extracts. Proper training to verify and use these hand pipettes is important, especially for people working in a non-laboratory environment.



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## Precision

- The precision achieved for all the aflatoxin and fumonisin test samples was within the GIPSA acceptance criteria.
- Relative standard deviations (RSD) > 20% were measured in four of the deoxynivalenol tests conducted.

## Accuracy

- The % recoveries of 11 of the 12 aflatoxin test kits' results were within the GIPSA range.
- The fumonisin % recoveries ranged from 77% - 121% , all within the GIPSA range.
- Higher % recoveries were measured for the DON determinations; seven results ranged within the GIPSA range. % recoveries as high as 162 – 176% were measured in four samples.



## *Available at the SAGL :*

- *SAGL Screening kits Report*
- *SAGL training courses - available upon request*
  - *Understand, find and measure mycotoxins*
  - *Use of a specific quantitative screening kit*



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