

MultiPlexed Quantitative Mycotoxin Analysis

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Background

Founded in 1982 in Co Antrim. Initial healthcare focus.

Currently employing over 1,400 people, including 400 scientists and engineers.

Randox manufacture 3.5 billion assays per year.

Every year over 370 million patients are diagnosed using our technology.

5th largest manufacturer of clinical chemistry reagents in the world. 10% of all cholesterols.

3rd in world in Quality Control and calibrators. World's largest PT scheme.

100,000 laboratories using Randox products.

R&D focus – up to 16% of turnover.



Some of Our Clients

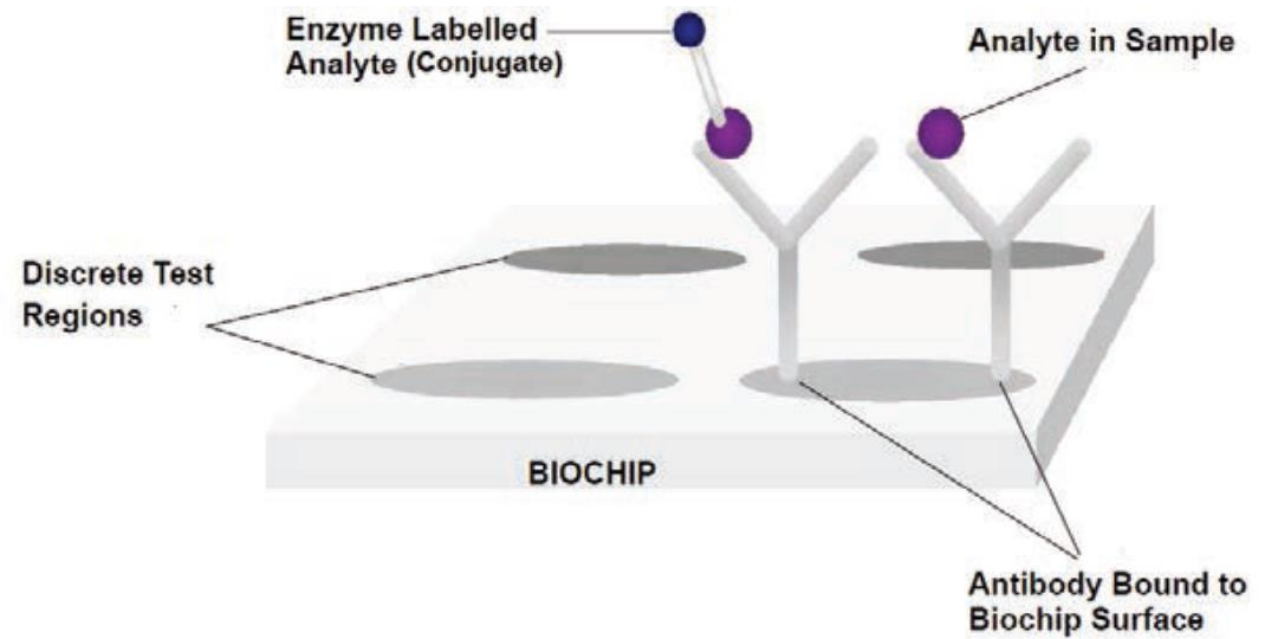


Harvard



Biochip Array Technology

- Biochip is a ceramic platform on which antibodies specific for various parameters are spotted on specific locations



Biochip Array Technology

Is it MS/MS????



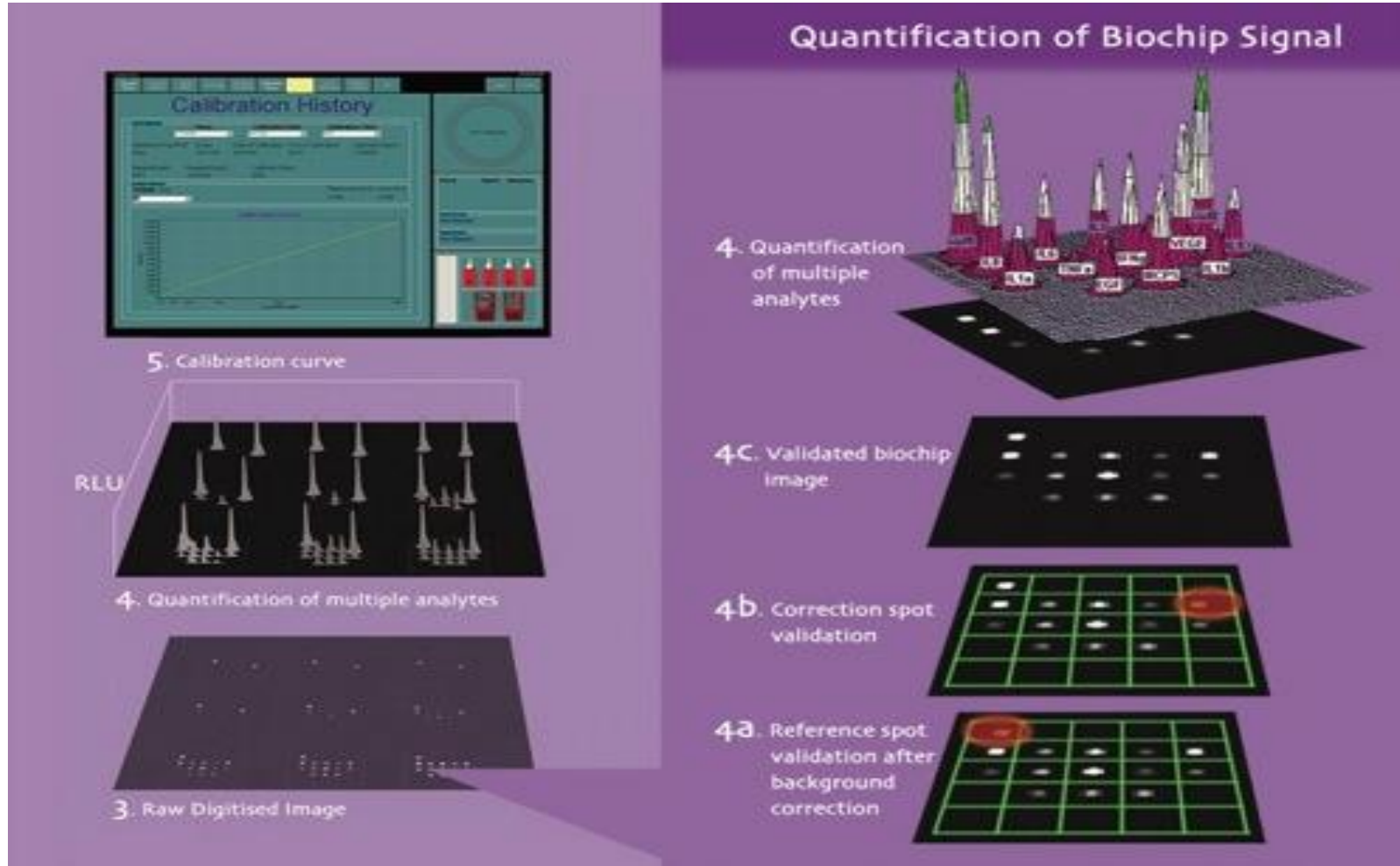
(1). Reference Spot, (2). Correction Spot, (10). Correction Spot (8). Aflatoxin B₁ (9). Zearalenone
(3). Fumonisin (4). Ochratoxin A (5). Aflatoxin G (6). Deoxynivalenol (7). T-2/HT-2 Toxin



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FOOD DIAGNOSTICS

Outstanding in its field

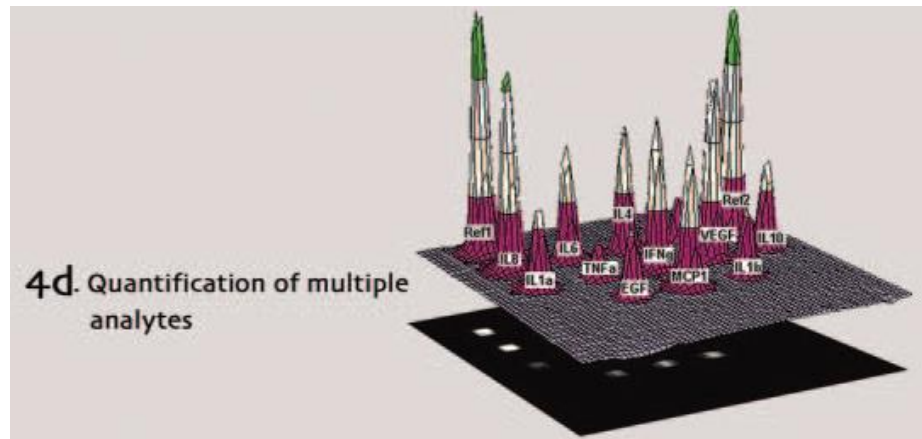
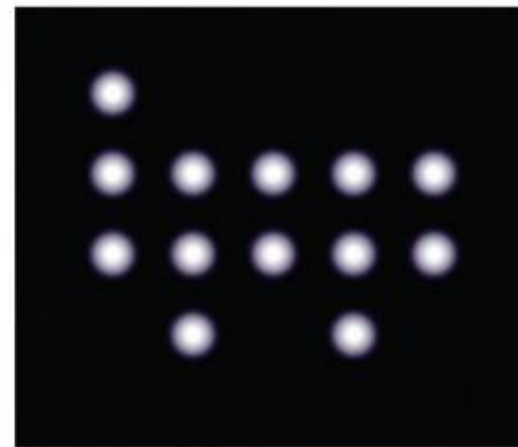
Assay Response



Biochip Array Technology

- (1). Reference Spot,
- (2). Correction Spot,
- (10). Correction Spot
- (8). Aflatoxin B₁
- (9). Zearalenone
- (3). Fumonisin
- (4). Ochratoxin A
- (5). Aflatoxin G
- (6). Deoxynivalenol
- (7). T-2/HT-2 Toxin
- (11). Diacetoxyscirpenol
- (12). Ergot Alkaloids

Is it MS/MS????



Mycotoxin Arrays (Myco 10, Myco 7, Myco 5, Myco 3 & MycoFlex)

Aflatoxin B₁/G₁ (0.5 ppb), FB₁/B₂/B₃ (10 ppb), OTA (0.25 ppb), T-2/HT-2 Toxin (5 ppb), ZEA (2.5 ppb), DON (100 ppb), DAS (25 ppb), Paxilline (50 ppb) and Ergot Alkaloids (10 ppb)

Aflatoxin B₁/B₂

Aflatoxin G₁/G₂

Ochratoxin A

Fumonisin

Deoxynivalenol

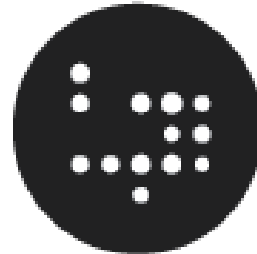
Disacetoxyisipenol

T2 toxin

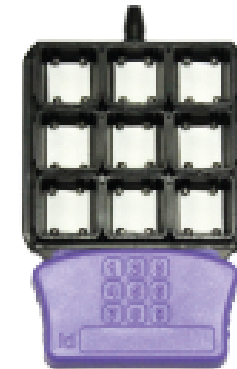
Ergot Alkaloids

Zearalenone

Paxilline



Your selection is spotted



Your MycoFlex Biochip

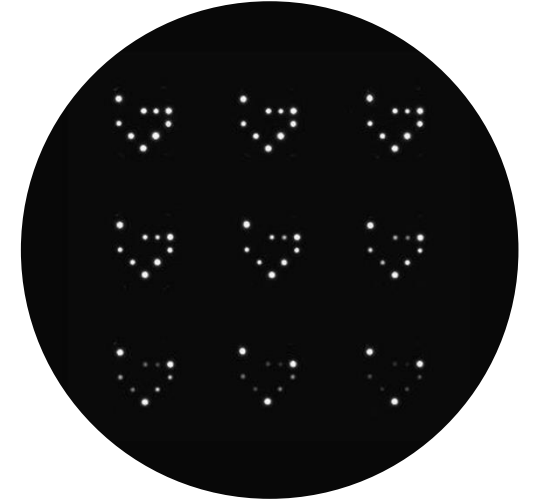


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Outstanding in its field

Biochip Array Technology - Endpoint measurement

- Mycotoxin-specific Antibodies spotted on the biochip
- Mycotoxin-HRP Conjugate
- Luminol and Peroxide generate light reaction
- Light is generated by Chemiluminescence
- Charge Coupled Device Camera captures images
- Investigator quantifies images using Relative Light Units (RLU) given off by each DTR
- Correction and Reference Spots used for image validation



Sample Preparation



1

5g of sample



2

Add 25ml of solvent
(Acetonitrile:Methanol:Water; 50:40:10, v/v/v)
Methanol/Water (80/20, v/v)
Ethanol/Water (80/20, v/v)



3

Vortexed and shaken for 10 minutes



4

Centrifuged for 10 minutes at 3000 rpm



5

Diluted 50µl sample in 150µl wash buffer



Assay Procedure

Add 100µL of working strength conjugate to each biochip



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1

Pipette 150 µl of Wash Buffer into each well



2

50 µl of Standards and samples were added to the Biochips



3

Incubation at 37°C for 30mins in a Thermoshaker



4

HRP-Conjugate was added & incubated for 1 hr at 37°C



5

Biochips were washed with Wash Buffer



6

Luminol/Peroxide (substrate) was added & incubated for 2 min in the dark

Outstanding in its field

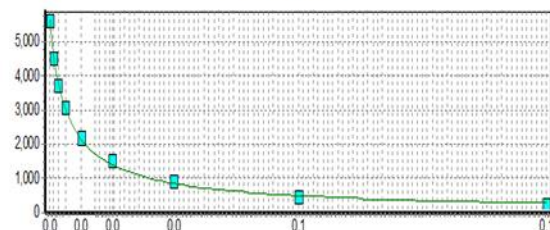
Results - Calibration

Analyte: AFLATOXINB1 Target Curve Fit(r): 0.95 Curve Fit(r): 0.999

Status: Pass

Calibration Range: 0-0.114 ppb

AFLATOXINB1 CURVE

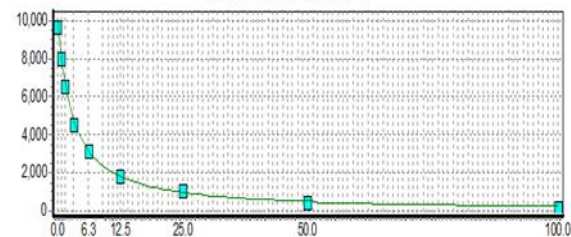


Analyte: DEOXYNIVALENOL Target Curve Fit(r): 0.95 Curve Fit(r): 0.999

Status: Pass

Calibration Range: 0-100 ppb

DEOXYNIVALENOL CURVE

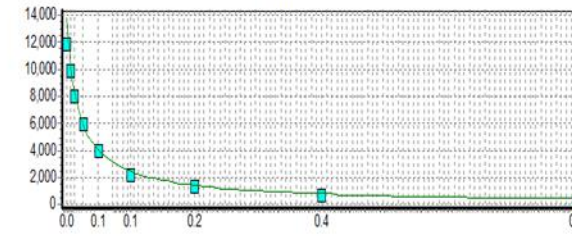


Analyte: OCHRATOXINA Target Curve Fit(r): 0.95 Curve Fit(r): 1.000

Status: Pass

Calibration Range: 0-0.8 ppb

OCHRATOXINA CURVE

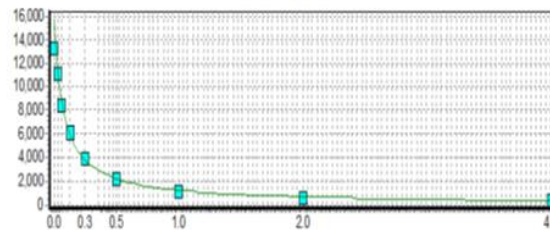


Analyte: TZTOWN Target Curve Fit(r): 0.95 Curve Fit(r): 1.000

Status: Pass

Calibration Range: 0-4 ppb

TZTOWN CURVE

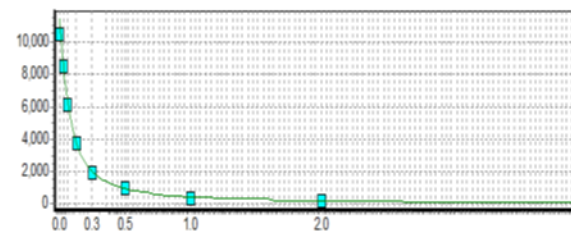


Analyte: FUMONISINS Target Curve Fit(r): 0.95 Curve Fit(r): 1.000

Status: Pass

Calibration Range: 0-4 ppb

FUMONISINS CURVE

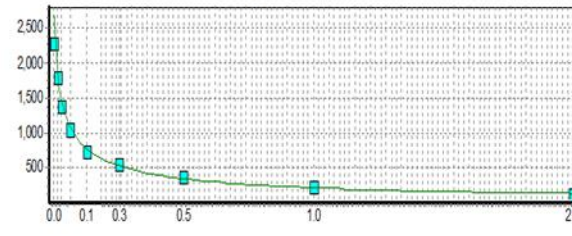


Analyte: ZEARELENONE Target Curve Fit(r): 0.95 Curve Fit(r): 0.998

Status: Pass

Calibration Range: 0-2 ppb

ZEARELENONE CURVE



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Outstanding in its field

Final Results Print-Off

Example: Myco 10

Absolute Concentrations

Array Details: Myco 10
Sample(s) Run By: g.
Batch Details: 7889
Date of Sample(s): 02/12/2016 14:17:23
Date of Calibration: 02/12/2016 02:11 PM
Calibrator Lot No: 7889

		ppb	ppb	ppb	ppb	ppb	ppb
SampleCode		(DF) PAX	FUM	OTA	AFG1	ERG	DAS
✓ 1	20	9.012	0.813	0.0608	0.1830	18.485	3.168

		ppb	ppb	ppb	ppb
SampleCode		(DF) DON	T2	AFB1	ZEA
✓ 1	20	72.694	5.819	0.0297	0.987



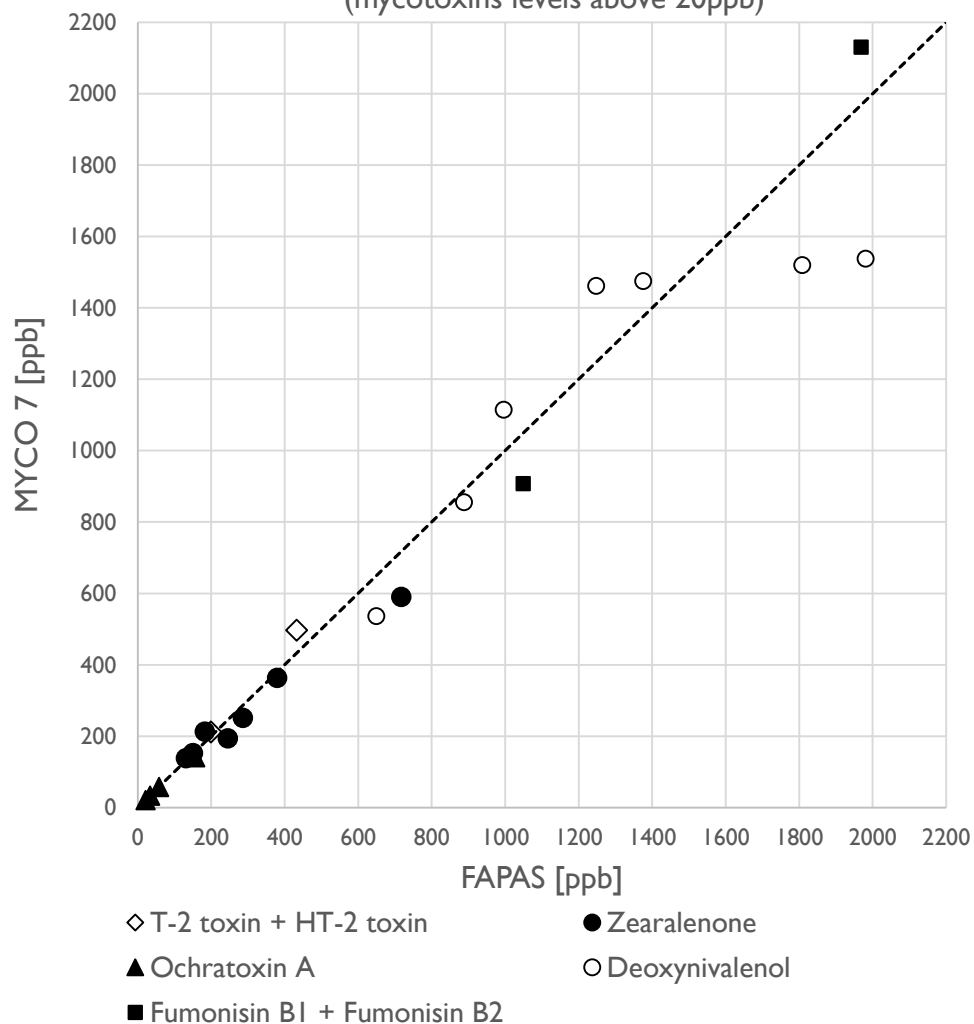
Results



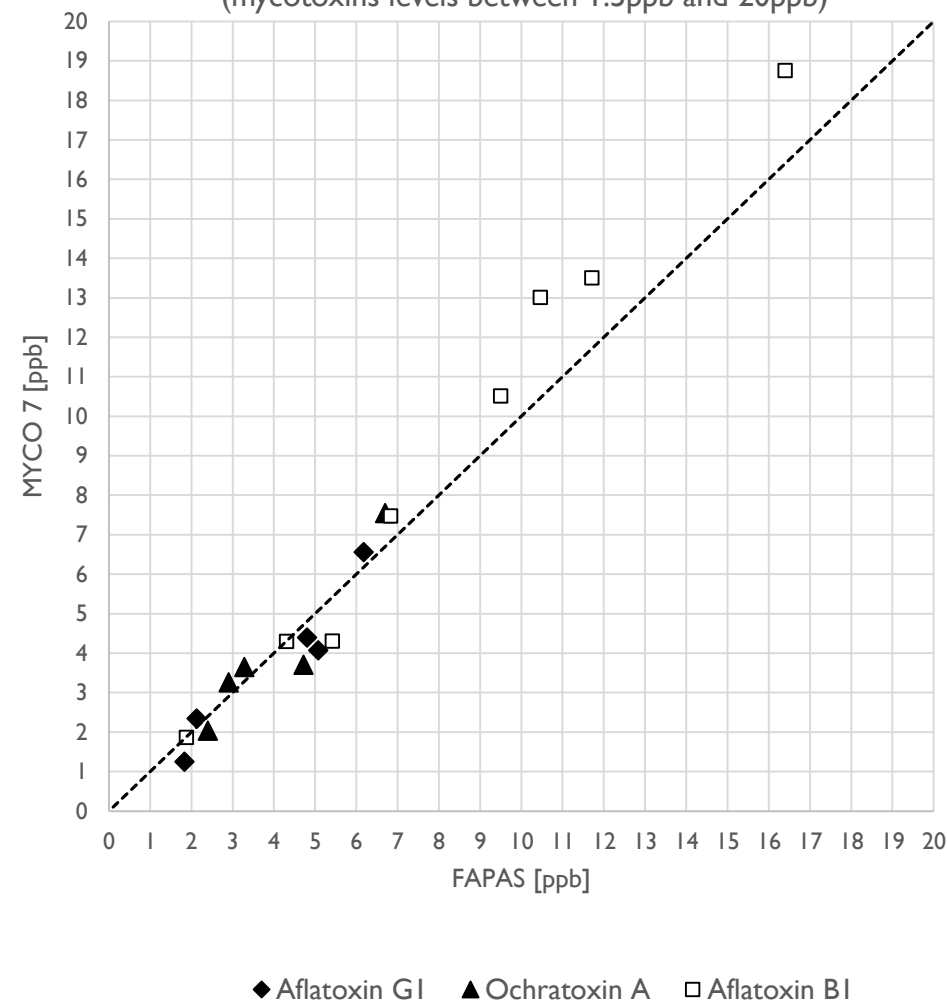
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Validation based on European Commission Decision 2002/657/EC for Myco 7 array: **Trueness**

Comparison of 22 mycotoxins concentrations detected by
Myco 7 assay with FAPAS assigned value
(mycotoxins levels above 20ppb)



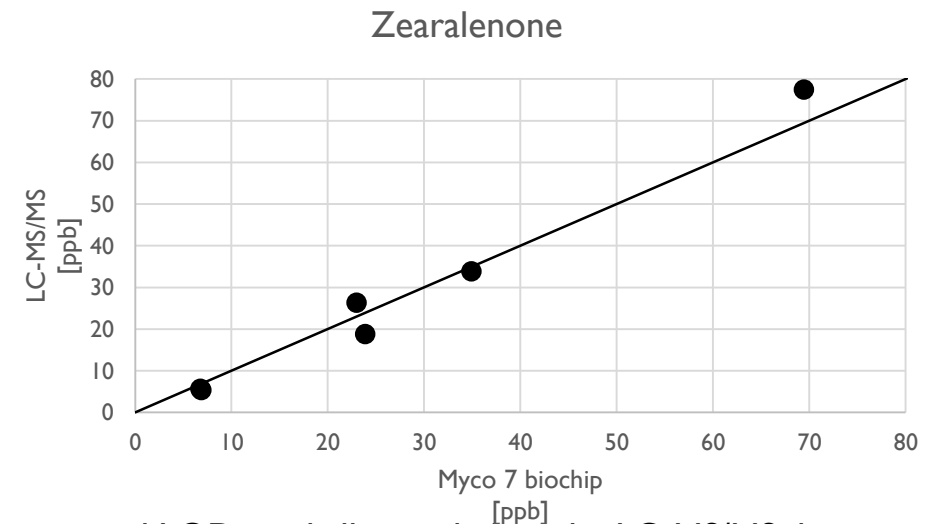
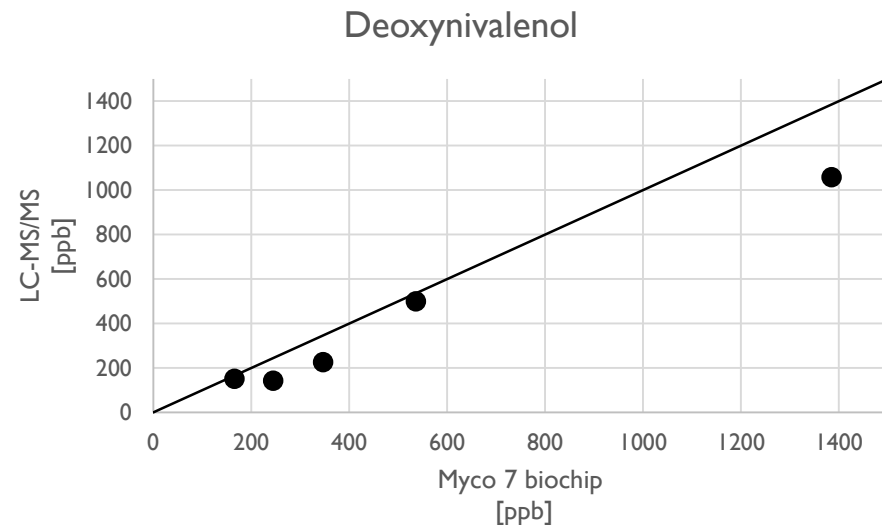
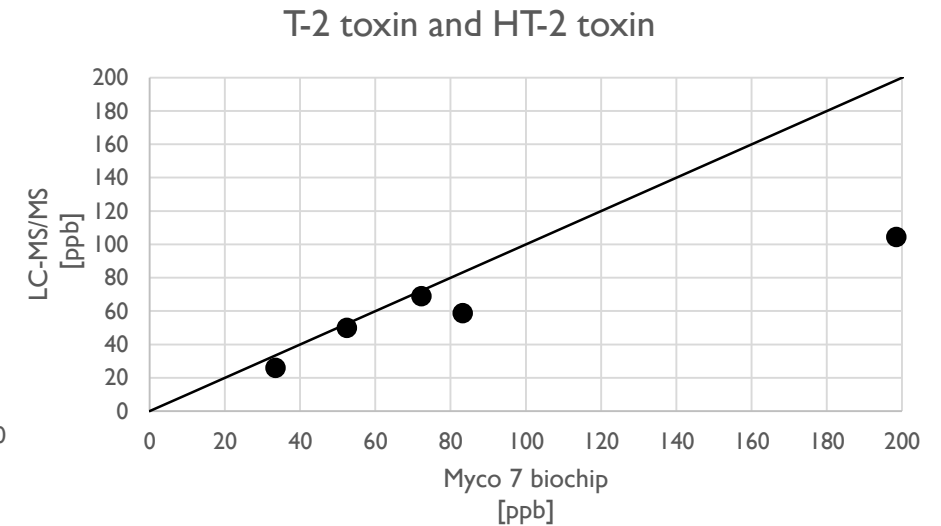
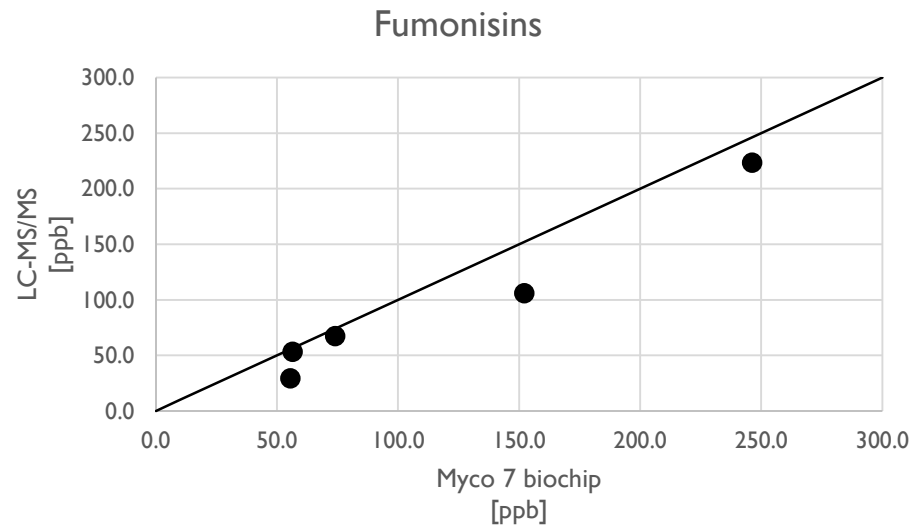
Comparison of 18 mycotoxins concentrations detected
by Myco 7 assay with FAPAS assigned value
(mycotoxins levels between 1.5ppb and 20ppb)



Validation based on European Commission Decision 2002/657/EC for Myco 7 array

Myco 7 Array

Eight authentic feed samples study confirmed by LC-MS/MS method



Aflatoxins and ochratoxin A levels detected by Myco 7 biochip were around LODs and all were below the LC-MS/MS detection limits as well as 2 more samples for zearalenone assays and 3 more samples for fumonisins, T-2 toxin and deoxynivalenol assays.



FAPAS Proficiency Results

FAPAS PROFICIENCY				
ANALYTE	LOD (µg/kg)	RESULT (µg/kg)	ASSIGNED VALUE (µg/kg)	Z-SCORE
OTA	0.25	3.71	4.72	-1.00
ZON	1.50	192.87	245	-1.10
DON	100	1537.94	1981	-1.60
AFB ₁	0.25	5.90	9.45	-1.70



Results

Maize



	Fumonisin	OTA	AFG	AFB ₁	DON	ZEA	T-2/HT-2	Ergot	DAS
1	<10	<0.25	<0.5	<0.25	284.13	<2.5	<5	<10	<25
2	10.96	<0.25	<0.5	<0.25	426.44	83.38	<5	<10	<25
3	<10	<0.25	<0.5	<0.25	118.19	<2.5	<5	<10	<25
4	253.4	<0.25	<0.5	<0.25	233.66	<2.5	18.65	<10	<25
5	163.34	<0.25	<0.5	<0.25	150.04	9.44	36.86	<10	<25
6	998.25	3.99	<0.5	0.40	544.93	10.62	194.72	<10	<25
7	<10	<0.25	<0.5	<0.25	338.83	23.89	106.26	<10	<25
8	2600.77	<0.25	<0.5	1.49	<100	<2.5	<5	<10	<25
9	3329.84	<0.25	<0.5	0.45	<100	<2.5	<5	<10	<25
10	3862.45	<0.25	<0.5	<0.25	<100	<2.5	<5	<10	<25
11	<10	<0.25	<0.5	<0.25	2531.46	336.8	7.41	<10	<25
12	24.12	<0.25	<0.5	0.63	724.28	266.35	5.99	15.73	<25
13	<10	<0.25	<0.5	0.36	309.12	33.75	77.18	<10	<25
14	308.48	<0.25	<0.5	<0.25	1884.81	307.41	7.00	<10	<25
15	42.76	<0.25	<0.5	<0.25	3449.45	171.36	6.93	<10	<25

Results

Wheat



	Fumonisin	OTA	AFG	AFB ₁	DON	ZEA	T-2/HT-2	Ergot	DAS
1	<10	<0.25	<0.5	<0.25	165.13	17.52	21.07	<10	<25
2	<10	<0.25	<0.5	<0.25	976.51	<2.5	7.55	<10	<25
3	<10	0.90	<0.5	<0.25	293.35	20.56	11.96	339.91	<25
4	<10	<0.25	<0.5	<0.25	181.30	17.45	<5	<10	<25
5	<10	<0.25	<0.5	<0.25	288.87	8.50	<5	<10	<25
6	<10	<0.25	<0.5	<0.25	683.49	<2.5	7.27	<10	<25
7	<10	<0.25	<0.5	<0.25	217.03	7.22	12.34	<10	<25
8	<10	0.30	<0.5	<0.25	471.74	15.48	<5	83.12	<25
9	<10	<0.25	<0.5	<0.25	2536.94	3.81	5.78	<10	<25
10	<10	0.64	<0.5	<0.25	277.83	<2.5	26.48	<10	<25
11	<10	<0.25	<0.5	<0.25	180.80	5.58	7.69	<10	<25
12	<10	<0.25	<0.5	<0.25	717.93	5.83	6.87	13.36	<25
13	<10	<0.25	<0.5	<0.25	910.21	10.19	<5	<10	<25
14	<10	<0.25	<0.5	<0.25	192.73	<2.5	<5	18.46	<25
15	<10	<0.25	<0.5	<0.25	394.19	10.25	147.96	<10	<25
16	<10	<0.25	<0.5	<0.25	359.54	61.96	54.41	<10	<25
17	<10	<0.25	<0.5	<0.25	215.20	7.28	16.09	<10	<25
18	<10	<0.25	<0.5	<0.25	100.66	367.26	14.43	<10	<25

Results

Feed

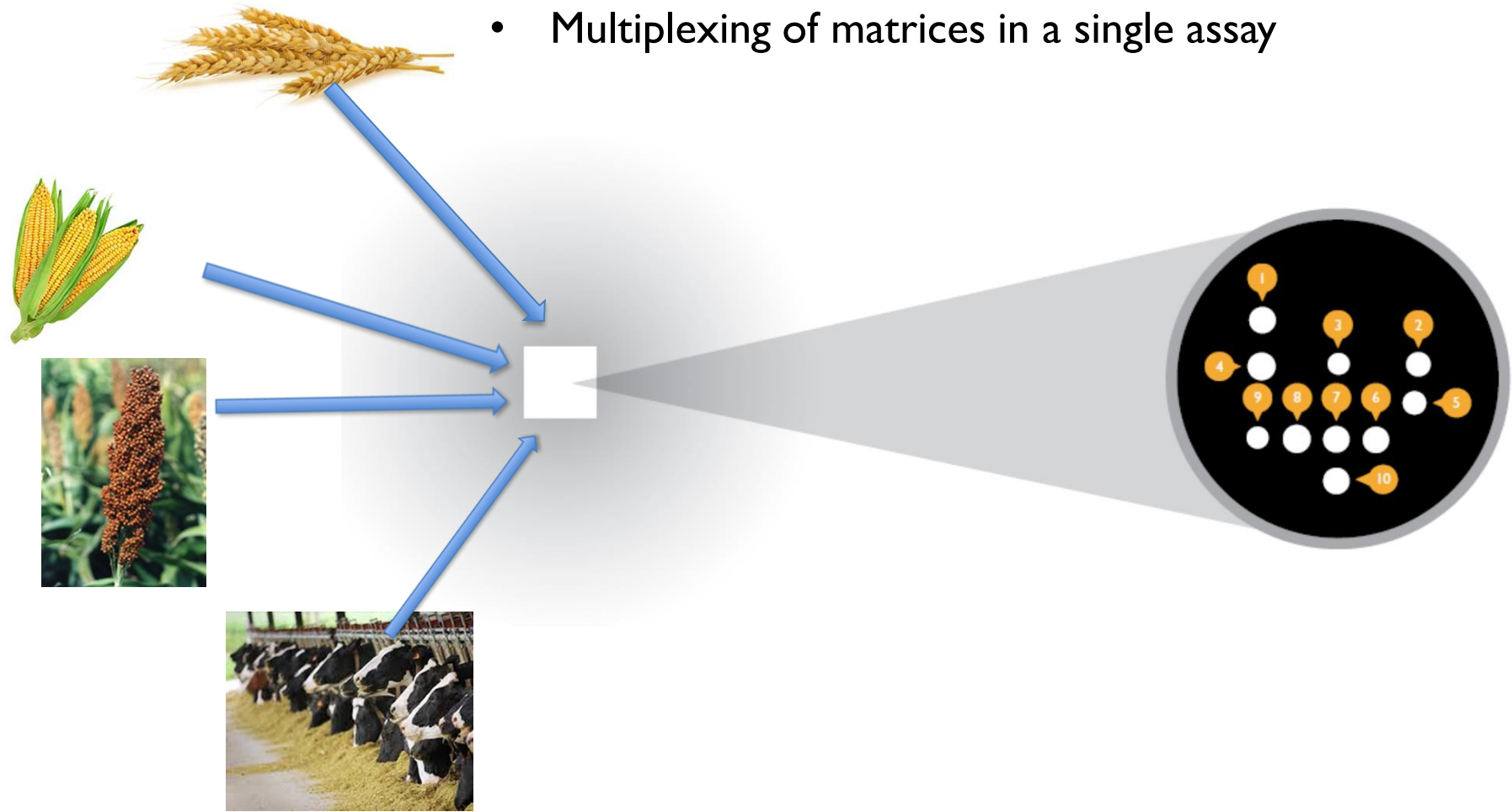


	Fumonisin	OTA	AFG	AFB ₁	DON	ZEA	T-2/HT-2	Ergot	DAS
1	26.49	0.87	0.58	0.32	248.70	10.01	10.18	133.05	<25
2	22.80	0.82	0.59	0.34	257.94	9.48	9.83	269.41	<25
3	15.45	0.82	<0.5	<0.25	186.59	8.07	7.82	<10	<25
4	71.25	0.86	<0.5	<0.25	240.32	6.28	8.38	<10	<25
5	1644.37	0.71	<0.5	0.51	163.20	33.63	<5	12.47	<25
6	2217.8	<0.25	<0.5	<0.25	431.31	246.11*	<5	11.6	<25
7	1329.15	<0.25	<0.5	<0.25	170.18	56.82	<5	11.39	<25
8	698.22	<0.25	<0.5	<0.25	<100	19.80	<5	55.18	<25
9	2282.10	0.93	1.12	3.89	241.35	13.73	8.75	19.78	<25
10	1680.00	0.26	<0.5	<0.25	209.97	68.37	<5	15.13	<25
11	19.79	0.72	<0.5	<0.25	297.17	105.29	11.26	236.04	<25
12	<10	<0.25	<0.5	<0.25	238.39	81.73	<5	118.16	<25
13	14.60	1.01	<0.5	<0.25	372.25	71.23	26.51	135.91	<25
14	18.79	1.15	0.72	0.34	528.94	82.67	24.71	121.56	<25
15	17.19	0.79	<0.5	<0.25	273.09	78.53	17.30	344.03	<25
16	10.54	0.72	<0.5	<0.25	504.44	>200	5.94	43.54	<25
17	17.91	0.27	<0.5	<0.25	263.91	21.93	<5	24.83	<25



Conclusion

- Multiplexing of mycotoxin detection & quantification
- Multiplexing of matrices in a single assay

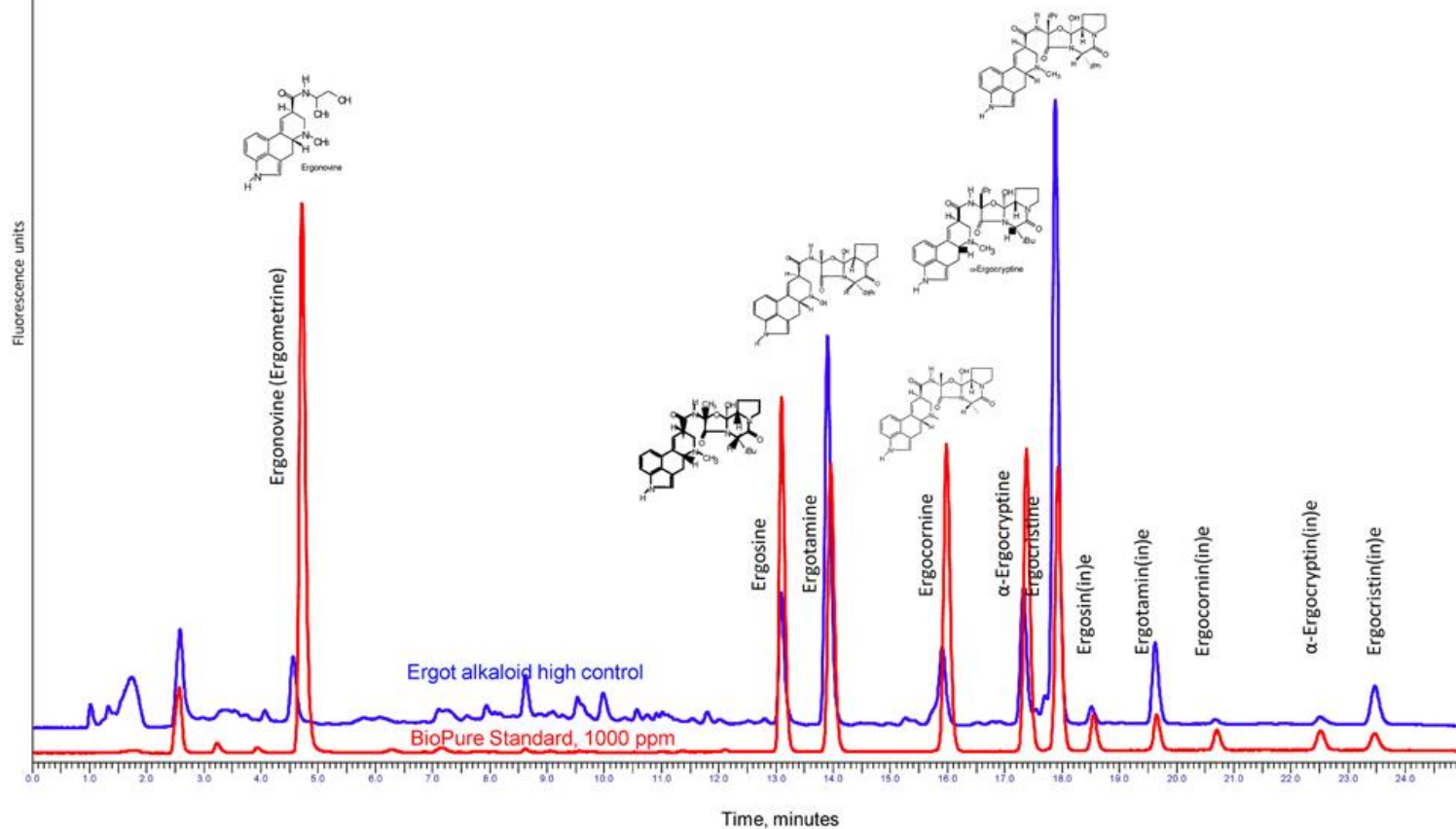


Ergot Alkaloids



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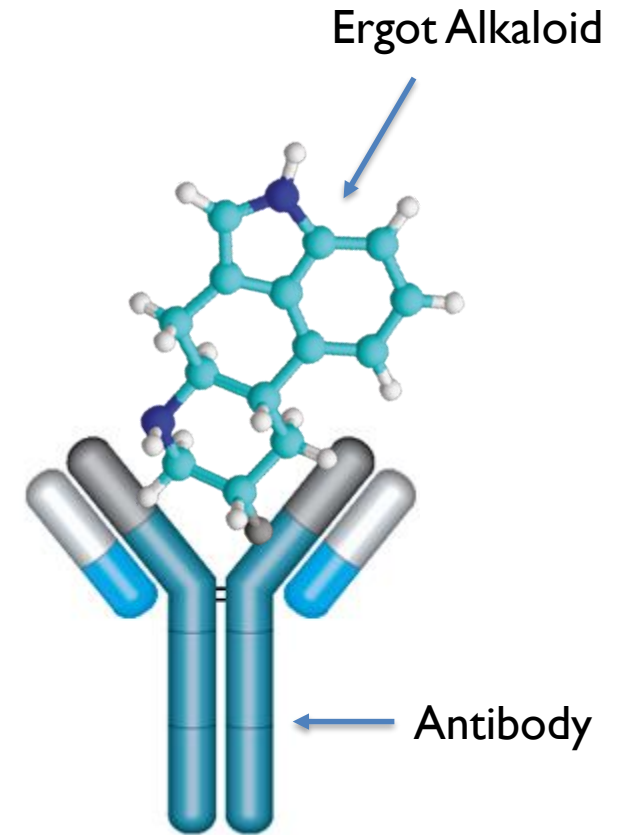
Ergot Alkaloids
YMC Triart C18 150X4.6mm, 3µm
Full scale = 700mv
Ammonium carbonate/acetonitrile gradient (80/20)
BioPure Standard, 1000 ppm
Ergot alkaloid high control



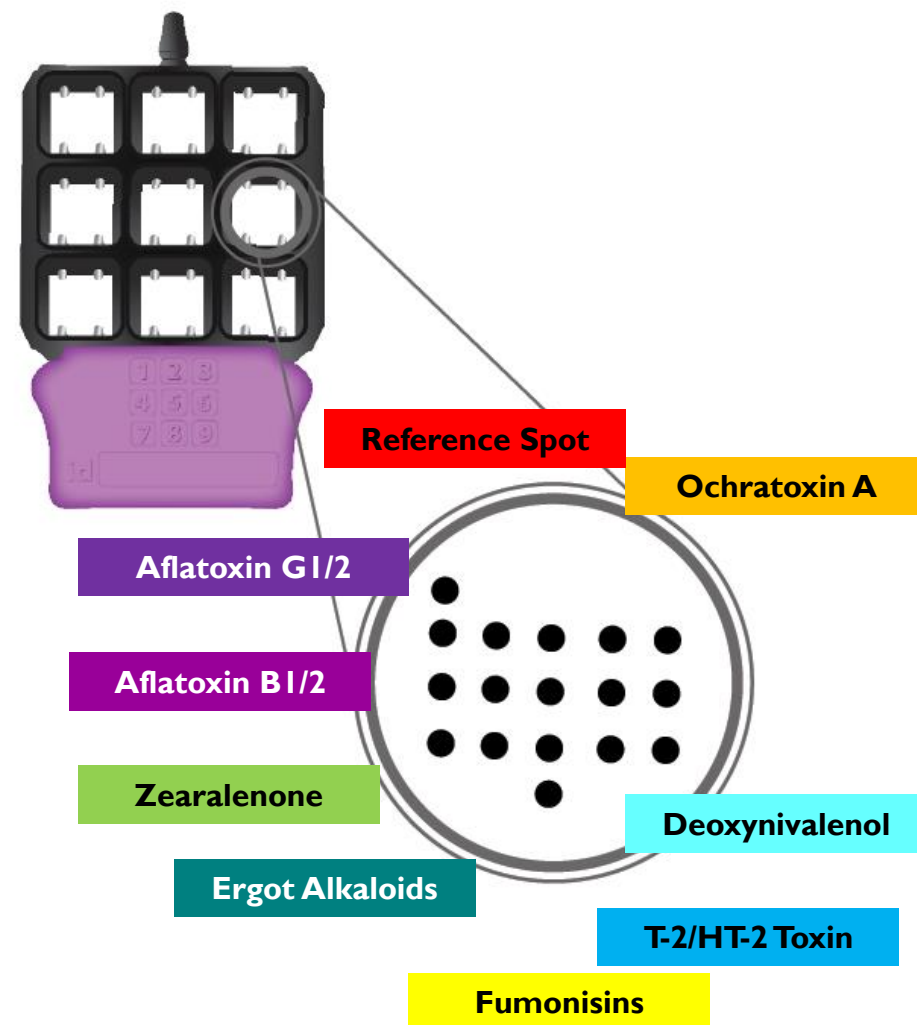
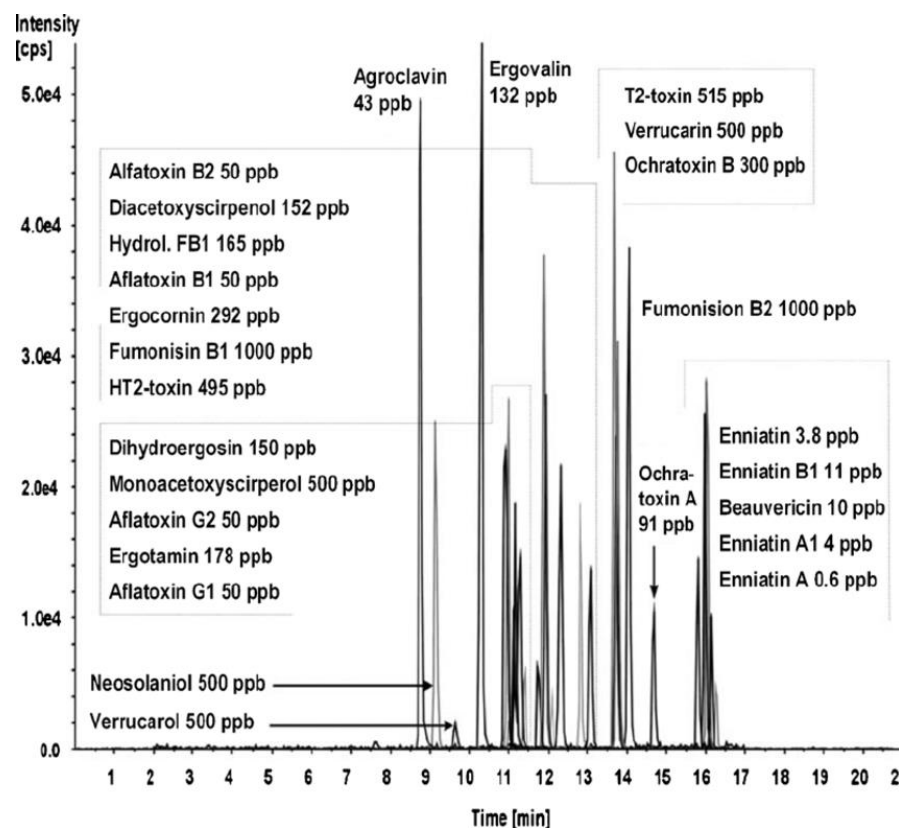
Outstanding in its field

Ergot Alkaloids – Quantitative Testing

Ergot Alkaloid	Cross-Reactivity
Ergometrine	475
Ergometrinine	350
Ergotamine	100
Ergotamine	80
Ergosine	138
Ergosinine	121
Ergocristine	85
Ergocristinine	43
Ergocryptine	87
Ergocryptinine	70
Ergocomine	180
Ergocominine	151



Comparisons



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Outstanding in its field

Comparisons

- **Chromatographic – Quantitative Methods**
 - LC-MS/MS runs multi-mycotoxin Biochip
 - sensitivity, repeatability and accuracy is comparable
 - sample preparation for chromatography is more expensive – involving immunoaffinity columns.

