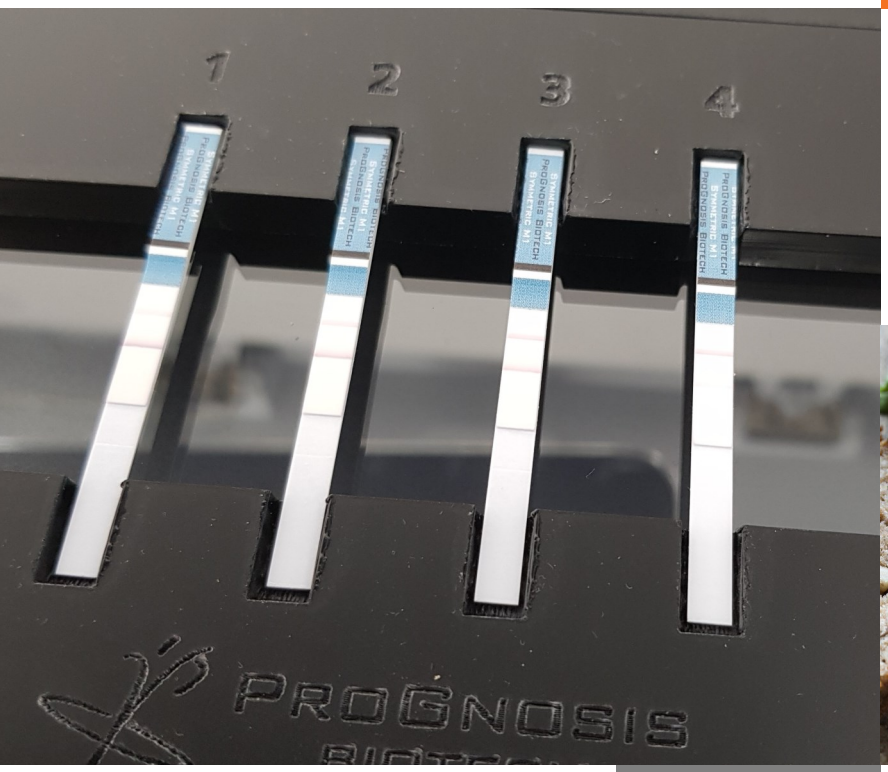
VALIDATION REPORT

SYMMETRIC FUMONISIN GREEN



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PROGNOSIS BIOTECH

Symmetric FUMONISIN Lateral Flow kit

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Symmetric FUMONISIN Lateral Flow kit

1. Immunoassay Specifications

2.1 General Specifications

- The LOD of the method is 100 ppb (Table 1)
- The LOQ of the method is 150 ppb (Table 1)
- **Matrices:** Barley, Beer Residue, Brown Rice, Buckwheat, Corn, Corn Flour, DDGS, Dried Brassica Integrifolia, Dried Gai Choy, Dried Palm, Malt, Millet, Oats, Pasta, Pop Corn, Rice Flour, Soybeans, Soy bean meal, Sunflower meal, Wheat, Wheat Flour, White Rice.

1.2 Specificity & Cross-reactivity

The cross-reaction of the anti-Fumonisin antibody with FB1, FB2 and FB3 is 100, 65 and 48% 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 Fumonisin-free maize powder samples (<150 ppb) were used (Table 1).

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

	Concentration (ppb)	
Sample (n=8)	**MEAN**	**SD**
Maize Powder A	7.16	49.70
Maize Powder B	5.75	49.81
	SUM	
MEAN	**6.46**	
SD	**49.76**	

The LOD and LOQ were defined as 2 x Standard Deviation and 3 x Standard Deviation of the Fumonisin-free maize powder blank samples, respectively. It was found that calculated LOD and LOQ is 100 ppb and 150 ppb, 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 Fumonisin 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 S7024/S7048 V23 chapter 9.

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

For the determination of Recovery at LOQ level (Table 2) Fumonisin-free maize powder B was used as blank and it has been spiked with Fumonisin Trilogy standard solution (TSL-204).

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

Sample (n=8)	Concentration (ppb)	Spike (ppb)	Recovery (%)	
MAIZE POWDER B	145.92	150.00	**97.28**	**Average 150 ppb**
			15.2	**SD**
			15.6	**CV%**

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

For the determination of Recovery at two different levels 450 and 1500 ppb), different Fumonisin-free matrices were spiked with Fumonisin Trilogy standard solution (TSL-204) (Tables 3-24).

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

Spike 450 ppb		
Barley (n=8)	**438.75**	**Average**
	9.93	**SD**
	2.26	**CV(%)**
	97.5	**Recovery (%)**
Spike 1500 ppb		
Barley (n=8)	**1457.31**	**Average**
	45.12	**SD**
	3.09	**CV(%)**
	97.15	**Recovery (%)**
	97.32	**Mean Recovery (%)**

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

Spike 450 ppb		
Beer Residue (n=8)	**463.75**	**Average**
	5.16	**SD**
	1.11	**CV(%)**
	103.05	**Recovery (%)**
Spike 1500 ppb		
Beer Residue (n=8)	**1492.12**	**Average**
	43.1	**SD**
	2.88	**CV(%)**
	99.47	**Recovery (%)**
	101.26	**Mean Recovery (%)**

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

Spike 450 ppb		
Brown Rice (n=8)	463.25	Average
	8.88	SD
	1.9	CV(%)
	102.9	Recovery (%)
Spike 1500 ppb		
Brown Rice (n=8)	1461	Average
	67.22	SD
	4.6	CV(%)
	97.4	Recovery (%)
	100.15	Mean Recovery (%)

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

Spike 450 ppb		
Buckwheat (n=8)	461.3	Average
	10.23	SD
	2.21	CV(%)
	102.51	Recovery (%)
Spike 1500 ppb		
Buckwheat (n=8)	1463.5	Average
	79.21	SD
	3.22	CV(%)
	97.56	Recovery (%)
	100.03	Mean Recovery (%)

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

Spike 450 ppb		
Corn (n=8)	461.25	Average
	25.52	SD
	5.53	CV(%)
	102.5	Recovery (%)
Spike 1500 ppb		
Corn (n=8)	1479.51	Average
	75.81	SD
	5.1	CV(%)
	98.63	Recovery (%)
	100.56	Mean Recovery (%)

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

Spike 450 ppb		
Corn **flour (n=8)**	481	Average
	9.08	SD
	1.88	CV(%)
	106.88	Recovery (%)
Spike 1500 ppb		
Corn **flour (n=8)**	1565.2	Average
	75.21	SD
	4.8	CV(%)
	104.3	Recovery (%)
	105.59	Mean Recovery (%)

Table 9. Recovery of DDGS sample at two different levels.

Spike 450 ppb		
Corn **flour (n=8)**	451.75	Average
	6.68	SD
	1.48	CV(%)
	100.4	Recovery (%)
Spike 1500 ppb		
Corn **flour (n=8)**	1492.12	Average
	57.11	SD
	3.82	CV(%)
	99.47	Recovery (%)
	99.93	Mean Recovery (%)

Table 10. Recovery of Dried Brassica Intergrifolia sample at two different levels.

Spike 450 ppb		
Dried Brassica Intergrifolia **(n=8)**	383.87	Average
	20.23	SD
	5.27	CV(%)
	85.3	Recovery (%)
Spike 1500 ppb		
Dried Brassica Intergrifolia **(n=8)**	1439.5	Average
	65.83	SD
	4.57	CV(%)
	95.96	Recovery (%)
	90.63	Mean Recovery (%)

Table 11. Recovery of Dried Gai Choy sample at two different levels.

Spike 450 ppb		
Dried Gai Choy **(n=8)**	**386.12**	**Average**
	20.24	**SD**
	5.24	**CV(%)**
	85.80	**Recovery (%)**
Spike 1500 ppb		
Dried Gai Choy **(n=8)**	**1475.2**	**Average**
	54.15	**SD**
	3.67	**CV(%)**
	97.14	**Recovery (%)**
	91.47	**Mean Recovery (%)**

Table 12. Recovery of Dried Palm sample at two different levels.

Spike 450 ppb		
Dried Palm **(n=8)**	**388.12**	**Average**
	19.2	**SD**
	4.95	**CV(%)**
	86.25	**Recovery (%)**
Spike 1500 ppb		
Dried Palm **(n=8)**	**1448.33**	**Average**
	57.1	**SD**
	3.94	**CV(%)**
	96.55	**Recovery (%)**
	91.4	**Mean Recovery (%)**

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

Spike 450 ppb		
Malt **(n=8)**	**447.94**	**Average**
	21.05	**SD**
	4.7	**CV(%)**
	99.54	**Recovery (%)**
Spike 1500 ppb		
Malt **(n=8)**	**1556.1**	**Average**
	46.32	**SD**
	2.97	**CV(%)**
	103.74	**Recovery (%)**
	101.64	**Mean Recovery (%)**

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

Spike 450 ppb		
Millet**(n=8)**	**433.87**	**Average**
	7.68	**SD**
	1.77	**CV(%)**
	96.4	**Recovery (%)**
Spike 1500 ppb		
Millet **(n=8)**	**1458.55**	**Average**
	61.23	**SD**
	4.19	**CV(%)**
	97.23	**Recovery (%)**
	96.81	**Mean Recovery (%)**

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

Spike 450 ppb		
Oats **(n=8)**	**506.7**	**Average**
	4.6	**SD**
	0.9	**CV(%)**
	112.6	**Recovery (%)**
Spike 1500 ppb		
Oats **(n=8)**	**1582.1**	**Average**
	88.92	**SD**
	5.6	**CV(%)**
	105.4	**Recovery (%)**
	110.5	**Mean Recovery (%)**

Table 16. Recovery of Pasta sample at two different levels.

Spike 450 ppb		
Pasta **(n=8)**	**471**	**Average**
	8	**SD**
	1.7	**CV(%)**
	104.66	**Recovery (%)**
Spike 1500 ppb		
Pasta **(n=8)**	**1445.21**	**Average**
	55.56	**SD**
	3.84	**CV(%)**
	96.34	**Recovery (%)**
	100.5	**Mean Recovery (%)**

Table 17. Recovery of Pop corn sample at two different levels.

Spike 450 ppb		
Pop Corn **(n=8)**	**477**	**Average**
	8.86	**SD**
	1.86	**CV(%)**
	106	**Recovery (%)**
Spike 1500 ppb		
Pop Corn **(n=8)**	**1472**	**Average**
	55	**SD**
	3.7	**CV(%)**
	98.13	**Recovery (%)**
	103.41	**Mean Recovery (%)**

Table 18. Recovery of Rice Flour sample at two different levels.

Spike 450 ppb		
Rice Flour **(n=8)**	**406.9**	**Average**
	14.5	**SD**
	3.56	**CV(%)**
	90.4	**Recovery (%)**
Spike 1500 ppb		
Rice Flour **(n=8)**	**1437.2**	**Average**
	45.82	**SD**
	3.18	**CV(%)**
	95.81	**Recovery (%)**
	93.1	**Mean Recovery (%)**

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

Spike 450 ppb		
Soybeans **(n=8)**	**424.12**	**Average**
	7.86	**SD**
	1.85	**CV(%)**
	94.25	**Recovery (%)**
Spike 1500 ppb		
Soybeans **(n=8)**	**1445.14**	**Average**
	55.35	**SD**
	38.13	**CV(%)**
	96.34	**Recovery (%)**
	95.3	**Mean Recovery (%)**

Table 20. Recovery of Soybean Meal sample at two different levels.

Spike 450 ppb		
Soybean Meal **(n=8)**	**430.25**	**Average**
	10.64	**SD**
	2.47	**CV(%)**
	95.6	**Recovery (%)**
Spike 1500 ppb		
Soybean Meal **(n=8)**	**1486.2**	**Average**
	87.22	**SD**
	5.86	**CV(%)**
	99.08	**Recovery (%)**
	97.34	**Mean Recovery (%)**

Table 21. Recovery of Sunflower Meal sample at two different levels.

Spike 450 ppb		
Sunflower Meal **(n=8)**	**454.87**	**Average**
	11.67	**SD**
	2.56	**CV(%)**
	101.1	**Recovery (%)**
Spike 1500 ppb		
Sunflower Meal **(n=8)**	**1512.28**	**Average**
	65.57	**SD**
	4.33	**CV(%)**
	100.8	**Recovery (%)**
	100.95	**Mean Recovery (%)**

Table 22. Recovery of Wheat sample at two different levels.

Spike 450 ppb		
Wheat **(n=8)**	**434.6**	**Average**
	9.64	**SD**
	2.22	**CV(%)**
	96.58	**Recovery (%)**
Spike 1500 ppb		
Wheat (n=8)	**1488.3**	**Average**
	54.2	**SD**
	3.64	**CV(%)**
	99.22	**Recovery (%)**
	97.9	**Mean Recovery (%)**

Table 23. Recovery of Wheat Flour sample at two different levels.

Spike 450 ppb		
Wheat Flour **(n=8)**	**475.75**	**Average**
	9.86	**SD**
	2.07	**CV(%)**
	105.7	**Recovery (%)**
Spike 1500 ppb		
Wheat Flour (n=8)	**1487.9**	**Average**
	63.12	**SD**
	4.24	**CV(%)**
	99.19	**Recovery (%)**
	102.44	**Mean Recovery (%)**

Table 24. Recovery of White Rice sample at two different levels.

Spike 450 ppb		
White Rice **(n=8)**	**468**	**Average**
	6.44	**SD**
	1.37	**CV(%)**
	96.15	**Recovery (%)**
Spike 1500 ppb		
White Rice **(n=8)**	**1462.1**	**Average**
	57.8	**SD**
	3.95	**CV(%)**
	97.47	**Recovery (%)**
	96.81	**Mean Recovery (%)**

Table 25. Mean Recovery (%) of all ground matrices

Matrix	Mean Recovery (%)
Barley	97.32
Beer Residue	101.26
Brow Rice	100.15
Buckwheat	100.03
Corn	100.56
Corn Flour	105.59
DDGS	99.93
Dried Brassica Integrifolia	90.63
Dried Gai Choy	91.47
Dried Palm	91.4
Malt	101.64
Millet	96.81
Oats	110.5
Pasta	100.5
Pop Corn	103.41
Rice Flour	93.1
Soybeans	95.3
Soybean Meal	97.34
Sunflower Meal	100.95
Wheat	97.9
Wheat Flour	102.44
White Rice	96.81
MEAN	**98.86**

3.3 Reproducibility

The coefficients of variation of reproducibility of the concentrations (ppb) (Table 26) of two different samples ran eight times in 8 different tests are reported:

Table 26. Coefficients of Variation of the concentration (ppb) of two different samples ran in eight different tests.

	Concentration (ppm)	
Sample (n=8)	**MEAN**	**CV(%)**
FAPAS MAIZE T04411QC	965.45	3.95
FAPAS MAIZE T04384QC	719.5	1.72

3.4 Performance Evaluation

i. Reference Materials

Table 27. Recovery on samples prepared by FAPAS.

Reference material	Lot number	Certified value (µg/kg)	Uncertainty (µg/kg)	Result (µg/kg)	Recovery (%)
FAPAS Maize T04411QC	1	962	310	965.45	100.36
FAPAS Maize T04384QC	87	752	251	719.5	104.5

4. References

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