SAGL REPORT OF PROJECT FUNDED BY MAIZE TRUST

EVALUATION OF COMMERCIAL SCREENING KITS FOR THE DETERMINATION OF MYCOTOXINS IN MAIZE IN NON-LABORATORY ENVIRONMENTS

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1. Introduction

The results of the mycotoxin surveys on maize performed by the SAGL with funding from the Maize Trust over the past 4 – 5 seasons confirmed that different patterns of occurrence of fumonisins and deoxynivalenol exist depending on the season, production region and class and grade of maize [1]. The SAGL received a request from the Maize Trust Steering Committee to submit a proposal for funding to the Maize Trust for the selection and validation of commercial screening kits for the quantitative determination of mycotoxin levels in maize in non–laboratory environments such as at silos. The request arises from the need of the Maize Industry for fast, reliable testing of mycotoxins in all the stages of intake and storage of maize. The industry has to make informed decisions on compliance of their unprocessed maize and maize products according to the South African mycotoxin Regulations [2, 3, 4] or commercial standards and/or to verify the efficacy of food safety management systems.

To be able to establish the magnitude of mycotoxin contamination in all stages of production, storage and processing, more mycotoxin testing including reliable screening kits in non-laboratory environments are required. These test kits must be fit for the intended purpose. There are a wide variety of commercially available rapid test kits for different mycotoxins and a variety of testing matrices and the challenge for commercial mycotoxin test kits to accurately determine the mycotoxin concentration is huge. The results of test kits have been widely used in the trade of products such as maize and the international food industry was the driving force for the development of the GIPSA and AOAC International evaluation programs to help standardize the performance criteria for testing kits. Many research publications identified the pros and cons of the different test kits; one example is the CODA-CERVA evaluation of test kits for DON in cereals [32]. They indicated that cross reactivity of the antibodies often lead to overestimated individual mycotoxin concentration.

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. Hundred and ninety one commercial test kits were identified by Wei Li in 2014 [10] but a smaller number of kits have been certified by the Grain Inspection, Packers and Stockyards Administration Federal Grain Inspection Service (GIPSA) in the latest update of Performance Verified Mycotoxin Test Kits effective from 16 December 2016 [9].

The screening kits that are in use at the moment in South Africa are not necessarily selected nor certified according to the selection criteria as described in the GIPSA Design Criteria and Test performance Specifications [8, 9].

This project to evaluate rapid test kits for the quantitative analyses of aflatoxins (Afla), fumonisins (FUM) and deoxynivalenol (DON) in maize samples in a non-laboratory environment was conducted in collaboration with the suppliers of the test kits in South Africa. The project was conducted in two phases where phase one focused on the following activities:

- Selection: The selection of quantitative screening kits according to set criteria as described in Section 2 *Selection of test kits*.
- Collaboration: The collaboration was with suppliers in South Africa who are the agents for the selected kits and who were willing to supply the kits and readers to the SAGL for **independent** verification of the kits. Kits from four different manufacturers were identified and the suppliers of the kits in South Africa agreed to supply the kits and readers to the SAGL. The test kits provided by the suppliers are described in Section 3 T*est kits provided.*
- Sample collection: The collection of 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 (see section 4) and the results of the samples selected are summarised in Section 5.2 Multi-*mycotoxin Results.*

In the second phase of the project, the screening kits supplied by the agents were verified with the selected contaminated maize samples according to predefined verification parameters. The verification parameters are described in Section 6.1. Not all the information needed to evaluate the test kits was available in the test kit operating procedures and information available in the GIPSA reports was used. The test kit information of the different test kits is summarised in this report in Sections 6.2 – 6. 5.

2. 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. [5,6,7] 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 in the second phase of the project. Test kits for quantitative analysis were selected from the list provided by GIPSA: GIPSA performance verified mycotoxin test kits – Effective date: 22 April 2016 [8]. The tests kits for fumonisins and deoxynivalenol from Romer Labs were GIPSA approved later in 2016 [9] and were also evaluated.

The suppliers of these kits in South Africa were invited to participate and a detail project layout was made available to four suppliers.

3. Test kits provided

The suppliers willing to participate were requested to supply the following:

- GIPSA certificates of conformance for the different test kits.
- Instrumentation and consumables to conduct the tests in the SAGL laboratory.
- At least 25 – 35 test strips / mycotoxin.
- Complete instructions for the use of the test kits.
- Any related validation reports and application notes available on these test kits.

The test kits and readers that were received from four suppliers of different manufacturers are summarised in table 1.

All the rapid tests kits use lateral flow strip assay technology to quantitatively determine a specific mycotoxin in a sample extract. The lateral flow tests are user-friendly, very fast, relatively inexpensive and suitable for field applications [10]. Quantitative analyses are in most cases based on lot specific calibration curves.

Table 1 Test kits supplied

Manufacturer	SA Supplier and contact person	Mycotoxin and concentration range	Test kit	Part Number	Extraction	Detection method	Remarks
Charm Sciences	Anatech Instruments	Aflatoxin 5 – 100 ppb [8]	ROSA WET-S5 Aflatoxin Quantitative test	LF-AFQ-WET-S5	Water based	ROSA-M Reader	-
		Deoxynivalenol 0.5 – 5 ppm [8]	ROSA DON WET-S5 Quantitative test	LF-DONQ-WETS5	Water based		
		Fumonisins 0.5 – 5 ppm [9]	ROSA WET-S5 Fumonisin Quantitative test	LF-FUMQ-WETS5	Water based		
Envirologix	Stargate Scientific	Aflatoxin 5 – 100 ppb [8]	Quick Tox Kit Afla Free	AQ 209 BG	Water based	Quick Scan	-
		Deoxynivalenol 0.5 – 5 ppm [8]	Quick Tox Kit DON3	AQ 254 BG	Water based		-
		Fumonisins 0.5 – 5 ppm [9]	Quick Tox Kit Fumonisin Flex	AQ 311 BG	Water based		-
Neogen Corporation	Analytical & Diagnostic Products CC	Aflatoxin 5 – 100 ppb [9]	Reveal Q+ Max for Aflatoxin	8088	Water based	AccuScan Gold Reader	-
		Deoxynivalenol 0.5 – 5 ppm [9]	Reveal Q+ for DON	8385	Water based		
		Fumonisins	-	--	Water based		Not yet available, expected 2017
Romer Labs	Biomin South Africa	Aflatoxin 5 – 100 ppb [9]	Agrastrip Total Aflatoxin WATEX	COKAS1600W	Water based	AgraVision Reader	-
		Deoxynivalenol 0.25 – 6 ppm	Agrastrip Deoxynivalenol WATEX	COKAS4000W	Water based		Submitted for GIPSA approval
		Fumonisin Total (B_1, B_2 and B_3) 0.5 – 5 ppm [9]	Agrastrip Total Fumonisin WATEX	COKAS3000W	Water based		-

4. Multi-mycotoxin analysis with LCMSMS

The SAGL In-house method 026 is a validated method for the analysis of 13 different mycotoxins in cereals and related products with UPLC-MS/MS. The method is an accredited method under the requirements of ISO 17025:2005 and the SANAS Certificate of Accreditation for the SAGL; Facility number T0116 is available on the SANAS and SAGL web pages [11]. The mycotoxins that are included in the test method, the concentration ranges, the limit of quantitation (LOQ) and limit of detection (LOD) are given in table 2.

Maize samples are extracted with the extraction solution, diluted and analysed with the UPLC-MS/MS. The mycotoxins are separated on a reversed-phase UPLC column and analysed with positive electrospray (EI) ionisation in the multiple reaction monitoring (MRM) mode. For each compound, one precursor and two product ions were monitored, one product ion for quantification and one for confirmation.

The reference materials purchased from Sigma Aldrich, Biopure and Cape Town University of Technology (CPUT) were used for the preparation of separate stock solutions of each mycotoxin. Matrix-matched working standards containing a mixture of the 13 mycotoxins were prepared regularly to draw a calibration curve for each mycotoxin with at least five calibration levels. A blank maize sample was analysed with every batch of samples to confirm that no contamination was present in the laboratory and spiked maize samples to verify the sample preparation process.

Table 2. Limit of quantitation and limit of detection

Mycotoxin	Limit of quantitation, (LOQ), µg/kg	Limit of detection, (LOD), µg/kg	Concentration range, µg/kg
Aflatoxin B_1	2	1	1.25 – 80
Aflatoxin B_2	2	1	1.25 - 80
Aflatoxin G_1	2	1	1.25 - 80
Aflatoxin G_2	5	2.5	1.25 - 80
Deoxynivalenol	100	50	50 - 4000
15 Acetyl deoxynivalenol	100	50	50 - 4000
Fumonisin B_1	20	10	10 - 4000
Fumonisin B_2	20	10	10 - 4000
Fumonisin B_3	20	10	10 - 4000
Ochratoxin A	5	2.5	1.25 - 80
T-2 Toxin	20	10	10 - 4000
H-T2 toxin	20	10	10 - 4000
Zearalenone	20	10	10 - 4000

General laboratory performance is verified by successful participation in the bimonthly Bipea and several FAPAS international proficiency testing programs. They provided samples of different commodity types and mycotoxins.

5. Maize samples

5.1. Preparation of the whole maize samples

Enough commercially available maize with naturally contaminated fumonisins (FUM), deoxynivalenol (DON) and / or aflatoxins (AFLA) were sourced to cover the concentration ranges indicated in table 1. A minimum

of 1 kg maize sample / mycotoxin / concentration level was needed for the verification experiments that were conducted in phase two.

Before the analyses were conducted, the samples were milled to a particle size < 1 mm. Each sample was mixed in a bin with ceramic balls on a roller mill for at least an hour to ensure homogeneity of the samples. Samples were labelled with unique codes, fully randomized, before the five replicate LCMSMS analyses and the testing of the kits were commenced.

It must be noted that none of the test kit information about speed of analysis include the time that it takes to collect a representative sample and to do the milling and mixing before the quick tests are conducted. This may give the impression that a mycotoxin result may be available within a few minutes once the whole maize sample is received but the milling and mixing process may take one to two hours, depending on the sample size and the available milling facilities.

It is critically important for every test facility to ensure that representative samples are taken. The samples must be milled to the correct particle size specifications and mixed well to ensure that the sample extraction is complete and the subsample used for the analysis does represent the maize. A small sample of whole maize kernels is definitely not representative of a maize consignment.

GIPSA used 50 g sample for all these test kit evaluations. Three of the suppliers also supply kits that use a smaller sample size for the extraction. It is therefore even more important to have a representative maize sample, milled to the required particle size and mixed well to ensure homogeneity.

5.2. Multi-mycotoxin results

Five replicate subsamples of each of these maize samples were analysed with the UPLC MS/MS. Ten different samples were selected at three different concentration levels for Afla and/or FUM and/or DON, for the phase 2 verification process.

The LCMSMS results of the ten selected samples are summarised in table 3. One of the samples that were selected to evaluate the aflatoxin test kits included aflatoxin B_1 and G_1 and one sample aflatoxin B_1 and B_2. The samples selected to verify the fumonisin test kits had FUM B_1, B_2 and B_3. The metabolite 15-Acetyl deoxynivalenol (15-ADON) was measured in the samples with DON residues.

The RSD of the aflatoxin results was ≤ 18%; for the fumonisin results, the RSD was between 1-6% and the RSD of the deoxynivalenol results was ≤ 8%.

Table 3 LCMSMS Mycotoxin results of samples used for the verification of the kits

Sample number		Multi-Mycotoxin Mean Results, µg/kg (ppb) (SAGL In-House Method 26 with UPLC-MS/MS)														
		Aflatoxin					Fumonisin				DON	15-Acetyl-DON	OTA	ZON	T2-toxin	HT-2 -toxin
		B_1	B_2	G_1	G_2	Total	B_1	B_2	B_3	Total						
		LOQ = 2	*LOQ = 2*	*LOQ = 2*	*LOQ = 5*		*LOQ = 20*	*LOQ = 20*	*LOQ = 20*		*LOQ = 100*	*LOQ = 100*	*LOQ = 5*	*LOQ = 20*	*LOQ = 20*	*LOQ = 20*
MSK 17	Mean	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MSK 6	Mean	59.90	4.79	ND	ND	64.68	5319	1177	823	7318	ND	ND	ND	ND	ND	ND
	RSD	16%	12%	-	-	15%	9%	13%	15%	10%	-	-	-	-	-	-
MSK 7	Mean	42.75	<LOQ	13.68	ND	56.43	4882	1167	751	6799	ND	ND	ND	ND	ND	ND
	RSD	7%	-	18%	-	6%	7%	9%	7%	7%	-	-	-	-	-	-
MSK 13	Mean	11.72	ND	ND	ND	11.72	3627	963	397	4987	ND	ND	ND	ND	ND	ND
	RSD	17%	-	-	-	17%	5%	7%	4%	5%	-	-	-	-	-	-
MSK 5	Mean	ND	ND	ND	ND	-	3615	1099	217	4931	ND	ND	ND	ND	ND	ND
	RSD	-	-	-	-	-	2%	2%	2%	1%	-	-	-	-	-	-
MSK 10	Mean	ND	ND	ND	ND	-	1727	386	114	2227	144	ND	ND	<LOQ	ND	ND
	RSD	-	-	-	-	-	5%	4%	5%	4%	12%	-	-	-	-	-
MSK 11	Mean	ND	ND	ND	ND	-	803	197	40	1040	459	<LOQ	ND	89	ND	ND
	RSD	-	-	-	-	-	5%	2%	6%	4%	4%	-	-	-	-	-
MSK 4	Mean	ND	ND	ND	ND	-	30190	13390	3782	47362	1476	146	ND	325	ND	38
	RSD	-	-	-	-	-	7%	8%	7%	7%	7%	16%	-	12%	-	41%
MSK 8	Mean	ND	ND	ND	ND	-	181	55	ND	236	739	<LOQ	ND	17	ND	ND
	RSD	-	-	-	-	-	19%	13%	ND	18%	8%	16%	-	16%	-	-
MSK 14	Mean	ND	ND	ND	ND	-	ND	ND	ND	ND	4007	719	ND	92	ND	ND
	RSD	-	-	-	-	-	-	-	-	-	7%	11%	-	8%	-	-

6. Verification of the test kits

The verification of the tests kits commenced in August 2016 and was completed in November 2016.

Each supplier had the opportunity to demonstrate the use of the test kits to ensure that the equipment and kits are in working order and that the SAGL analysts are familiar with the operation. Anomalies encountered during the verification process were discussed with the specific supplier and manufacturer and where necessary retesting was conducted.

6.1. Verification parameters

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

The speed and ease of use of the tests kits are summarised in the Test Kit information tables, see tables 5 – 8 in Section 6.2 – 6.5.

Specificity over the concentration range was evaluated with samples containing the different combinations of Afla B_1, Afla B_2 and Afla G_1, FUM B_1, FUM B_2, FUM B_3 and / or deoxynivalenol as well as related mycotoxins e.g. 15-ADON. Cross reactivity is also discussed in the Kit Information tables.

Accuracy was determined by comparing the test kit results with the LCMSMS results obtained with the SANAS accredited SAGL In-House method. The % recovery was calculated as the specific mycotoxin concentration measured with test kit / LCMSMS mycotoxin concentration x 100. The acceptable range of % recoveries calculated from the GIPSA Acceptable range / concentration level is summarised in table 4.

Replicate samples were tested to determine the precision (repeatability and reproducibility) and the corresponding GIPSA acceptance criteria (5,6,7) summarised in table 4 were used for the evaluations. A minimum of 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.

Table 4 GIPSA acceptance criteria

Mycotoxin	**Concentration**	**Maximum RSD (%)** (GIPSA acceptance criteria)	**Standard deviation**	**Acceptable range**	**Calculated range of % Recovery**
Total Aflatoxin [5]	5.0 µg/kg	25	1.25	2.5 – 7.5 µg/kg	50 – 150%
	20 µg/kg	20	4	12 – 28 µg/kg	60 – 140 %
	100 µg/kg	16	16	68 – 130 µg/kg	68 – 130 %
	300 µg/kg	16	48	200 – 400 µg/kg	67 – 133 %
Total Fumonisin [6]	0.50 mg/kg	18	0.09	0.32 – 0.68mg/kg	64 – 136 %
	1.00 mg/kg	16	0.16	0.68 – 1.3 mg/kg	68 – 130 %
	2.00 mg/kg	14	0.28	1.4 – 2.6 mg/kg	70 – 130 %
	5.0 mg/kg	13	0.65	3.7 – 6.3 mg/kg	74 – 126 %
Deoxynivalenol [7]	0.50 mg/kg	20	0.1	0.3 – 0.7 mg/kg	60 – 140 %
	1.00 mg/kg	16	0.16	0.68 – 1.3 mg/kg	68 – 130 %
	2.00 mg/kg	12	0.24	1.5 – 2.5 mg/kg	75 - 125 %
	5.0 mg/kg	10	0.50	4.0 – 6.0 mg/kg	80 - 120%

6.2. Charm Sciences (ROSA) Test kits

6.2.1. Use of the ROSA test kits

Table 5

ROSA TEST KIT INFORMATION			
Parameter	**Aflatoxin**	**Fumonisins**	**Deoxynivalenol**
Test kit Name	Rosa Aflatoxin WETS5 Quantitative test [12, 15]	Rosa Fumonisin WETS5 Quantitative test [13, 16]	Rosa DONQ-WETS5 Quantitative test [14]
Catalogue number	LF-AFQ-WETS5-20K	LF- FUMQ-WETS5-20K	LF-DONQ-WETS5-20K
Test kit lot number	AFQ-WETS5-003B A003007B-07-EZ	FUMQ-WETS5-001I A0010021-16-EZ	DONQ-WETS5-001J A001002J-10-EZ
Expiry date	December 2016	January 2017	January 2017
Concentration range	5 – 100 ppb [15]	0.5 – 5.4 ppm & 5 – 30 ppm [16]	0.5 – 5 ppm
Approved commodities	Maize and 17 other commodities	Maize and 7 other commodities	Maize, barley and wheat
Incubator	ROSA incubator @ 45 ^{0}C		
Reader	ROSA- M Reader		
Storage conditions	Store strips, dilution buffer, positive control refrigerated (0 – 7 ^{0}C) WET-S Extraction powder at room temperature		
Precautions	Keep strips in closed containers, avoid high humidity		
Quality assurance	Positive and negative control and calibration strips for the reader		
Sample processing requirements	Ground, particle size not specified		
Material provided in the kit	• 20 Test strips, • Aflatoxin Dilution buffer, • Aflatoxin B_1 positive control	• 20 Test strips, • Fumonisin Dilution buffer, • Fumonisin positive control	•20 Test strips, •DON Dilution buffer, •Deoxynivalenol positive control
Material needed (not provided)	• WET-S extraction powder, • extraction containers, • 300 – 600 µL pipette and tips, • filter paper, • distilled water, • measuring cylinder, • balance, • mill, • Reader, • Incubator	• WET-S extraction powder, • extraction containers, • 100 – 900 µL pipette and tips, • filter paper, • distilled water, • measuring cylinder, • balance, • mill, • Reader, • Incubator	• WET-S extraction powder, • extraction containers, • 100 – 900 µL pipette and tips, • filter paper, • distilled water, • measuring cylinder, • balance, • mill, • Reader, • Incubator
EXTRACTION PROCEDURE AND READING			
Sample size	50 g	50 g	50 g
Extraction method summary	• Shake vigorously 50 g sample with one packet WET-S extraction powder and 150 mL distilled water for 1.5 minutes Filter within 30 minutes • Settle extract and filter to clarify. Use within 2 hours	Same procedure as for aflatoxins, may use the same sample extract for fumonisin testing	Same procedure as for aflatoxins, may use the same sample extract for DON testing
Dilution	• Add 300 µL extract to 600 µL AFQ dilution buffer, shake well for 5 seconds (may keep 6 hours)	• Add 100 µL extract to 900 µL FUMQ WETS5 dilution buffer, mix well for 5 seconds. • Add 100 µl of this diluted extract to 900 µL FUMQ WETS5 dilution buffer, mix well for 5 seconds (may keep 6 hours)	• Add 50 µL extract to 950 µL DONQ dilution buffer, mix well for 5 seconds (may keep 6 hours)

ROSA TEST KIT INFORMATION			
Parameter	**Aflatoxin**	**Fumonisins**	**Deoxynivalenol**
Incubation	• Strip in incubator @ 45 ^{0}C • Pipette slowly 300 uL diluted extract • Incubate 5 minutes • Read within 1 minute in Reader	• Strip in incubator @ 45 ^{0}C • Pipette slowly 300 uL of second diluted extract • Incubate 5 minutes • Read within 1 minute in Reader	• Strip in incubator @ 45 ^{0}C • Pipette slowly 300 uL diluted extract • Incubate 5 minutes • Read within 1 minute in Reader
Setup of Reader and calibration curve selection	Reader programmed with different calibration curves, user must select the correct maize calibration curve for the specific mycotoxin		
Visual inspection	Control line C, must be visible. T1 and T2 lines not smeared, uneven, obscured		
Speed of extraction and reading	1.5-minute extraction + filtration + 1 – 2-minute dilution + 5 minutes incubation + reading	1.5-minute extraction + filtration + 1 – 2-minute dilution x 2 dilutions + 5 minutes incubation + reading	1.5-minute extraction + filtration + 1 – 2-minute dilution + 5 minutes incubation + reading
Cross reactivity	Test detects 100% Afla B_1. Afla B_2 = 10%, Afla G_1 = 12% Afla G_2 = 1% [communication received from Charms]	Not mentioned in procedure	Cross reactive with 3-Acetyl DON

6.2.2 Comments

The same initial sample extraction can be used to test the sample for aflatoxin, fumonisin and deoxynivalenol; this is convenient and more cost effective because less sample extraction powder is used.

For quality assurance, these test kits include dry positive control samples to verify the concentration ranges of the tests kits. The procedure for the preparation of both the positive control and negative control samples are described in the test kit procedures and the dilutions may be kept frozen in small subsamples (-15 ^{0}C) for up to 2 months.

Calibration strips are provided for the calibration verification of the reader.

The aflatoxin calibration range, 5 – 100 ppb, is covered with only one sample dilution and one strip, this make the test more cost effective. For the quantification of fumonisin, two dilutions are initially prepared and one strip is incubated to cover the concentration range of 0.5 – 5.4 ppm. An additional dilution is made to cover the concentration range from 5.4 – 30 ppm [16].

The Rosa-M reader does recognize the strip information when a strip is inserted in the reader, the operator must just check that the correct calibration curve is used for the quantification.

Safety precautions can be described better in the procedures provided with the kits.

6.3. Envirologix Test kits

6.3.1. Use of the Envirologix test kits

Table 6

ENVIROLOGIX TEST KIT INFORMATION			
Parameter	**Aflatoxin**	**Fumonisins**	**Deoxynivalenol**
Test kit Name	QuickTox kit for QuickScan Aflatoxin Free [17, 20]	QuickTox kit for QuickScan Fumonisin Flex [18, 21]	QuickTox kit for QuickScan DON3 [19]
Catalogue number	AQ 209 BG	AQ 211 BG	AQ 254 BG
Test kit lot number	753-16 and re-testing with 993-16	844-6	713-6
Expiry date	September 2017	March 2017	February 2017
Concentration range	5 – 30 ppb and 30 -100 ppb with dilution [17]	0.5 – 3 ppm and >3 ppm – 18 ppm with dilution [18]	0.29 – 12 ppm and >12 ppm – 30 ppm with dilution [19]
Approved commodities	Corn (maize) [20]	Corn (maize) [21]	Corn (maize) [19]
Incubator	In cup at room temperature		
Reader	QuickScan System, Software version 4.7 Update 2 or later		
Storage conditions	Store strips and Buffers refrigerated @ 2- 8 ^{0}C The extraction powder for aflatoxin is stored refrigerated @ 2- 8 ^{0}C		
Precautions	Store strips in a moisture-resistant canister, avoid direct sunlight Read strips wet after incubation time The buffers are lot specific		
	Do not use bleach solution to treat extraction lab ware. EB17 extraction powder considered flammable and an irritant, avoid inhaling powder Avoid incomplete mixing when diluting	Avoid incomplete mixing when diluting	Avoid incomplete mixing when diluting
Quality assurance	No controls are provided with the kits		
Sample processing requirements	Grind samples such that 95% passes through a 20-mesh sieve. Detailed instruction in procedures		
Material provided in the kit	• 50 Test strips, • DB5 buffer, • 50 EB17 extraction powder, • 50 reaction vials, • pipette tips • Multi-matrix Barcode Card, kit lot specific	• 50 Test strips, • DB6 buffer kit lot specific, • 100 reaction vials, • 100 pipette tips, • Multi-matrix Barcode Card, kit lot specific	• 50 Test strips, • DB6 buffer kit lot specific, • 100 reaction vials, • 100 pipette tips,
Material needed (not provided)	• extraction cups, • 100 µL pipette, • distilled water, • filter paper, • measuring cylinder, • balance , • Mill, • Reader	• extraction cups, • 200 µL pipette • 1.5 mL pipette, • distilled water, • filter paper, • measuring cylinder, • balance, • Mill, • Reader	• extraction cups, • 200 µL and 800 µL pipette, • distilled water, • filter paper, • measuring cylinder, • balance, • Mill, • Reader
EXTRACTION PROCEDURE AND READING			
Sample size	25 g	20 g	25 g
Extraction method summary	• Shake vigorously 25 g sample with one packet EB17 extraction powder and 75 mL distilled water for 2 minutes	• Shake vigorously 20 g sample and 100 mL distilled water for 2 minutes	• Shake vigorously 25 g sample and 125 mL distilled water for 30 seconds

ENVIROLOGIX TEST KIT INFORMATION			
Parameter	**Aflatoxin**	**Fumonisins**	**Deoxynivalenol**
	• Settle extract to clarify and filter	• Settle extract to clarify and filter	• Settle extract to clarify and filter
Dilution	• Add 100 µL clarified extract to 100 µL DB5 buffer in the reaction vial, • Mix 10x with pipette, • If > 30 ppb Aflatoxin, do an additional dilution and read second strip, select correct dilution level on reader (1:A)	• Add 200 µL extract to 1.5 mL DB6 buffer in blue dilution tube, mix well with pipette tip, •Transfer 200 µL of this diluted extract into the reaction vial, •If > 3 ppm, do two additional dilutions and read second strip, select correct dilution level on reader	•Add 200 µL extract to 800 µL DB6 buffer in blue dilution tube, mix well with pipette tip, •Transfer 200 µL of this diluted extract into the reaction vial, •If > 12 ppm, do an additional dilution and read second strip, select correct dilution level
Incubation	• No incubator, • Add strip to reaction vial for 4 minutes, • Immediately cut strip to discard bottom pad, • Insert in Quickscan reader immediately	• No incubator, • Add strip to reaction vial for 5 minutes, • Immediately cut strip to discard bottom pad • Insert in Quickscan reader immediately	• No incubator, • Add strip to reaction vial for 3 minutes, • Immediately cut strip to discard bottom pad, • Insert in Quickscan reader immediately
Setup of Reader and calibration curve selection	On strip matrix group calibration or multi-matrix bar code chart with kit	On strip matrix group calibration	On strip matrix group calibration
Visual inspection	Control line must be visible. Test line visible		
Speed of extraction and reading	2 minutes extraction + settling and filter time + 1 - 2 minutes dilution + 4 minutes reaction + reading	2 minutes extraction + settling and filter time + 1 – 2 minutes dilution + 5 minutes reaction + reading	30 seconds extraction + settling time + 1 minute dilution + 4 minutes reaction + reading
Cross reactivity	No false results for DON, FUM B_1, OTA and ZON [17]	No false results for Afla B_1, DON, OTA and ZON [18]	No false results for Afla B_1, FUM B_1, OTA and ZON [19]

6.3.2 Comments

No controls are provided for quality assurance, test facilities may use maize samples with specific mycotoxins as quality control samples when analysing samples.

The Test kit procedures do not provide clear and detailed instructions, e.g. the dilution procedure for the aflatoxin concentrations >30 ppm is not clearly described and the fumonisin procedure is only available in the summary guide table A.

For aflatoxin concentrations > 30 ppb, a second 1:6 dilution is made and a second strip is used to quantify the aflatoxin content. A dilution factor must be selected on the QuickScan reader for the higher aflatoxin concentrations and this reader factor did not correspond with the factor described in the procedure. [17] Feedback received from Envirologix confirmed that the dilution factor is lot specific and may vary from lot to lot. The kits that will be provided in future will have a hidden dilution factor labelled as 1:A.

For fumonisin concentrations > 3 ppm, a second 1:6 dilution is made and a second strip is used to quantify the fumonisin content. The dilution factor selected on the scanner was 1:A.

The development (incubation) is at room temperature in the cup, an incubator is not necessary.

The QuickScan reader does recognize the strip information when a strip is inserted in the reader, the operator must just select the correct dilution factor for the quantification.

Safety precautions can be described better in the procedures provided with the kits.

6.4. Neogen Test kits

6.4.1. Use of the Neogen test kits

Table 7

NEOGEN TEST KIT INFORMATION			
Parameter	**Aflatoxin**	**Fumonisins**	**Deoxynivalenol**
Test kit Name	Reveal Q+ Max for aflatoxin [22, 24]	Not yet available	Reveal Q for DON [23, 25]
Catalogue number	8088	-	8385
Test kit lot number	222930	-	223224
Expiry date	February 2017	-	April 2017
Concentration range	5 – 300 ppb	-	0.3 – 6 ppm
Approved commodities	Maize and 12 other commodities [24]	-	Maize and 8 other commodities [25]
Incubator	In cup at room temperature		
Reader	AccuScan Gold reader		
Storage conditions	Store strips, diluent and cups at room temperature (18 – 30 ^{0}C)		
Precautions	Keep strips dry in container, Avoid high humidity		
Quality assurance	No controls submitted with strips		
Sample processing requirements	Grind samples such that 75% passes through a 20-mesh sieve		
Material provided in the kit	• 25 Test strips, • 25 MAX aqueous extraction powder, • 25 red sample dilution cups, • 25 clear sample cups, • 1 bottle diluent, • lot specific QR code	-	• 25 Test strips, • 25 red sample dilution cups, • 25 clear sample cups, • 2 bottles diluent, • lot specific QR code
Material needed (not provided)	• extraction containers, • 100 – 1000 µL pipette • tips, • filter paper, • distilled water , • measuring cylinder, • balance, • mill, • reader	-	• extraction containers, • 100 – 1000 µL pipette • tips, • filter paper, • distilled water , • measuring cylinder • balance, • mill, • reader
EXTRACTION PROCEDURE AND READING			
Sample size	10 g	-	10 g
Extraction method summary	• Shake vigorously 10 g sample with one packet MAX extraction powder and 50 mL distilled water for 3 minutes • Settle extract and filter to clarify	-	• Shake/ vortex vigorously 10 g sample with 100 mL distilled water for 3 minutes • Settle extract and filter to clarify
Dilution	•Add 100 µL extract to 100 µL sample diluent to red dilution cup, •mix 5x with pipette	-	•Add 100 µL extract to 1000 µL sample diluent to red dilution cup, •mix 5x with pipette, •If > 6 ppm, do an additional dilution and read second strip

NEOGEN TEST KIT INFORMATION			
Parameter	**Aflatoxin**	**Fumonisins**	**Deoxynivalenol**
Incubation	• Pipette 100 uL diluted extract into clear sample cup, • Place RevealQ Max for Afla strip in cup for 6 minutes, • Place strip in cartridge in reader, • Read within 6 minutes in Reader	-	• Pipette 100 uL diluted extract into clear sample cup, • Place DON strip in cup for 3 minutes, • Place strip in cartridge in reader, • Read within 1 minute in Reader
Setup of Reader and calibration curve selection	Scan lot specific QR code. The device lot number and curve details must match the LOT ID number selected on the reader		
Visual inspection	-		
Speed of extraction and reading	3 minutes extraction + filtration + 1 - 2 minutes dilution + 6 minutes development + reading	-	3 minutes extraction + filtration + 1 – 2 minutes dilution + 3 minutes development + reading
Cross reactivity	Afla B_1 = 100% Afla B_2 = 91%, Afla G_1 = 87% Afla G_2 = 64% [22]	-	DON = 100% 3Ac-DON = 105% 15Ac-DON = 7% DON3-glucoside = 26% Fusarenon-X = 0.4% Nivalenol = 3.8% T-2 Toxin = < 0.042% [26]

6.4.2 Comments

No controls are provided for quality assurance, test facilities may use maize samples with specific mycotoxins as quality control samples when analysing samples.

The development (incubation) is at room temperature in the cup, an incubator is not necessary.

The aflatoxin calibration range 5 – 300 ppb is covered with only one sample dilution and one strip; this is more cost effective [22].

The AccuScan Gold reader does not recognize the strip information when a strip is inserted in the reader, the operator must scan the QR code received with the kit and check that the kit lot number and curve details match the lot ID number selected on the reader.

Safety precautions can be described better in the procedures provided with the kits.

6.5. Romer Test kits

6.5.1. Use of the Romer Test Kits

Table 8

ROMER TEST KIT INFORMATION			
Parameter	**Aflatoxin**	**Fumonisins**	**Deoxynivalenol**
Test kit Name	AgraStrip Total Aflatoxin Quantitative Test WATEX [27, 30]	AgraStrip Total Fumonisin Quantitative Test WATEX [28, 31]	AgraStrip Deoxynivalenol (DON) Quantitative Test WATEX [29]
Catalogue number	COKAS1600WS	COKAS3000WS	COKAS4000WS
Test kit lot number	16D1088	16G1193	16E1125
Expiry date	April 2017	July 2017	March 2017
Concentration range	5 – 100 ppb [30]	0.5 – 5.0 ppm [28, 31] and >5.0 – 30 ppm [28]	0.5 – 5.0 ppm and > 5 ppm – 30 ppm [29]
Approved commodities	Maize and 6 other commodities [30]	Maize and 3 other commodities [31]	Maize
Incubator	AgraStrip heat block @ 45 ^{0}C		
Reader	Agra Vision Reader		
Storage conditions	Store strips, dilution buffer conjugated wells at room temperature (18 – 25 ^{0}C)	Store strips, dilution buffer conjugated wells refrigerated (2 – 8 ^{0}C)	Store strips, dilution buffer conjugated wells refrigerated (2 – 8 ^{0}C)
Precautions	Keep strips dry in original tubes, do not freeze, do not leave in direct sunlight Keep conjugate microwells inside original tubes Do not mix components from different lot numbers		
Quality assurance	No controls submitted with strips, are available from supplier		
Sample processing requirements	Grind samples such that 75% passes through a 20-mesh sieve		
Material provided in the kit	• 24 Test strips, • 1 tube with 24 Aflatoxin conjugate wells, • 24 extraction buffer bags, • 1 bottle (30 mL) Aflatoxin dilution buffer, • pipette tips, • 24 filter Whirl-Pak Bags, • 24 dilution tubes, • 1 tube holder, • tweezers, • SD card for the reader	• 24 Test strips, • 1 tube with 24 Fumonisin conjugate wells, • 24 extraction buffer bags, • 1 bottle (30 mL) Aflatoxin dilution buffer, • pipette tips, • 24 filter Whirl-Pak Bags, • 24 dilution tubes, • 1 tube holder, • tweezers, • SD card for the reader	• 24 Test strips, • 1 tube with 24 DON conjugate wells, • 24 extraction buffer bags, • 1 bottle (30 mL) Aflatoxin dilution buffer, • pipette tips, • 24 filter Whirl-Pak Bags, • 24 dilution tubes, • 1 tube holder, • tweezers, • SD card for the reader
Material needed (not provided)	• 50, 100 and 1000 µL pipettes, • distilled water, • measuring cylinder, • balance, • mill, • Incubator, • Reader	• 50, 100 and 1000 µL pipettes, • distilled water, • measuring cylinder, • balance, • mill, • Incubator, • Reader	• 50, 100 and 1000 µL pipettes, • distilled water, • measuring cylinder, • balance, • mill, • Incubator, • Reader
EXTRACTION PROCEDURE AND READING			
Sample size	10 g	10 g	10 g
Extraction method summary	• Shake vigorously 10 g sample with one Extraction buffer bag (dissolves completely) and 30 mL distilled water for 3 minutes in a filter Whirl-Pak bag, • Settle extract for 2 minutes (max 3 minutes),	• Shake vigorously 10 g sample with one Extraction buffer bag (dissolves completely) and 30 mL distilled water for 3 minutes in a filter Whirl-Pak bag, • Settle extract for 2 minutes (max 3 minutes),	• Shake vigorously 10 g sample with one Extraction buffer bag (dissolves completely) and 30 mL distilled water for 3 minutes in a filter Whirl-Pak bag, • Settle extract for 2 minutes (max 3 minutes),

ROMER TEST KIT INFORMATION			
Parameter	**Aflatoxin**	**Fumonisins**	**Deoxynivalenol**
	• Aspirate from opposite side of corn in filter bag	• Aspirate from opposite side of corn in filter bag	Extract pulled from opposite side of corn in filter bag.
Dilution	• Add 50 µL extract to 1000 µL dilution buffer in the dilution tube, • mix 5x with pipette, • Extract stable for maximum of 1 hour	• Add 50 µL extract to 1000 µL dilution buffer in the dilution tube, • mix 5x with pipette, • Extract stable for maximum of 1 hour, • If > 5 ppm, do a second dilution	• Add 50 µL extract to 1000 µL dilution buffer in the dilution tube, • mix 5x with pipette, • Extract stable for maximum of 1 hour
Incubation	• Put microwell in incubator heat block @ 45 ^{0}C, • Pipette 100 µL diluted extract into microwell, • Mix 10x with pipette, • Insert a strip in microwell and cover heat block, • Incubate 3 minutes, • Wipe strip and place strip in holder in reader, • Read within 1 minute in Reader	• Put microwell in incubator heat block @ 45 ^{0}C, • Pipette 100 µL diluted extract into microwell, • Mix 10x with pipette, • Insert a strip in microwell and cover heat block, • Incubate 3 minutes, • Wipe strip and place strip in holder in reader, • Read within 1 minute in Reader	• Put microwell in incubator heat block @ 45 ^{0}C, • Pipette 100 µL diluted extract into microwell, • Mix 10x with pipette, • Insert a strip in microwell and cover heat block, • Incubate 3 minutes, • Wipe strip and place strip in holder in reader, • Read within 1 minute in Reader
Setup of Reader and calibration curve selection	Scan lot specific SD card. The device lot number and curve details must match the LOT ID number selected on the reader		
Visual inspection	Control line C must be visible. 2 Test lines visible		
Speed of extraction and reading	3 minutes extraction + 2 minutes settling + 1 – 2 minutes dilution + 3 minutes incubation + reading	3 minutes extraction + 2 min settling + 1 – 2 minutes dilution + 3 minutes incubation + reading	3 minutes extraction + 2 minutes settling + 1 – 2 minutes dilution + 3 minutes incubation + reading
Cross reactivity	Presence of Total aflatoxin quantified (B_1, B_2, G_1 and G_2) [27]	Presence of Total fumonisin quantified (B_1, B_2, and B_3) [28]	Not mentioned

6.5.2 Comments

No controls are provided for quality assurance but are available from Romer Labs. Test facilities may also use maize samples with specific mycotoxins as quality control samples when analysing samples.

These are the only kits where all the consumables that is needed to perform the tests are included in the kits. The extraction Whirl-Pak bags supplied with the test kits are divided into two sections; the clear extract is aspirated from the second section after the settling time. No additional filtration is necessary.

The development (incubation) is in a heat block at 45 ^{0}C.

The aflatoxin calibration range 5 – 100 ppb is covered with only one sample dilution and one strip, this is more cost effective [27].

The Agra Vision reader does not recognize the strip information when a strip is inserted in the reader, the operator must scan the SD card received with the kit.

Other commodities are mentioned but not specified in the Romer Labs test kit instructions, corn is specified in the Aflatoxin and Fumonisin instructions. The DON test kit refers to grain and grain products.

The Agra Vision Reader was not that easy to operate.
Safety precautions can be described better in the procedures provided with the kits.

7. RESULTS

7.1. Charm Sciences (ROSA) test kit results

Table 9

Charm Rosa Test kit Aflatoxin results					
	Sample number	MSK 17	MSK 6	MSK 7	MSK 13
Rosa M Aflatoxin results	Mean (n=5), µg/kg	0	46	41	16
	RSD, %	-	7.0	11.0	2.5
	% Recovery of Afla B_1	-	77	96	137
LCMSMS Aflatoxin B_1 results	Mean (n=5), µg/kg	ND	59.90	42.75	11.72
LCMSMS Aflatoxin total results	Mean (n=5), µg/kg	ND	64.68	56.43	11.72

Charm Rosa Test kit Fumonisin results					
	Sample number	MSK 17	MSK 5	MSK 10	MSK 11
Rosa M Fumonisin results	Mean (n=5), mg/kg (ppm)	0	3.9	2.1	1.0
	RSD, %	-	10.3	14.3	10.0
	% Recovery	-	79	94	96
LCMSMS total Fumonisin results	Mean (n=5), mg/kg (ppm)	ND	4.931	2.227	1.040

Charm Rosa Test kit Deoxynivalenol results					
	Sample number	MSK 17	MSK 4	MSK 8	MSK 14
Rosa M Deoxynivalenol results	Mean (n=5), mg/kg (ppm)	0	2.5	0.80	4.9
	RSD, %	-	24	13.8	6.1
	% Recovery	-	169	108	122
LCMSMS Deoxynivalenol results	Mean (n=5), mg/kg (ppm)	ND	1.476	0.739	4.007

When the Rosa test kit results are compared with the GIPSA criteria, the aflatoxin and fumonisin % recoveries and the RSDs of all three samples are within the GIPSA acceptable ranges. Comparing to the GIPSA criteria higher % recoveries were measured on two of the DON samples and the RSD of one of these samples was > 14 % . This sample, number MSK 4, contained very high concentrations of all three fumonisins.

7.2. Envirologix test kit results

Table 10

Envirologix Test kit Aflatoxin results					
	Sample number	MSK 17	MSK 6	MSK 7	MSK 13
Envirologix Aflatoxin results	Mean (n=5), µg/kg	0	51	52	15
	RSD, %	-	6.7	7.7	24.0
	% Recovery of Afla B_1	-	85	122	128
LCMSMS Aflatoxin B_1 results	Mean (n=5), µg/kg	ND	59.90	42.75	11.72
LCMSMS Aflatoxin total results	Mean (n=5), µg/kg	ND	64.68	56.43	11.72

Envirologix Test kit Fumonisin results					
	Sample number	MSK 17	MSK 5	MSK 10	MSK 11
Envirologix Fumonisin results	Mean (n=5), mg/kg (ppm)	0	5.3	2.4	1.2
	RSD, %	-	11.3	8.3	8.3
	% Recovery	-	107	108	115
LCMSMS total Fumonisin results	Mean (n=5), mg/kg (ppm)	ND	4.931	2.227	1.040

Envirologix Test kit Deoxynivalenol results					
	Sample number	MSK 17	MSK 4	MSK 8	MSK 14
Envirologix Deoxynivalenol results	Mean (n=5), mg/kg (ppm)	0	2.6	1.2	6.9
	RSD, %	-	3.8	8.3	4.3
	% Recovery	-	176	162	172
LCMSMS Deoxynivalenol results	Mean (n=5), mg/kg (ppm)	ND	1.476	0.739	4.007

When the results of the Envirologix kits are compared with the GIPSA criteria, the aflatoxin and fumonisin % recoveries and the RSDs of all three samples are within the GIPSA acceptable ranges. Comparing to the GIPSA criteria higher % recoveries were measured on all three the DON samples, but the RSDs of the three samples were lower than the maximum RSD set by GIPSA. One of these samples, number MSK 4, contained very high concentrations of all three fumonisins.

7.3. Neogen test kit results

Table 11

Neogen Test kit Aflatoxin results					
	Sample number	MSK 17	MSK 6	MSK 7	MSK 13
Neogen Aflatoxin results	Mean (n=5), µg/kg	0	43.6	48.5	13.5
	RSD, %	-	9.4	4.7	5.2
	% Recovery of Afla B_1	-	73	113	115
LCMSMS Aflatoxin B_1 results	Mean (n=5), µg/kg	ND	59.90	42.75	11.72
LCMSMS Aflatoxin total results	Mean (n=5), µg/kg	ND	64.68	56.43	11.72

Neogen Test kit Deoxynivalenol results					
	Sample number	MSK 17	MSK 4	MSK 8	MSK 14
Neogen Deoxynivalenol results	Mean (n=5), mg/kg (ppm)	0	1.7	0.9	4.2
	RSD, %	-	23.5	11.1	4.8
	% Recovery	-	115	122	105
LCMSMS Deoxynivalenol results	Mean (n=5), mg/kg (ppm)	ND	1.476	0.739	4.007

Comparing the Neogen kits results with the GIPS criteria, the aflatoxin % recoveries and the RSDs of all three samples are within the GIPSA acceptable ranges. The % recoveries measured for the DON samples were all within the allowable range set by GIPSA, only one RSD (sample number MSK 4) was higher than the GIPSA maximum RSD.

7.4. Romer test kit results

Table 12

Romer labs Test kit Aflatoxin results					
	Sample number	MSK 17	MSK 6	MSK 7	MSK 13
Romer Aflatoxin results	Mean (n=5), µg/kg	<2	66.4	82.5	11.6
	RSD, %	-	6.8	4.7	19.0
	% Recovery of Total Aflatoxin	-	103	146	99
LCMSMS Aflatoxin total results	Mean (n=5), µg/kg	ND	64.68	56.43	11.72

Romer labs Test kit Fumonisin results					
	Sample number	MSK 17	MSK 5	MSK 10	MSK 11
Romer Fumonisin results	Mean (n=5), mg/kg (ppm)	<0.25	3.8	2.7	1.3
	RSD, %	-	10.5	11.1	15.4
	% Recovery	-	77	121	120
LCMSMS total Fumonisin results	Mean (n=5), mg/kg (ppm)	ND	4.931	2.227	1.040

Romer labs Test kit Deoxynivalenol results					
	Sample number	MSK 17	MSK 4	MSK 8	MSK 14
Romer Deoxynivalenol results	Mean (n=5), mg/kg (ppm)	<0.25	2.0	0.8	5.0
	RSD, %	-	20.0	25.0	22.6
	% Recovery	-	136	108	125
LCMSMS Deoxynivalenol results	Mean (n=5), mg/kg (ppm)	ND	1.476	0.739	4.007

When the results of the Romer kits are compared with the GIPSA criteria, the aflatoxin % recoveries of two of the samples are within the GIPSA acceptable range and the RSDs of all three samples are within the GIPSA acceptable ranges. The fumonisin % recoveries and the RSDs of all three samples are within the GIPSA acceptable ranges. The % recoveries measured for two of the DON samples were within the allowable range set by GIPSA, the sample MSK 4, measured a higher % recovery than the GIPSA maximum. The RSD values of all three samples were higher than the allowable limits set by GIPSA.

8. Discussion

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 with all the test kits 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.

The precision achieved for all the aflatoxin and fumonisin test samples was within the GIPSA acceptance criteria as described in table 4. Relative standard deviations (RSD) > 20% were measured in four of the deoxynivalenol tests conducted.

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 between 107 – 125% recovery, within the GIPSA range. % recoveries as high as 162 – 176% were measured in three samples.

9. Conclusion and recommendations

This study confirmed that all the test kits evaluated might be used in a non-laboratory environment to test maize for aflatoxins, fumonisins and deoxynivalenol. Potential users may use the information provided in this report to evaluate the specific features of the test kits and identify a kit that will be suitable for their requirements. The outlier results obtained may be investigated during further collaboration with the test kit suppliers. Proper training to use the kits is highly recommended.

10. References

1. SAGL South African Maize Crop Quality Report, 2014/2015 Season.
2. SA Act 54 of 1972 Food stuffs, Cosmetics and Disinfectants, Regulation No. R. 1145, dated 8 October 2004 specifies the allowable levels of total aflatoxin.
3. SA Regulation No. 987, dated 5 September 2016 specifies the allowable levels of fumonisins (B_1 + B_2) and deoxynivalenol for human consumption.
4. SA Amendment No. R 70, dated 12 February 2010 of Act 36 of 1947 Fertilizers, Farm Feeds, Agricultural and Stock Remedies describes the maximum allowable content of different mycotoxins in farm feeds is described in.
5. GIPSA Criteria and Specifications: Design Criteria and Test performance Specifications for Quantitative Aflatoxin Test kits. January 01, 2016.
6. GIPSA Criteria and Specifications: Design Criteria and Test performance Specifications for Quantitative Fumonisin Test kits. July 09, 2010.
7. GIPSA Criteria and Specifications: Design Criteria and Test performance Specifications for Quantitative Deoxynivalenol Test kits. July 09, 2010.
8. GIPSA Performance Verified Mycotoxin Test Kits – Effective 04/22/2016.
9. GIPSA Performance Verified Mycotoxin Test Kits – Effective date.
10. Wei Li, S. Powers and S.Y. Dai, Using commercial immunoassay kits for mycotoxins: 'joys and sorrows'? World Mycotoxin Journal, 2014; 7(4): 417 – 430.
11. www.sagl.co.za Select Accreditation.
12. CHARM Operator's Manual: ROSA WET-S5 Aflatoxin Quantitative Test for Feed and Grain, 26 May 2016, OM-615-006.
13. CHARM Operator's Manual: ROSA WET-S5 Fumonisin Quantitative Test for Feed and Grain, 6 November 2015, OM-646-003.
14. CHARM Operator's Manual : ROSA WET-S5 DON Quantitative Test for Feed and Grain, 17 June 2016, OM-648-004.
15. GIPSA-AFQ-WETS5 Test Kit instruction, 3 February 2016, Revision 3.
16. GIPSA-LFFUMQ-WETS5 Test kit Instruction, 19 September 2016, Revision 0.
17. EnviroLogix Procedure for QuickTox Kit for QuickScan Aflatoxin Free, Rev. 10-17-16.
18. EnviroLogix Procedure for QuickTox Kit for QuickScan Fumonisin Flex, Rev. 02-02-16.
19. EnviroLogix Procedure for QuickTox Kit for QuickScan DON3 (Vomitoxin), Rev. 05-28-15.
20. GIPSA QuickScan Aflatoxin Free Test kit Instructions, 8 August 2016, Revision 0.
21. GIPSA QuickScan Fumonisin Flex Test kit Instructions, 2 August 2016, Revision 0
22. Neogen Reveal Q+ MAX for aflatoxins Product Training document.
23. Neogen Reveal Q+ for DON Instructions supplied with Test kit.
24. GIPSA Neogen Reveal Q+ MAX for aflatoxin Test kit Instructions, 25 July 2016, Revision 0.
25. GIPSA Neogen Reveal Q+ for DON Test kit Instructions, 8 August 2015, Revision 0.
26. Neogen Validation Report for Reveal Q+ for DON (#8385), January 2012, Revision 1.
27. Romer Labs Procedure for AgraStrip Total Aflatoxin Quantitative Test WATEX, PI_COKAS1600W(S) _PI_V3, Finalised 22 October 2014.
28. Romer Labs Procedure for AgraStrip Total Fumonisin Quantitative Test WATEX, PI_COKAS3000W(S) _PI_V2, 23 September 2016.
29. Romer Labs Procedure for AgraStrip Deoxynivalenol Quantitative Test WATEX, PI_COKAS3000W(S) _PI_V1, 10 June 2015.
30. GIPSA AgraStrip Total Aflatoxin Quantitative Test WATEX Test kit Instructions, 28 July 2015, Revision 5.
31. GIPSA AgraStrip Total Fumonisin Quantitative Test WATEX Test kit Instructions, 8 June 2016, Revision 0.

32. CODA-CERVA Report “administrative and experimental evaluation of fast commercial test kits for the determination of deoxynivalenol in cereals”, National Reference Laboratory for Mycotoxins, March 2009.