VALIDATION REPORT
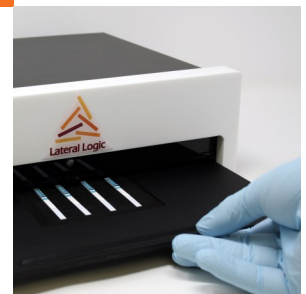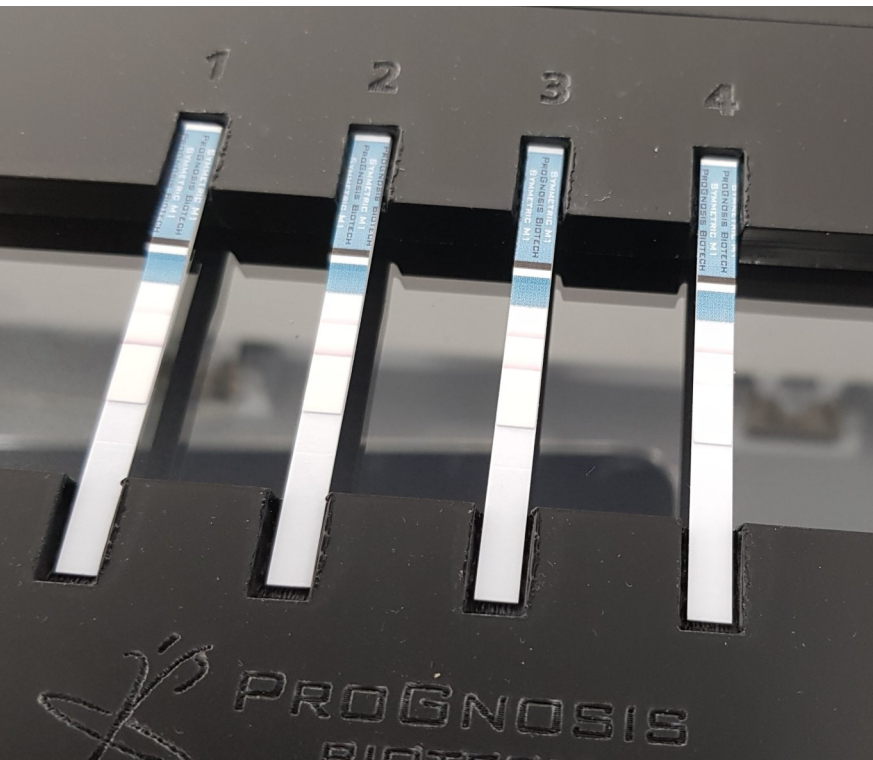## SYMMETRIC TOTAL GREEN 0-30

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Symmetric TOTAL Green 0-30 Lateral Flow kit

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Symmetric TOTAL Green 0-30 Lateral Flow kit

1. Immunoassay Specifications

1.1 General Specifications

The LOD and LOQ of the 0-30ppb method are 0.9ppb and 1.3ppb respectively (Type I & II, Table 1)
The LOD and LOQ of the 0-50ppb method are 1.7ppb and 2.5ppb respectively (Type III & IV, Table 2)

Matrices:

Type I: Corn, Corn Flour, Barley, Oat, Soy flour, Soy beans, Almond, Hazelnut, Pistachio, Sunflower seeds, Walnut, Roasted Chickpeas, Sorghum, Quinoa, Red lentils

Type II: Wheat, Wheat flour, Sesame

Type III: Brown rice, Millet

Type IV: Peanut, Cashews, White rice

1.2 Specificity & Cross-reactivity

The cross-reaction of the anti-Aflatoxin antibody with Aflatoxin B1. B2. G1 and G2 is 100. 81. 73 and 55% respectively.

2. Validation

2.1 Determination of the Limit of Detection LOD and the Limit of Quantification LOQ

The LOD and LOQ were defined as 2 x Standard Deviation and 3 x Standard Deviation of the Aflatoxin-free maize powder blank samples, respectively. For the determination of LOD and LOQ, four Aflatoxin-free maize powder were used: Samples A & B (<1.3ppb) for 0-30ppb method (Table 1) and samples C & D (<2.5ppb) for 0-50ppb method (Table 2) .

Table 1. Aflatoxin-free maize powder samples for the determination of LOD and LOQ 0-30 ppb (Type I & II)

Sample (n=8)	Concentration (ppb)	
	MEAN	SD
Maize Powder A	1.05	0.44
Maize Powder B	1.13	0.48
	SUM	
MEAN	1.09	
SD	0.46	

Table 2. Aflatoxin-free maize powder samples for the determination of LOD and LOQ 0-50 ppb (Type III & IV)

Sample (n=8)	Concentration (ppb)	
	MEAN	SD
Maize Powder C	2.03	0.85
Maize Powder D	2.16	0.83
	SUM	
MEAN	2.09	
SD	0.84	

It was found that calculated LOD and LOQ is 0.9ppb and 1.3ppb (0-30ppb), 1.7 and 2.5 ppb (0-50ppb), respectively.

2.2 Determination of Recovery (%)

Spike Protocol

All the samples were spiked according to the United States Department of Agriculture (USDA). Agricultural Marketing Service. GIPSA's Federal Grain Inspection Service (FGIS) protocol. More particularly. all spike experiments were carried out by spiking the individual pre-weighted test portion with a concentration adjusted solution to maintain the spiking volume at 100μl. Liquid spike prepared in 100% methanol was added with a positive displacement syringe and dried for 30 minutes at 37°C prior to extraction. unless stated otherwise. A known amount of Aflatoxin B1 is added to the solid sample to be tested using a standard solution. The dried spiked sample was then extracted and analyzed according to the manual S3424/S3448 V32 chapter 9.

i. Determination of Recovery (%) at the LOQ level

For the determination of Recovery at LOQ level (Table 2) Aflatoxin-free maize powder B was used as blank and it has been spiked with Fermentek Aflatoxin B1 standard solution (SSAF004A).

Table 3. Recovery at LOQ level. Maize Powder B was spiked with Aflatoxin B1.

Sample (n=8)	Concentration (ppb)	Spike (ppb)	Recovery (%)	
MAIZE POWDER A	1.29	1.3	**99.23**	**Average 1.3 ppb**
			5.05	**SD**
			5.09	**CV%**
MAIZE POWDER C	2.48	2.5	**99.2**	**Average 2.5 ppb**
			4.55	**SD**
			4.58	**CV%**

ii. Determination of Recovery (%) for all matrices at two different levels

For the determination of Recovery at two different levels (5 and 10ppb). different Aflatoxin-free matrices were spiked with Fermentek Aflatoxin B1 standard solution (SSAF004A) (Tables 3-25).

Table 4. Recovery of Almond sample at two different levels.

	Spike 5 ppb	
Almond (n=8)	5.21	**Average**
	0.16	**SD**
	3.07	**CV(%)**
	104.2	**Recovery (%)**
	Spike 10 ppb	
Almond (n=8)	10.12	**Average**
	0.28	**SD**
	2.77	**CV(%)**
	101.2	**Recovery (%)**
	102.7	**Mean Recovery (%)**

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Table 5. Recovery of Barley sample at two different levels.

Spike 5 ppb		
Barley (n=8)	**4.73**	**Average**
	0.15	**SD**
	3.17	**CV(%)**
	94.61	**Recovery (%)**
Spike 10 ppb		
Barley (n=8)	9.84	**Average**
	0.52	**SD**
	5.28	**CV(%)**
	98.4	**Recovery (%)**
	96.51	**Mean Recovery (%)**

Table 6. Recovery of Brown rice sample at two different levels.

Spike 5 ppb		
Brown Rice (n=8)	4.72	**Average**
	0.21	**SD**
	4.45	**CV(%)**
	94.4	**Recovery (%)**
Spike 10 ppb		
Brown Rice (n=8)	9.78	**Average**
	0.54	**SD**
	5.52	**CV(%)**
	97.8	**Recovery (%)**
	96.1	**Mean Recovery (%)**

Table 7. Recovery of Cashews sample at two different levels.

Spike 5 ppb		
Cashews (n=8)	4.94	**Average**
	0.21	**SD**
	4.25	**CV(%)**
	98.8	**Recovery (%)**
Spike 10 ppb		
Cashews (n=8)	10.01	**Average**
	0.29	**SD**
	2.90	**CV(%)**
	100.01	**Recovery (%)**
	99.41	**Mean Recovery (%)**

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Table 8. Recovery of Corn sample at two different levels.

Spike 5 ppb		
Corn (n=8)	4.71	Average
	0.25	SD
	5.30	CV(%)
	94.20	Recovery (%)
Spike 10 ppb		
Corn (n=8)	9.95	Average
	0.26	SD
	2.61	CV(%)
	99.50	Recovery (%)
	96.85	Mean Recovery (%)

Table 9. Recovery of Corn flour at two different levels.

Spike 5 ppb		
Corn flour (n=8)	4.84	Average
	0.17	SD
	3.51	CV(%)
	96.8	Recovery (%)
Spike 10 ppb		
Corn flour (n=8)	9.92	Average
	0.22	SD
	2.22	CV(%)
	99.2	Recovery (%)
	98.00	Mean Recovery (%)

Table 10. Recovery of Hazelnut sample at two different levels.

Spike 5 ppb		
Hazelnut (n=8)	4.42	Average
	0.22	SD
	4.98	CV(%)
	88.4	Recovery (%)
Spike 10 ppb		
Hazelnut (n=8)	9.45	Average
	0.31	SD
	3.28	CV(%)
	94.5	Recovery (%)
	91.45	Mean Recovery (%)

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Table 11. Recovery of Millet sample at two different levels.

Spike 5 ppb		
Millet (n=8)	4.65	**Average**
	0.24	**SD**
	5.16	**CV(%)**
	93	**Recovery (%)**
Spike 10 ppb		
Millet (n=8)	9.54	**Average**
	0.31	**SD**
	3.25	**CV(%)**
	95.4	**Recovery (%)**
	94.2	**Mean Recovery (%)**

Table 12. Recovery of Oats sample at two different levels.

Spike 5 ppb		
Oat (n=8)	4.62	**Average**
	0.23	**SD**
	4.98	**CV(%)**
	92.4	**Recovery (%)**
Spike 10 ppb		
Oat (n=8)	9.75	**Average**
	0.46	**SD**
	4.71	**CV(%)**
	97.5	**Recovery (%)**
	94.95	**Mean Recovery (%)**

Table 13. Recovery of Peanut sample at two different levels.

Spike 5 ppb		
Peanut (n=8)	4.39	**Average**
	0.19	**SD**
	4.32	**CV(%)**
	87.5	**Recovery (%)**
Spike 10 ppb		
Peanut (n=8)	9.42	**Average**
	0.25	**SD**
	2.65	**CV(%)**
	94.2	**Recovery (%)**
	90.85	**Mean Recovery (%)**

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Table 14. Recovery of Pistachio sample at two different levels.

Spike 5 ppb		
Pistachio (n=8)	4.78	Average
	0.17	SD
	3.56	CV(%)
	95.6	Recovery (%)
Spike 10 ppb		
Pistachio (n=8)	9.89	Average
	0.36	SD
	3.64	CV(%)
	98.9	Recovery (%)
	97.25	Mean Recovery (%)

Table 15. Recovery of Quinoa sample at two different levels.

Spike 5 ppb		
Quinoa (n=8)	5.16	Average
	0.24	SD
	4.65	CV(%)
	103.2	Recovery (%)
Spike 10 ppb		
Quinoa (n=8)	10.21	Average
	0.42	SD
	4.11	CV(%)
	102.1	Recovery (%)
	102.65	Mean Recovery (%)

Table 16. Recovery of Red lentil sample at two different levels.

Spike 5 ppb		
Red lentil (n=8)	4.52	Average
	0.22	SD
	4.87	CV(%)
	90.4	Recovery (%)
Spike 10 ppb		
Red lentil (n=8)	8.56	Average
	0.47	SD
	5.49	CV(%)
	85.6	Recovery (%)
	88	Mean Recovery (%)

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Table 17. Recovery of Rice sample at two different levels.

Spike 5 ppb		
Rice (n=8)	4.63	Average
	0.22	SD
	4.75	CV(%)
	92.6	Recovery (%)
Spike 10 ppb		
Rice (n=8)	9.52	Average
	0.42	SD
	4.41	CV(%)
	95.2	Recovery (%)
	93.9	Mean Recovery (%)

Table 18. Recovery of Roasted chickpeas sample at two different levels.

Spike 5 ppb		
Roasted chickpeas (n=8)	4.86	Average
	0.21	SD
	4.32	CV(%)
	97.2	Recovery (%)
Spike 10 ppb		
Roasted chickpeas (n=8)	10.02	Average
	0.17	SD
	1.70	CV(%)
	100.2	Recovery (%)
	98.7	Mean Recovery (%)

Table 19. Recovery of Sesame sample at two different levels.

Spike 5 ppb		
Sesame (n=8)	4.44	Average
	0.14	SD
	3.15	CV(%)
	88.8	Recovery (%)
Spike 10 ppb		
Sesame (n=8)	9.35	Average
	0.17	SD
	1.82	CV(%)
	93.5	Recovery (%)
	91.15	Mean Recovery (%)

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Table 20. Recovery of Sorghum sample at two different levels.

Spike 5 ppb		
Sorghum (n=8)	4.59	Average
	0.22	SD
	4.79	CV(%)
	91.8	Recovery (%)
Spike 10 ppb		
Sorghum (n=8)	9.86	Average
	0.42	SD
	4.25	CV(%)
	98.6	Recovery (%)
	95.2	Mean Recovery (%)

Table 21. Recovery of Soy bean sample at two different levels.

Spike 5 ppb		
Soy bean (n=8)	5.37	Average
	0.26	SD
	4.84	CV(%)
	107.4	Recovery (%)
Spike 10 ppb		
Soy bean (n=8)	10.65	Average
	0.32	SD
	3	CV(%)
	106.5	Recovery (%)
	106.95	Mean Recovery (%)

Table 22. Recovery of Soy flour sample at two different levels.

Spike 5 ppb		
Soy flour (n=8)	4.67	Average
	0.25	SD
	5.35	CV(%)
	93.4	Recovery (%)
Spike 10 ppb		
Soy flour (n=8)	9.86	Average
	0.50	SD
	5.07	CV(%)
	98.6	Recovery (%)
	96	Mean Recovery (%)

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Table 23. Recovery of Sunflower seeds sample at two different levels.

Spike 5 ppb		
Sunflower seeds (n=8)	4.24	Average
	0.20	SD
	4.72	CV(%)
	84.8	Recovery (%)
Spike 10 ppb		
Sunflower seeds (n=8)	9.32	Average
	0.44	SD
	4.72	CV(%)
	93.2	Recovery (%)
	89	Mean Recovery (%)

Table 24. Recovery of Walnut sample at two different levels.

Spike 5 ppb		
Walnut (n=8)	5.26	Average
	0.26	SD
	4.94	CV(%)
	105.2	Recovery (%)
Spike 10 ppb		
Walnut (n=8)	11.01	Average
	0.37	SD
	3.36	CV(%)
	110.1	Recovery (%)
	107.65	Mean Recovery (%)

Table 25. Recovery of Wheat sample at two different levels.

Spike 5 ppb		
Wheat (n=8)	4.85	Average
	0.23	SD
	4.74	CV(%)
	97	Recovery (%)
Spike 10 ppb		
Wheat (n=8)	9.73	Average
	0.52	SD
	5.34	CV(%)
	97.3	Recovery (%)
	97.15	Mean Recovery (%)

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Table 26. Recovery of Wheat flour sample at two different levels.

Spike 5 ppb		
Wheat flour(n=8)	4.51	Average
	0.24	SD
	5.32	CV(%)
	90.2	Recovery (%)
Spike 10 ppb		
Wheat flour (n=8)	9.34	Average
	0.51	SD
	5.46	CV(%)
	93.4	Recovery (%)
	91.8	Mean Recovery (%)

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Table 27. Mean Recovery (%) of all ground matrices.

Matrix	Mean Recovery (%)
Almond	102.7
Barley	96.51
Brown rice	96.1
Cashews	99.41
Corn	96.85
Corn flour	98
Hazelnut	91.45
Millet	94.2
Oats	94.95
Peanut	90.85
Pistachio	97.25
Quinoa	102.65
Red Lentil	88
Rice	93.9
Roasted chickpeas	98.7
Sesame	91.15
Sorghum	95.2
Soybeans	106.95
Soy flour	96
Sunflower seeds	89
Walnut	107.65
Wheat	97.15
Wheat flour	91.8
MEAN	96.37

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2.3 Reproducibility

The coefficients of variation of reproducibility of the concentrations (ppb) (Table 31) of two different samples ran eight times in 8 different tests are reported:

Table 28. Coefficients of Variation of the concentration (ppb) of two different samples ran in eight different tests.

Sample (n=8)	Concentration (ppb)	
	MEAN	CV(%)
FAPAS MAIZE T04439QC	**5.61**	5.01
FAPAS MAIZE T04411QC	**5.28**	5.62
FAPAS Wheat Flour T22187QC	**6.62**	4.84

2.4 Performance Evaluation
***i**. Reference Materials*

Table 29. Recovery on samples prepared by FAPAS.

Reference material	Lot number	Certified value (µg/kg)	Uncertainty (µg/kg)	Result (µg/kg)	Recovery (%)
FAPAS Maize T04439QC	90	5.55	2.44	5.61	101.1
FAPAS MAIZE T04411QC	193	5.35	2.35	5.28	98.7
FAPAS Wheat flour T22187QC	62	6.69	2.95	6.62	98.9

3. References

[1] M. Gkanas. Ch. Chatzoglou. K. Badra. Ch. Tsaridou. A.N. Ntantasios. G. Papageorgiou and S.D. Athanasiou. Uso di solventi non organici nell'analisi delle micotossine. Seminario AIA Laboratori e 20o ARAL SATA. 30-31 January 2018. Milan. Italy.

[2] Drakouli S, Skliris A, Voulgari DL, Angeli E, Ntantasios AN, Papageorgiou G and Athanassiou SD, Estrazione unica in acqua, per la quantificazione di nove Micotossine usando la tecnologia Symmetric lateral flow. VI Congresso Nazionale: Micotossine e Tossine Vegetali nella filiera agro-alimentare,10-12 June, 2019 Rome, Italy.

[3] Drakouli S, Skliris A, Tziortziou M, Iliopoulou S, Natsaridis N, Papageorgiou G, Ntantasios AN and Athanassiou SD, Quantification of all Mycotoxins, using Symmetric lateral flow technology and one step multitoxin aqueous extraction. The World Mycotoxin Forum and the IUPAC International symposium on Mycotoxins, 14-16 October 2019, Belfast, Northern Ireland, UK.

[4] Skliris A, Drakouli S, Tziortziou M, Voulgari DL, Iliopoulou S, Papageorgiou G, Zaralis K and Athanassiou SD, Symmetric lateral flow technology with one step Multitoxin aqueous extraction for the quantification of all Mycotoxins. 9th International Symposium on Recent Advances in Food Analysis, November 5-8, 2019, Prague, Czech Republic.

[5] Papageorgiou G. Ntantasios AN. Voulgari D. Badra K. Gotsopoulos M and Athanasiou SD. An innovative symmetric lateral flow system for the quantification of Aflatoxin M1. 8th International Symposium on RAFA. 7-10 November 2017. Prague. Czech Republic.

[6] Ntantasios AN. Arampatzis A. Voulgari D. Badra K. Papageorgiou G. Athanasiou SD and Gotsopoulos M. Innovative lateral flow method for the quantification of Aflatoxin M1. IDF DAIRY SUMMIT. 29 October-03 November 2017. Belfast. Northern Ireland. UK.

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