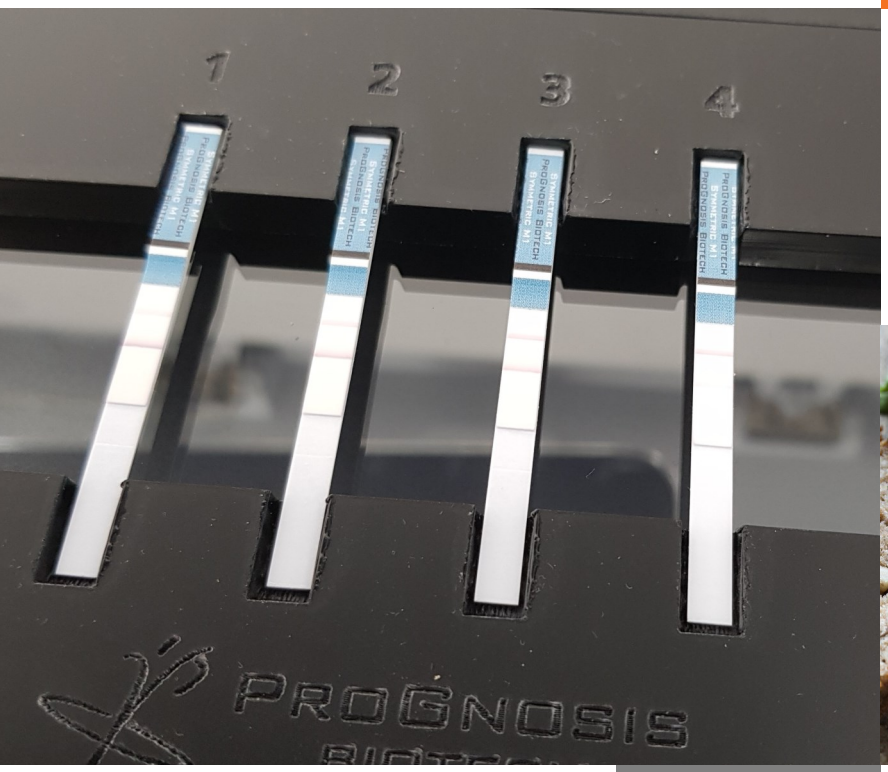
VALIDATION REPORT
SYMMETRIC ZON GREEN



Product code: S5024/S5048
Document no: S50[V10]
Date: 09 July 2024

PROGNOSIS BIOTECH

Symmetric ZON Lateral Flow kit

INDEX

Symmetric ZON Lateral Flow kit

1. Immunoassay Specifications

1.1 General Specifications

The LOD and LOQ of the 0-800ppb method are 24ppb and 35ppb respectively. (Type I ,II & III, Table 1)

Matrices:

Type I: Corn, Corn flour, Barley, Oats, Soy flour, Malt, Brown rice, Buck-wheat, Millet, Dried Brassica Integrifolia, Dried Gai Choy, Dried Palm.

Type II: Wheat, Wheat flour.

Type III: White rice.

1.2 Specificity & Cross-reactivity

The cross-reaction of the anti-ZON antibody with Zearalenone, α-zearalenol, β-zearalenol, Zearalanone, α-zearalanol, β-zearalanol is 100, 85, 47, 96, 80 and 82% 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. For the determination of LOD and LOQ, two ZON-free maize powder samples A & B (<35ppb) were used (Table 1).

Table 1. ZON-free maize powder samples for the determination of LOD and LOQ (Type I ,II & III)

	Concentration (ppb)	
Sample (n=8)	**MEAN**	**SD**
Maize Powder A	**25.32**	**11.52**
Maize Powder B	**12.10**	**11.88**
	SUM	
MEAN	**18.71**	
SD	**11.7**	

It was found that calculated LOD and LOQ is 24ppb and 35ppb, 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 100 µL. 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 ZON 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 S5024/S5048 V19 chapter 9.

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

For the determination of Recovery at LOQ level (Table 2) ZON-free maize powder B was used as blank and it has been spiked with ZON Trilogy standard solution (TSL-401).

Table 2. Recovery at LOQ level. Maize Powder B was spiked with ZON.

Sample (n=8)	Concentration (ppb)	Spike (ppb)	Recovery (%)	
MAIZE POWDER B	34.74	35	99.25	Average 35ppb
			3.71	SD
			3.74	CV%

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

For the determination of Recovery at two different levels (100 and 300 ppb), different ZON-free matrices were spiked with ZON Trilogy standard solution (TSL-401) (Tables 3-17).

Table 3. Recovery of Barley sample at two different levels.

Spike 100 ppb		
Barley (n=8)	96.8	Average
	5.30	SD
	5.48	CV(%)
	96.8	Recovery (%)
Spike 300 ppb		
Barley (n=8)	289.62	Average
	9.68	SD
	3.34	CV(%)
	96.54	Recovery (%)
	96.67	Mean Recovery (%)

Table 4. Recovery of Brown Rice sample at two different levels.

Spike 100 ppb		
Brown Rice (n=8)	92.59	Average
	5.47	SD
	5.91	CV(%)
	92.59	Recovery (%)
Spike 300 ppb		
Brown Rice (n=8)	270.03	Average
	9.83	SD
	3.64	CV(%)
	90.01	Recovery (%)
	91.30	Mean Recovery (%)

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

Spike 100 ppb		
Buckwheat (n=8)	99.3	Average
	5.84	SD
	5.88	CV(%)
	99.3	Recovery (%)
Spike 300 ppb		
Buckwheat (n=8)	295.47	Average
	8.69	SD
	2,94	CV(%)
	98.49	Recovery (%)
	98.9	Mean Recovery (%)

Table 6. Recovery of Corn sample at two different levels.

Spike 100 ppb		
Corn (n=8)	88.56	Average
	4.97	SD
	5.61	CV(%)
	88.56	Recovery (%)
Spike 300 ppb		
Corn (n=8)	266.67	Average
	7.51	SD
	2.87	CV(%)
	88.89	Recovery (%)
	88.73	Mean Recovery (%)

Table 7. Recovery of Corn Flour sample at two different levels.

Spike 100 ppb		
Corn Flour(n=8)	94.2	Average
	5.42	SD
	5.75	CV(%)
	94.2	Recovery (%)
Spike 300 ppb		
Corn Flour (n=8)	271.04	Average
	6.78	SD
	2.50	CV(%)
	90.34	Recovery (%)
	92.27	Mean Recovery (%)

Table 8. Recovery of Dried Brassica Integrifolia sample at two different levels.

Spike 100 ppb		
Dried Brassica Intergrofolia(n=8)	81.96	Average
	4.43	SD
	5.41	CV(%)
	81.96	Recovery (%)
Spike 300 ppb		
Dried Brassica Intergrofolia(n=8)	268	Average
	12.10	SD
	4.51	CV(%)
	89.33	Recovery (%)
	85.65	Mean Recovery (%)

Table 9. Recovery of Dried Gai Choy sample at two different levels.

Spike 100 ppb		
Dried Gai Choy (n=8)	106.3	Average
	6.23	SD
	5.86	CV(%)
	106.3	Recovery (%)
Spike 300 ppb		
Dried Gai Choy (n=8)	295.79	Average
	11.01	SD
	3.72	CV(%)
	98.6	Recovery (%)
	102.45	Mean Recovery (%)

Table 10. Recovery of Dried Palm sample at two different levels.

Spike 100 ppb		
Dried Palm (n=8)	87.61	Average
	4.32	SD
	4.93	CV(%)
	87.61	Recovery (%)
Spike 300 ppb		
Dried Palm (n=8)	280.45	Average
	10.92	SD
	3.89	CV(%)
	93.48	Recovery (%)
	90.55	Mean Recovery (%)

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

Spike 100 ppb		
Malt (n=8)	93.3	Average
	5.21	SD
	5.58	CV(%)
	93.3	Recovery (%)
Spike 300 ppb		
Malt (n=8)	276	Average
	9.58	SD
	3.47	CV(%)
	92	Recovery (%)
	92.65	Mean Recovery (%)

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

Spike 100 ppb		
Millet (n=8)	87.72	Average
	4.44	SD
	5.06	CV(%)
	87.72	Recovery (%)
Spike 300 ppb		
Millet (n=8)	291.31	Average
	10.79	SD
	3.70	CV(%)
	97.1	Recovery (%)
	92.41	Mean Recovery (%)

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

Spike 100 ppb		
Oats (n=8)	98.2	Average
	5.56	SD
	5.66	CV(%)
	98.2	Recovery (%)
Spike 300 ppb		
Oats (n=8)	269.89	Average
	12.61	SD
	4.67	CV(%)
	89.96	Recovery (%)
	94.08	Mean Recovery (%)

Table 14. Recovery of Soy Flour sample at two different levels.

Spike 100 ppb		
Soy Flour (n=8)	94.8	Average
	4.97	SD
	5.24	CV(%)
	94.8	Recovery (%)
Spike 300 ppb		
Soy Flour (n=8)	291.91	Average
	11.23	SD
	3.85	CV(%)
	97.30	Recovery (%)
	96.05	Mean Recovery (%)

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

Spike 100 ppb		
Wheat (n=8)	92.29	Average
	5.42	SD
	5.87	CV(%)
	92.29	Recovery (%)
Spike 300 ppb		
Wheat (n=8)	299.63	Average
	12.31	SD
	4.11	CV(%)
	99.88	Recovery (%)
	96.09	Mean Recovery (%)

Table 16. Recovery of Wheat Flour sample at two different levels.

Spike 100 ppb		
Wheat Flour(n=8)	87.77	Average
	4.92	SD
	5.61	CV(%)
	87.77	Recovery (%)
Spike 300 ppb		
Wheat Flour(n=8)	269.85	Average
	8.56	SD
	3.17	CV(%)
	89.95	Recovery (%)
	88.86	Mean Recovery (%)

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

Spike 100 ppb		
White Rice (n=8)	94.71	Average
	5.63	SD
	5.94	CV(%)
	94.71	Recovery (%)
Spike 300 ppb		
White Rice (n=8)	287.65	Average
	10.16	SD
	3.53	CV(%)
	95.88	Recovery (%)
	95.30	**Mean Recovery (%)**

Table 18. Mean Recovery (%) of all ground matrices

Matrix	Mean Recovery (%)
Barley	96.67
Brown Rice	91.30
Buckwheat	98.9
Corn	88.73
Corn Flour	92.27
Dried Brassica Integrifolia	85.65
Dried Gai Choy	102.45
Dried Palm	90.55
Malt	92.65
Millet	92.41
Oats	94.08
Soy Flour	96.05
Wheat	96.09
Wheat Flour	88.86
White Rice	95.30
MEAN	**93.46**

2.3 Reproducibility

The coefficients of variation of reproducibility of the concentrations (ppb) (Table 19) of two different samples ran eight times in 8 different tests are reported:

Table 19. Coefficients of Variation of the concentration (ppb) of two different samples ran in eight different tests.

	Concentration (ppb)	
Sample (n=8)	**MEAN**	**CV(%)**
FAPAS MAIZE T04439QC	**97.1**	4.76
FAPAS MAIZE T04411QC	**108.55**	3.89
FAPAS MAIZE T22177QC	**65.63**	5.53

2.4 Performance Evaluation

i. Reference Materials

Table 20. Recovery on samples prepared by FAPAS.

Reference material	Lot number	Certified value (µg/kg)	Uncertainty (µ,g/kg)	Result (µg/kg)	Recovery (%)
FAPAS MAIZE T04439QC	90	94.3	41.5	97.1	103
FAPAS MAIZE T04411QC	1	105	46	108.55	103.3
FAPAS MAIZE T22177QC	62	75.6	33.3	65.6	86.8

3. References

[1] 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.

[2] 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.

[3] 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.

[4] 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.

[5] Tsaridou C, Badra K, Natsaridis N, Nikolopoulou E, Ntantasios AN, Papageorgiou G and Athanassiou SD, Estrazione unica in acqua, per la quantificazione di nove micotossine usando la tecnologia Bio-Shield Elisa. VI Congresso Nazionale: Micotossine e Tossine Vegetali nella filiera agro-alimentare,10-12 June, 2019 Rome, Italy.

[6] 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.

[7] 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

Antonios Ntantasios
Managing Director

PROGNOSIS BIOTECH

PROGNOSIS
BIOTECH

www.prognosis-biotech.com
E: info@prognosis-biotech.com

T: +30 2410 623922
Farsalon 153 | 41335 Larissa, Greece