VALIDATION REPORT
SYMMETRIC DON
GREEN
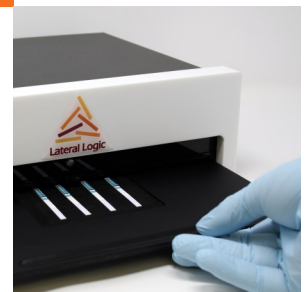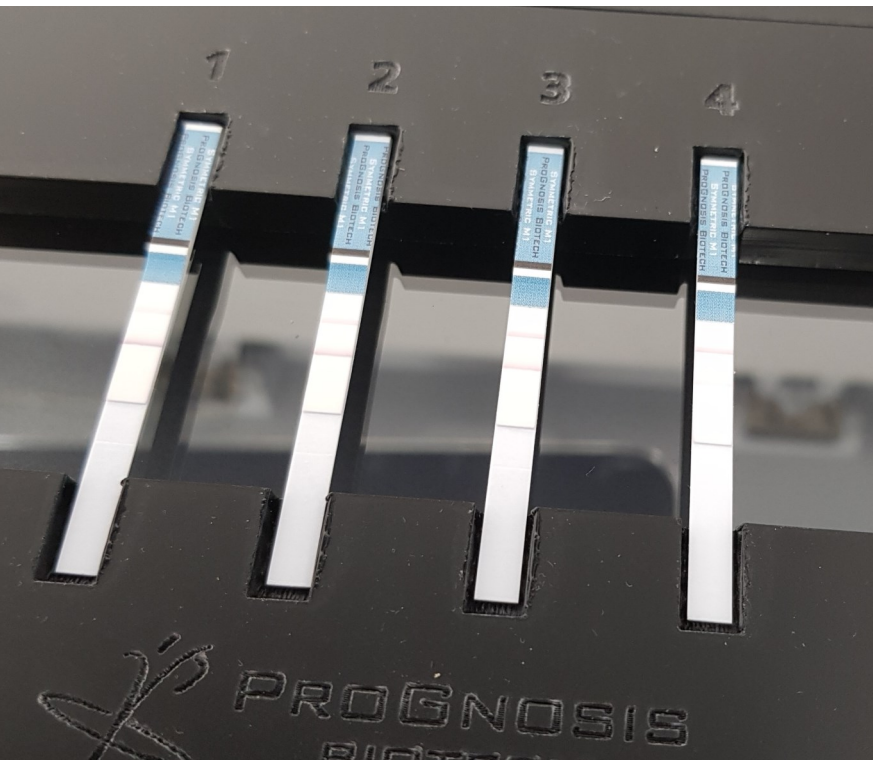
Product code: S4024/S4048
Document no: S40[V14]
Date: 09 July 2024

ProGnosis
BIOTECH

Symmetric DON Lateral Flow kit

INDEX

Symmetric DON Lateral Flow kit

1. Immunoassay Specifications

1.1 General Specifications
- The LOD of the method is 0.1ppm (Table 1)
- The LOQ of the method is 0.15ppm (Table 1)
- **Matrices:** Barley, Beer Residue, Brown Rice, Buckwheat, Corn, Corn Germ, Corn Germ Meal, Corn Flour, Cotton-seed, DDGS, DDGS Molasse, Dried Brassica Integrifolia, Dried Gai Choy, Dried Palm, Malt, Millet, Oats, Pasta, Pea flour, Rice flour, Rye, Rye Flour, Silage, Sorghum, Soybeans, Soybean meal, Sunflower Meal, Toasted wheat germ, Wheat, Wheat Flour, Wheat Germ, White Rice.

1.2 Specificity & Cross-reactivity
The cross-reaction of the anti-DON antibody with 15-acetyl-DON, DON and 3-acetyl-DON is >100, 100, <0.1% respectively.

2. Validation

2.1 Determination of the Limit of Detection LOD and the Limit of Quantification LOQ
For the determination of LOD (2xSD) and LOQ (3xSD), two DON-free maize powder samples (<0.15ppm) were used (Table 1).

Table 1. DON-free maize powder samples for the determination of LOD and LOQ

Sample (n=8)	Concentration (ppm)	
	MEAN	**SD**
Maize Powder A	0.070	0.052
Maize Powder B	0.046	0.039
	SUM	
MEAN	**0.06**	
SD	**0.05**	

The LOD and LOQ were defined as 2x Standard Deviation and 3x Standard Deviation of the DON-free maize powder blank samples, respectively. It was found that calculated LOD and LOQ is 0.1ppm and 0.15ppm, 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 DON 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 S4024/B4048 V24 chapter 9.

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

For the determination of Recovery at LOQ level (Table 2) DON-free maize powder B was used as blank and it has been spiked with DON Trilogy standard solution (TSL-317).

PROGNOSIS BIOTECH

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

Sample (n=8)	Concentration (ppm)	Spike (ppm)	Recovery (%)	
MAIZE POWDER B	0.155	0.15	103.75	Average 0.15ppm
			6.49	SD
			6.27	CV%

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

For the determination of Recovery at two different levels (0.5 and 1.5 ppm), different DON-free matrices were spiked with DON Trilogy standard solution (TSL-317) (Tables 3-33).

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

Spike 0.5 ppm		
Barley (n=8)	0.46	Average
	0.026	SD
	5.6	CV(%)
	92.0	Recovery (%)
Spike 1.5 ppm		
Barley (n=8)	1.54	Average
	0.07	SD
	4.54	CV(%)
	102.6	Recovery (%)
	97.3	Mean Recovery (%)

Table 4. Recovery of Beer Residue sample at two different levels.

Spike 0.5 ppm		
Beer Residue (n=8)	0.44	Average
	0.023	SD
	5.20	CV(%)
	88.0	Recovery (%)
Spike 1.5 ppm		
Beer Residue (n=8)	1.42	Average
	0.03	SD
	2.11	CV(%)
	94.66	Recovery (%)
	89.83	Mean Recovery (%)

Table 5. Recovery of Brown Rice sample at two different levels.

Spike 0.5 ppm		
Brown Rice (n=8)	0.46	Average
	0.03	SD
	6.52	CV(%)
	92	Recovery (%)
Spike 1.5 ppm		
Brown Rice (n=8)	1.45	Average
	0.08	SD
	5.51	CV(%)
	96.53	Recovery (%)
	94.26	Mean Recovery (%)

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

Spike 0.5 ppm		
Buckwheat (n=8)	0.46	Average
	0.02	SD
	4.34	CV(%)
	92	Recovery (%)
Spike 1.5 ppm		
Buckwheat (n=8)	1.23	Average
	0.03	SD
	2.43	CV(%)
	82	Recovery (%)
	87	Mean Recovery (%)

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

Spike 0.5 ppm		
Corn (n=8)	0.50	Average
	0.015	SD
	3	CV(%)
	100	Recovery (%)
Spike 1.5 ppm		
Corn (n=8)	1.47	Average
	0.034	SD
	2.31	CV(%)
	98	Recovery (%)
	99.58	Mean Recovery (%)

PROGNOSIS
BIOTECH

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

	Spike 0.5 ppm	
Corn Germ (n=8)	0.57	**Average**
	0.023	**SD**
	4.03	**CV(%)**
	114	**Recovery (%)**
	Spike 1.5 ppm	
Corn Germ (n=8)	1.51	**Average**
	0.05	**SD**
	3.31	**CV(%)**
	100,9	**Recovery (%)**
	107.46	**Mean Recovery (%)**

Table 9. Recovery of Corn Germ Meal sample at two different levels.

	Spike 0.5 ppm	
Corn Germ Meal (n=8)	0.59	**Average**
	0.04	**SD**
	6.77	**CV(%)**
	118	**Recovery (%)**
	Spike 1.5 ppm	
Corn Germ Meal (n=8)	1.65	**Average**
	0.08	**SD**
	4.84	**CV(%)**
	108.85	**Recovery (%)**
	113	**Mean Recovery (%)**

Table 10. Recovery of Corn Flour sample sample at two different levels.

	Spike 0.5 ppm	
Corn Flour (n=8)	0.43	**Average**
	0.03	**SD**
	6.98	**CV(%)**
	86	**Recovery (%)**
	Spike 1.5 ppm	
Corn Flour (n=8)	1.41	**Average**
	0.08	**SD**
	5.67	**CV(%)**
	94	**Recovery (%)**
	96	**Mean Recovery (%)**

PROGNOSIS
BIOTECH

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

Spike 0.5 ppm		
Cottonseed (n=8)	0.42	Average
	0.02	SD
	4.76	CV(%)
	84	Recovery (%)
Spike 1.5 ppm		
Cottonseed (n=8)	1.39	Average
	0.04	SD
	2.87	CV(%)
	92.67	Recovery (%)
	88.33	Mean Recovery (%)

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

Spike 0.5 ppm		
DDGS (n=8)	0.44	Average
	0.025	SD
	5,68	CV(%)
	88	Recovery (%)
Spike 1.5 ppm		
DDGS (n=8)	1.35	Average
	0.05	SD
	3.7	CV(%)
	90	Recovery (%)
	88	Mean Recovery (%)

Table 13. Recovery of DDGS Molasse sample at two different levels.

Spike 0.5 ppm		
DDGS (n=8)	0.59	Average
	0.03	SD
	5.08	CV(%)
	111	Recovery (%)
Spike 1.5 ppm		
DDGS (n=8)	1.67	Average
	0.08	SD
	4.79	CV(%)
	108	Recovery (%)
	109.5	Mean Recovery (%)

PrOGnosis BIOTECH

Table 14. Recovery of Dried Brassica Integrifolia sample at two different levels.

Spike 0.5 ppm		
Dried Brassica Integrifolia (n=8)	0.46	Average
	0.032	SD
	6.9	CV(%)
	92	Recovery (%)
Spike 1.5 ppm		
Dried Brassica Integrifolia (n=8)	1.45	Average
	0.04	SD
	2.75	CV(%)
	96.67	Recovery (%)
	94,33	Mean Recovery (%)

Table 15. Recovery of Dried Gai Choy sample at two different levels.

Spike 0.5 ppm		
Dried Gai Choy (n=8)	0.44	Average
	0.02	SD
	4.55	CV(%)
	88	Recovery (%)
Spike 1.5 ppm		
Dried Gai Choy (n=8)	1.56	Average
	0.02	SD
	1.28	CV(%)
	104	Recovery (%)
	96	Mean Recovery (%)

Table 16. Recovery of Dried Palm sample at two different levels.

Spike 0.5 ppm		
Dried Palm (n=8)	0.55	Average
	0.03	SD
	5.45	CV(%)
	110	Recovery (%)
Spike 1.5 ppm		
Dried Palm (n=8)	1.49	Average
	0.036	SD
	2.41	CV(%)
	99.33	Recovery (%)
	104.66	Mean Recovery (%)

PrOGNOSIS BIOTECH

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

	Spike 0.5 ppm	
Malt (n=8)	0.548	Average
	0.016	SD
	2.92	CV(%)
	109.6	Recovery (%)
	Spike 1.5 ppm	
Malt (n=8)	1.565	Average
	0.069	SD
	4.40	CV(%)
	104.33	Recovery (%)
	106.96	Mean Recovery (%)

Table 18. Recovery of Millet sample at two different levels.

	Spike 0.5 ppm	
Millet (n=8)	0.55	Average
	0.035	SD
	6.36	CV(%)
	110	Recovery (%)
	Spike 1.5 ppm	
Millet (n=8)	1.66	Average
	0.04	SD
	2.4	CV(%)
	111.23	Recovery (%)
	110.53	Mean Recovery (%)

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

	Spike 0.5 ppm	
Oat (n=8)	0.47	Average
	0.033	SD
	7.02	CV(%)
	94	Recovery (%)
	Spike 1.5 ppm	
Oat (n=8)	1.47	Average
	0.05	SD
	3.4	CV(%)
	98	Recovery (%)
	96	Mean Recovery (%)

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

Spike 0.5 ppm		
Pasta (n=8)	0.43	Average
	0.03	SD
	6.98	CV(%)
	86	Recovery (%)
Spike 1.5 ppm		
Pasta (n=8)	1.36	Average
	0.049	SD
	3.6	CV(%)
	90.67	Recovery (%)
	88.33	Mean Recovery (%)

Table 21. Recovery of Pea Flour sample at two different levels.

Spike 0.5 ppm		
Pea Flour (n=8)	0.41	Average
	0.036	SD
	8.78	CV(%)
	82	Recovery (%)
Spike 1.5 ppm		
Pea Flour (n=8)	1.43	Average
	0.059	SD
	4.12	CV(%)
	95.33	Recovery (%)
	89.66	Mean Recovery (%)

Table 22. Recovery of Rice Flour sample at two different levels.

Spike 0.5 ppm		
Rice Flour(n=8)	0.42	Average
	0.024	SD
	5,7	CV(%)
	93.75	Recovery (%)
Spike 1.5 ppm		
Rice Flour(n=8)	1.41	Average
	0.06	SD
	4.25	CV(%)
	94	Recovery (%)
	93.87	Mean Recovery (%)

PROGNOSIS
BIOTECH

Table 23. Recovery of Rye sample at two different levels.

	Spike 0.5 ppm	
	0,42	Average
Rye (n=8)	0.03	SD
	7.14	CV(%)
	84	Recovery (%)
	Spike 1.5 ppm	
	1.4	Average
Rye (n=8)	0,08	SD
	5,7	CV(%)
	93,3	Recovery (%)
	88.66	Mean Recovery (%)

Table 24. Recovery of Rye Flour sample at two different levels.

	Spike 0.5 ppm	
	0,39	Average
Rye Flour (n=8)	0.025	SD
	6.4	CV(%)
	93.3	Recovery (%)
	Spike 1.5 ppm	
	1.32	Average
Rye Flour (n=8)	0,08	SD
	6	CV(%)
	88	Recovery (%)
	83	Mean Recovery (%)

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

Ta-

	Spike 0.5 ppm	
	0.44	Average
Silage (n=8)	0.025	SD
	5,68	CV(%)
	88	Recovery (%)
	Spike 1.5 ppm	
	1.53	Average
Silage (n=8)	0.1	SD
	6.53	CV(%)
	102	Recovery (%)
	95	Mean Recovery (%)

Table 26. Recovery of Sorghum sample at two different levels.

	Spike 0.5 ppm	
Sorghum (n=8)	0.48	**Average**
	0.03	**SD**
	6.25	**CV(%)**
	96	**Recovery (%)**
	Spike 1.5 ppm	
Sorghum (n=8)	1.33	**Average**
	0.06	**SD**
	4.51	**CV(%)**
	88.67	**Recovery (%)**
	92.33	**Mean Recovery (%)**

Table 27. Recovery of Soybeans sample at two different levels.

	Spike 0.5 ppm	
Soybeans (n=8)	0.47	**Average**
	0.04	**SD**
	8.51	**CV(%)**
	94	**Recovery (%)**
	Spike 1.5 ppm	
Soybeans (n=8)	1.455	**Average**
	0.043	**SD**
	2.96	**CV(%)**
	96.67	**Recovery (%)**
	95.33	**Mean Recovery (%)**

Table 28. Recovery of Soybean meal sample at two different levels.

	Spike 0.5 ppm	
Soybean meal (n=8)	0.44	**Average**
	0.029	**SD**
	6.6	**CV(%)**
	88	**Recovery (%)**
	Spike 1.5 ppm	
Soybean meal (n=8)	1.55	**Average**
	0.09	**SD**
	5.7	**CV(%)**
	103.8	**Recovery (%)**
	95.9	**Mean Recovery (%)**

Table 29. Recovery of Sunflower Meal sample at two different levels.

Spike 0.5 ppm		
Sunflower Meal (n=8)	0.536	**Average**
	0.038	**SD**
	7.09	**CV(%)**
	107.25	**Recovery (%)**
Spike 1.5 ppm		
Sunflower Meal (n=8)	1.488	**Average**
	0.049	**SD**
	3.31	**CV(%)**
	99.2	**Recovery (%)**
	103.25	**Mean Recovery (%)**

Table 30. Recovery of Toasted Wheat Germ sample at two different levels.

Spike 0.5 ppm		
Toasted Wheat Germ (n=8)	0.48	**Average**
	0.036	**SD**
	7.5	**CV(%)**
	0.96	**Recovery (%)**
Spike 1.5 ppm		
Toasted Wheat Germ (n=8)	1.43	**Average**
	0.095	**SD**
	6.66	**CV(%)**
	95.3	**Recovery (%)**
	95.66	**Mean Recovery (%)**

Table 31. Recovery of Wheat sample at two different levels.

Ta-

Spike 0.5 ppm		
Wheat (n=8)	0.49	**Average**
	0.03	**SD**
	6.12	**CV(%)**
	98	**Recovery (%)**
Spike 1.5 ppm		
Wheat (n=8)	1.43	**Average**
	0.06	**SD**
	4.2	**CV(%)**
	95.3	**Recovery (%)**
	97.36	**Mean Recovery (%)**

PROGNOSIS BIOTECH

Table 32. Recovery of Wheat Flour sample at two different levels.

	Spike 0.5 ppm	
	0.48	Average
Wheat Flour (n=8)	0.01	SD
	2.08	CV(%)
	96.0	Recovery (%)
	Spike 1.5 ppm	
	1.33	Average
Wheat Flour (n=8)	0.04	SD
	3	CV(%)
	88.67	Recovery (%)
	92.33	Mean Recovery (%)

Table 33. Recovery of Wheat Germ sample at two different levels.

	Spike 0.5 ppm	
	0.40	Average
Wheat Germ (n=8)	0.03	SD
	7.5	CV(%)
	80	Recovery (%)
	Spike 1.5 ppm	
	1.319	Average
Wheat Germ (n=8)	0.043	SD
	3.26	CV(%)
	87.33	Recovery (%)
	83.66	Mean Recovery (%)

Table 34. Recovery of White Rice sample at two different levels.

	Spike 0.5 ppm	
	0.468	Average
White Rice (n=8)	0.029	SD
	6.2	CV(%)
	93.6	Recovery (%)
	Spike 1.5 ppm	
	1.43	Average
White Rice (n=8)	0.07	SD
	4.89	CV(%)
	95.33	Recovery (%)
	94.46	Mean Recovery (%)

PROGNOSIS
BIOTECH

Table 35. Mean Recovery (%) of all ground matrices

Matrix	Mean Recovery (%)
Barley	97.3
Beer Residue	89.83
Brown Rice	94.26
Buckwheat	87.00
Corn	99.58
Corn Germ	107.46
Corn Germ Meal	113
Corn Flour	96
Cottonseeds	88.33
DDGS	88.00
DDGS Molasse	109.5
Dried Brassica integrifolia	94.33
Dried Gai Choy	96.00
Dried Palm	104.66
Malt	106.96
Millet	110.53
Oats	96.00
Pasta	88.33
Pea Flour	89.66
Rice Flour	93.87
Rye	88.66
Rye Flour	83
Silage	95.00
Sorghum	92.33
Soybeans	95.33
Soybean meal	95.9
Sunflower Meal	103.25
Toasted Wheat Germ	95.66
Wheat	97.36
Wheat Flour	92.33
Wheat Germ	83.66
White Rice	94.46
MEAN	**95.74**

ProGnosis BIOTECH

2.3 Reproducibility

The coefficients of variation of reproducibility of the concentrations (ppm) (Table 35) of three different samples ran eight times in 8 different tests are reported:

Table 36. Coefficients of Variation of the concentration (ppm) of three different samples ran in eight different tests.

Sample (n=8)	Concentration (ppm)	
	MEAN	CV(%)
FAPAS MAIZE T04439QC	1.03	5,3
FAPAS MAIZE T04411QC	1.15	5,8
FAPAS Wheat Flour T22187QC	0.708	5.65

2.4 Performance Evaluation

Table 37. 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	1018	325	1032	101.3
FAPAS Maize T04411QC	1	1125	354	1150	102.2
FAPAS Wheat flour T22187QC	62	769	256	708	92.06

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 Athanasiou 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

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

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