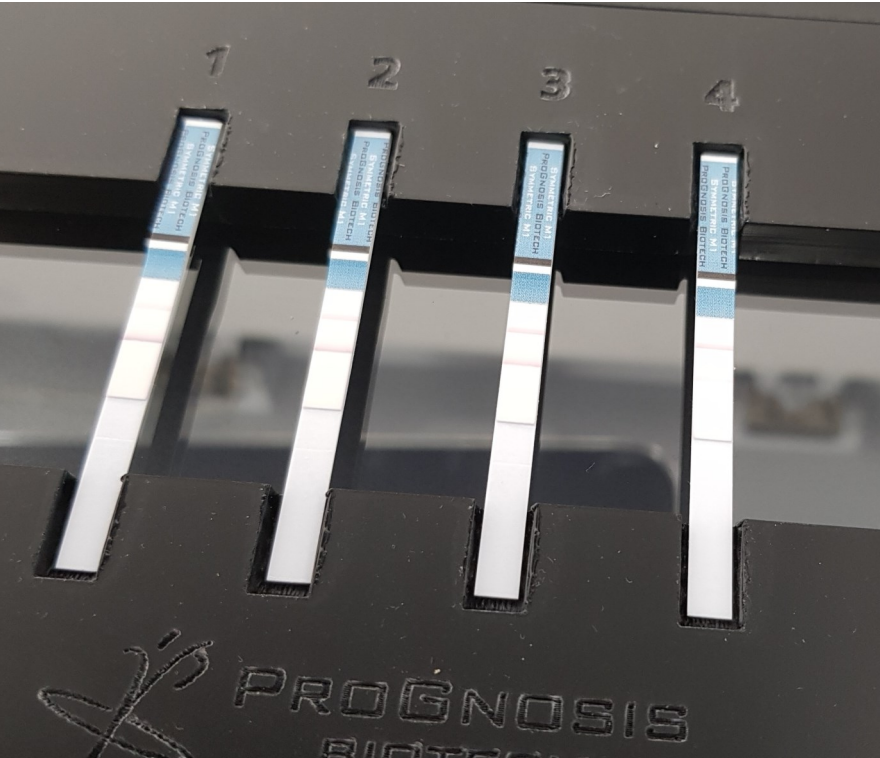


VALIDATION REPORT

SYMMETRIC T-2/HT-2

GREEN



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Symmetric T-2/HT-2 Green Lateral Flow kit

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Symmetric T-2/HT-2 Green Lateral Flow kit

1. Immunoassay Specifications

1.1 General Specifications

The LOD and LOQ of the 0-600ppb method are 26,5ppb and 40ppb respectively. (Type I & II, Table 1)

The LOD and LOQ of the 0-2000ppb method are 67ppb and 100ppb respectively. (Type III, Table 2)

Matrices:

Type I: Corn, Barley, Oats, White Rice, Brown Rice

Type II: Wheat.

Type III: Corn flour, Wheat flour, Soya, Millet. (as well as types I & II matrices that are greater than 600ppb)

1.2 Specificity & Cross-reactivity

The cross-reaction of the anti-T-2 antibody with HT-2, T-2 Triol and T-2 Tetrol is 80, 3.7 and <0.1% respectively.

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, T-2/HT-2-free samples (four maize powder samples and two wheat powder samples) were used: Samples mA-mB & wa-wB (<40ppb) for 0-600ppb (Table 1 & 2) and samples mC-mD (<100ppb) for 0-2000ppb (Table 3).

Table 1. T-2/HT-2-free maize powder samples for the determination of LOD and LOQ 0-600 ppb (TypeI)

Sample (n=8)	Concentration (ppb)	
	MEAN	SD
Maize Powder A	21	14.25
Maize Powder B	15	12.2
	SUM	
MEAN	18	
SD	13.2	

Table 2. T-2/HT-2-free wheat powder samples for the determination of LOD and LOQ 0-600 ppb (TypeII)

Sample (n=8)	Concentration (ppb)	
	MEAN	SD
Wheat Powder A	19	13.9
Wheat Powder B	28	12.7
	SUM	
MEAN	23.5	
SD	13.3	

Table 3. T-2/HT-2-free maize powder samples for the determination of LOD and LOQ 0-2000 ppb (Type III)

Sample (n=8)	Concentration (ppb)	
	MEAN	SD
Maize Powder C	38	36.5
Maize Powder D	67	30.3
	SUM	
MEAN	52.5	
SD	33.4	

It was found that calculated LOD and LOQ are 26,5ppb and 40ppb (0-600ppb), 67ppb and 100ppb (0-2000ppb), 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 μ L. 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 T-2/HT-2 (60:40) 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 S8024/S8048 vN3 chapter 9.

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

. Determination of Recovery (%) at the LOQ level (40ppb) – TYPE I

For the determination of Recovery at LOQ level (Table 4) T-2/HT-2-free maize powder A was used as blank and it has been spiked with Fermentek T-2 and HT-2 standard solution (SST004 and SSHT004).

Table 4. Recovery at LOQ level. Maize Powder A was spiked with T-2/HT-2.

Sample (n=8)	Concentration (ppb)	Spike (ppb)	Recovery (%)	
MAIZE POWDER A	40.9	40	102.25	Average 40 ppb
			5.26	SD
			5.14	CV%

. *Determination of Recovery (%) at the LOQ level (40ppb) – TYPE II*

For the determination of Recovery at LOQ level (Table 5) T-2/HT-2- free wheat powder A was used as blank and it has been spiked with Fermentek T-2 and HT-2 standard solution (SST004 and SSHT004).

Table 5. Recovery at LOQ level. Wheat Powder A was spiked with T-2/HT-2.

Sample (n=8)	Concentration (ppb)	Spike (ppb)	Recovery (%)	
WHEAT POWDER A	39.7	40	99.25	Average 40 ppb
			5.13	SD
			5.17	CV%

. *Determination of Recovery (%) at the LOQ level (100ppb) – TYPE III*

For the determination of Recovery at LOQ level (Table 6) T-2/HT-2-free maize powder C was used as blank and it has been spiked with Fermentek T-2 and HT-2 standard solution (SST004 and SSHT004).

Table 6. Recovery at LOQ level. Maize Powder C was spiked with T-2/HT-2.

Sample (n=8)	Concentration (ppb)	Spike (ppb)	Recovery (%)	
MAIZE POWDER C	102	100	102	Average 100 ppb
			4.95	SD
			4.85	CV%

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

For the determination of Recovery at two different levels (150 and 300ppb), different T-2/HT-2-free matrices were spiked with Fermentek T-2 and HT-2 standard solution (SST004 and SSHT004) (Tables 7-16).

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

Spike 150 ppb		
Barley (n=8)	142	Average
	5.81	SD
	4.09	CV(%)
	94.67	Recovery (%)
Spike 300 ppb		
Barley (n=8)	282	Average
	12.49	SD
	4.43	CV(%)
	94	Recovery (%)
	94.34	Mean Recovery (%)

Table 8. Recovery of Brown Rice sample at two different levels.

Spike 150 ppb		
Brown Rice (n=8)	142	Average
	5.43	SD
	3.82	CV(%)
	94.6	Recovery (%)
Spike 300 ppb		
Brown Rice (n=8)	295	Average
	12.79	SD
	4.34	CV(%)
	98.33	Recovery (%)
	96.47	Mean Recovery (%)

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

Spike 150 ppb		
Corn (n=8)	146	Average
	5.32	SD
	3.64	CV(%)
	97.33	Recovery (%)
Spike 300 ppb		
Corn (n=8)	291	Average
	10.94	SD
	3.76	CV(%)
	97	Recovery (%)
	97.17	Mean Recovery (%)

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

Spike 150 ppb		
Corn flour (n=8)	154	Average
	5.46	SD
	3.55	CV(%)
	102.67	Recovery (%)
Spike 300 ppb		
Corn flour (n=8)	308	Average
	16.01	SD
	5.20	CV(%)
	102.67	Recovery (%)
	101.99	Mean Recovery (%)

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

Spike 150 ppb		
Millet (n=8)	165	Average
	5.86	SD
	5.53	CV(%)
	110	Recovery (%)
Spike 300 ppb		
Millet (n=8)	316	Average
	11.70	SD
	3.70	CV(%)
	105.33	Recovery (%)
	107.67	Mean Recovery (%)

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

Spike 150 ppb		
Oat (n=8)	169	Average
	7.33	SD
	4.34	CV(%)
	112.67	Recovery (%)
Spike 300 ppb		
Oat (n=8)	317	Average
	11.1	SD
	3.50	CV(%)
	105.67	Recovery (%)
	109.17	Mean Recovery (%)

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

Spike 150 ppb		
Soybean (n=8)	132	Average
	5.55	SD
	4.20	CV(%)
	88	Recovery (%)
Spike 300 ppb		
Soybean (n=8)	273	Average
	13.45	SD
	4.93	CV(%)
	91	Recovery (%)
	89.5	Mean Recovery (%)

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

Spike 150 ppb		
Millet (n=8)	125	Average
	4.26	SD
	3.40	CV(%)
	83.33	Recovery (%)
Spike 300 ppb		
Millet (n=8)	261	Average
	11.23	SD
	4.30	CV(%)
	87	Recovery (%)
	85.17	Mean Recovery (%)

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

Spike 150 ppb		
Oat (n=8)	165	Average
	5.81	SD
	3.52	CV(%)
	110	Recovery (%)
Spike 300 ppb		
Oat (n=8)	326	Average
	18.59	SD
	5.70	CV(%)
	108.67	Recovery (%)
	109.34	Mean Recovery (%)

Table 16. Recovery of White rice sample at two different levels.

Spike 150 ppb		
White rice (n=8)	166	Average
	6.12	SD
	3.69	CV(%)
	110.67	Recovery (%)
Spike 300 ppb		
White rice (n=8)	319	Average
	9.94	SD
	3.12	CV(%)
	106.33	Recovery (%)
	108.5	Mean Recovery (%)

Table 17. Mean Recovery (%) of all ground matrices.

Matrix	Mean Recovery (%)
Barley	94.34
Brown rice	96.47
Corn	97.17
Corn flour	101.99
Millet	107.67
Oats	109.17
Soya	89.5
Wheat	85.17
Wheat flour	109.34
White rice	108.5
MEAN	99.93

2.3 Reproducibility

The coefficients of variation of reproducibility of the concentrations (ppb) (Table 18) of three different samples ran eight times in 8 different tests are reported:

Table 18. Coefficients of Variation of the concentration (ppb) of three different samples ran in eight different tests.

Sample (n=8)	Concentration (ppb)	
	MEAN	CV(%)
FAPAS MAIZE T04510QC	115	4.15

2.4 Performance Evaluation

i. Reference Materials

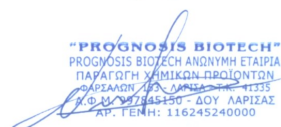
Table 19. Recovery on samples prepared by FAPAS.

Reference material	Lot number	Certified value (µg/kg)	Uncertainty (µg/kg)	Result (µg/kg)	Recovery (%)
FAPAS MAIZE T04510QC	111	111	49	115	103,6

3. References

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