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New Approaches to Mycotoxin Control Programs: Simultaneous Rapid Detection of Several Mycotoxins

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VICAM, A Waters Business

- Mycotoxins frequently contaminate animal feeds.

- More than 70% of feeds analyzed globally are contaminated with mycotoxins. 38% with more than one mycotoxin.

- The monitoring of mycotoxins in feed ingredients can be used as a valuable tool for a quality control program for mycotoxins and protection of animal health.

OVERVIEW

1. Traditionally, mycotoxins are determined and regulated at the level of feed ingredients, facing difficulties such as the lack of homogeneity in the contamination of the lot and the transfer of the problem into feed. At this level, rapid methods for simultaneous multiple analyzes in the field are an invaluable tool.

2. Monitoring of finished feeds confirms toxin has not been transferred into the feed.

3. Biomonitoring of the exposure of animals to mycotoxins using the determination of risk biomarkers is a complementary tool in the control programs of mycotoxins.

Rapid and Simple monitoring of feed ingredients

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With **one** short extraction step and **minimal** hands-on manipulation you can run **multiple** tests for **up to five** mycotoxins at once!

Rapid and Simple

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Myco 5in1 - Single Extraction

Weigh 5g of finely-ground sample into an extraction tube, then add 25 mL of AQUA Premix

→

Extract the sample by vortexing at high speed for 2 minutes

→

Filter the extract into a clean tube

Afla-V Ochra-V Fumo-V Zearala-V DON-V

Place Don-V strip into mini-incubator at 40°C and close the lid for 2-10 minutes.

Pipette 100 µL of extract into the sample well at 1 drop/second with the strip still inside the incubator

Pipette 100 µL of extract into the sample well at 1 drop/second

During the 5 minutes calibrate the Vertu by scanning the appropriate barcode

Incubate the strip for 5 minutes at 40°C

Place the strip on a flat surface for 5 minutes

Place the strip inside the Vertu and press the center key to read

Mycotoxin Level Low

Mycotoxin Level High

VICAM 5-in-1 Lateral Flow for Mycotoxin Diagnostics

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Mycotoxin	LOD	Range
Aflatoxin	2 ppb	0-40 ppb, (*High range method to 100 ppb)*
Deoxynivalenol	0.25 ppm	0-4 ppm (1st strip), to 16 ppm (2nd strip)
Fumonsin	0.2 ppm	0-20 ppm (1st strip), to 80 ppm (2nd strip)
Ochratoxin	2 ppb	0-30 ppb
Zearalenone	0.1 ppm (100 ppb)	0-5 ppm

- Rapid: from sample to results in less than 10 minutes
- Easy to use- anyone can be trained to run the test
- Inexpensive
- Excellent for screening at the point of collection
- Meets worldwide regulatory limits.
- Non toxic - Water based extraction solution

USDA-FGIS Certifications

- Afla-V AQUA
- Fumo-V AQUA
- DON-V

Real Results: Naturally Contaminated Corn

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	HPLC Results	Vertu Results					
		R1	R2	R3	Mean	SDr	RSDr%
Afla-V (ppb)	**9.45**	10	9.39	9.39	**9.46**	0.12	1.2
	18.3	17.1	18.9	18.9	**18.30**	1.0	5.6
DON-V (ppm)	**1.8**	1.62	1.82	1.80	**1.75**	0.11	6.28
	4.3	4.65	4.12	3.84	**4.20**	0.41	9.77
Fumo-V (ppm)	**0.50**	0.60	0.52	0.43	**0.52**	0.09	16.4
	4.8	4.08	4.56	4.52	**4.39**	0.27	6.0
Ochra-V (ppb)	**4.2**	5.48	4.46	5.81	**5.25**	0.70	13.4
	18.0	21.2	19.1	21.7	**20.6**	1.3	6.6
Zearala-V (ppm)	**0.42**	0.44	0.39	0.43	**0.42**	0.03	6.3
	1.36	1.42	1.51	1.45	**1.46**	0.05	3.1

- Mycotoxin testing in the field is best done by rapid quick in field assays such as lateral flow strip tests on incoming ingredients such as corn. Incoming grains can be rapidly screened before being made into a finished feed.

- Confirmation of mycotoxin results and testing of finished feeds and complex feed ingredients is best done by laboratory instrumental methods such as HPLC, UPLC or LC/MS/MS

Advantages of Immunoaffinity Chromatography clean up for HPLC, UPLC and LC/MS/MS for multiple mycotoxin analysis

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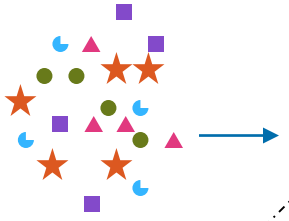
- Concentrates sample for lower limits of detection. Other methods make sample more dilute (dilute and shoot) or do not concentrate sample.
- Gives more toxin specific clean up for complex samples such as feeds.
- Reduces matrix materials so reduces background peaks and interferences.
- Reduces amount of cleaning needed for LC/MS/MS cone.
- Also reduces matrix enhancement or suppression on LC/MS/MS.

Inside an immunoaffinity column



1. Add sample extract to IAC

2. Wash away matrix

3. Add elution buffer

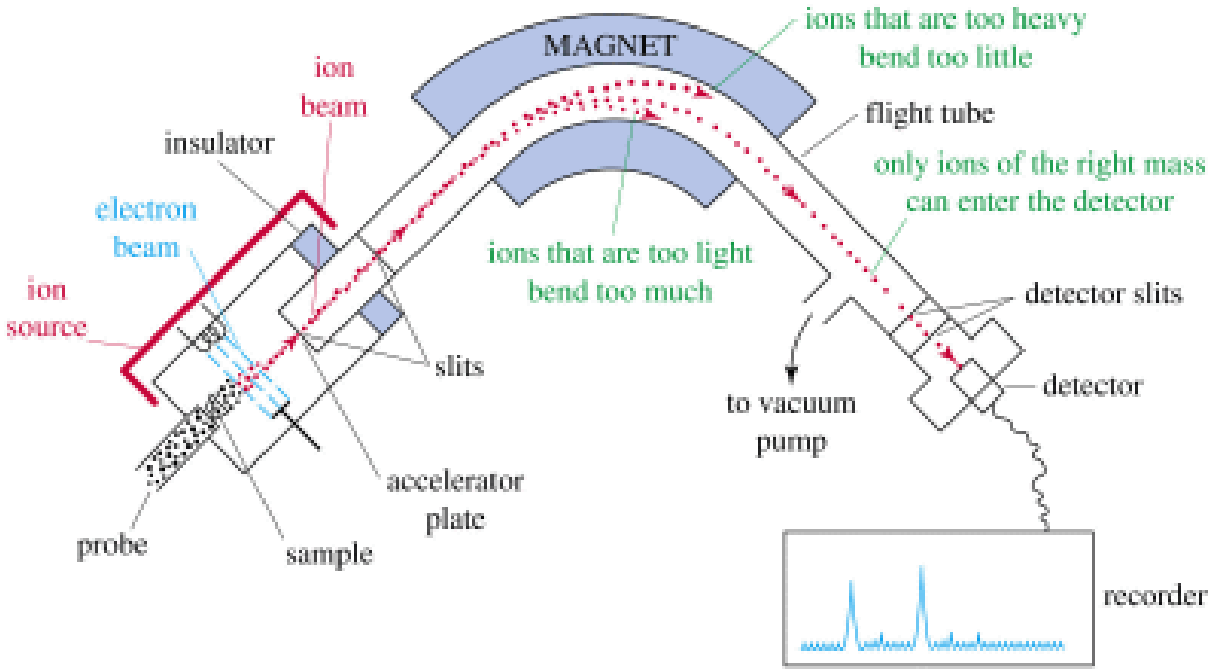
4. Analyze eluate by method of choice

Analyte

Non-retained compounds

Monoclonal Antibody

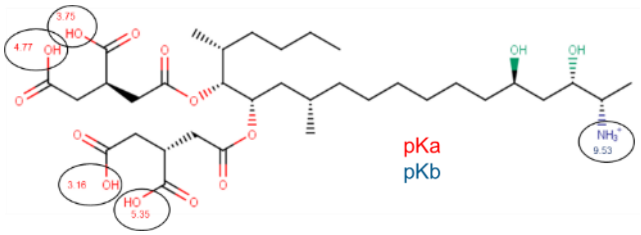
LC-MS (Liquid chromatography- Mass spectrometry)

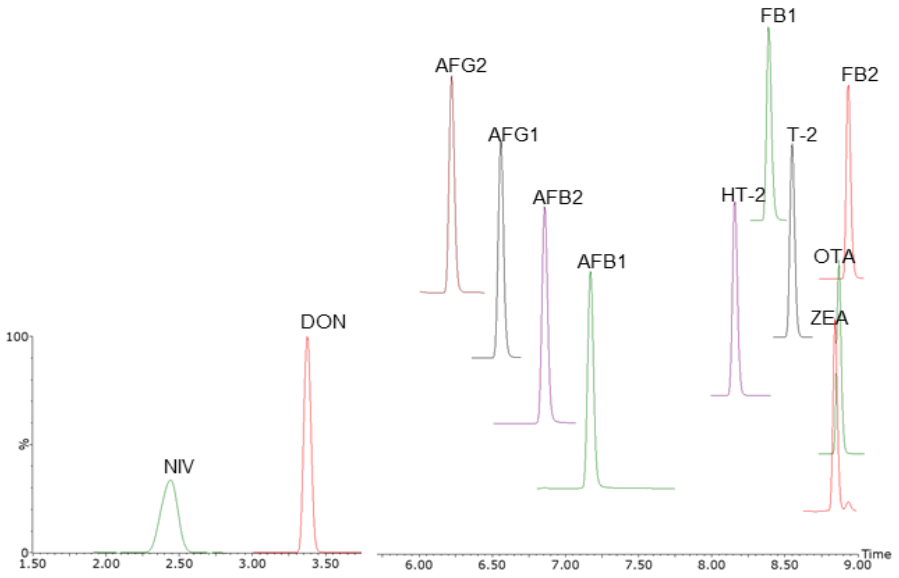


Advantage of MS- It can detect molecules that do not fluoresce or absorb

Waters UPLC conditions – multi-toxin LC-MS/MS

- Waters ACQUITY UPLC I-Class with FL injector operating in PLNO mode
- Needle: PEEK, 10µL
- Column: ACQUITY UPLC BEH-C_{18} column (2.1 × 100mm, 1.7µm)
- Mobile phase A: methanol +0.5% acetic acid +0.1% formic acid
- Mobile phase B: 1mM ammonium acetate in water +0.5% acetic acid +0.1% formic
- Weak needle wash: H_2O:MeCN 1:1 +0.125mM EDTA Strong needle wash: H_2O +20mM citric acid:MeOH:MeCN : IPA: acetone: DMSO 37:9:19:19:9:7
- Flow rate = 0.4 mL min^{-1}
- Column temperature: 40° C





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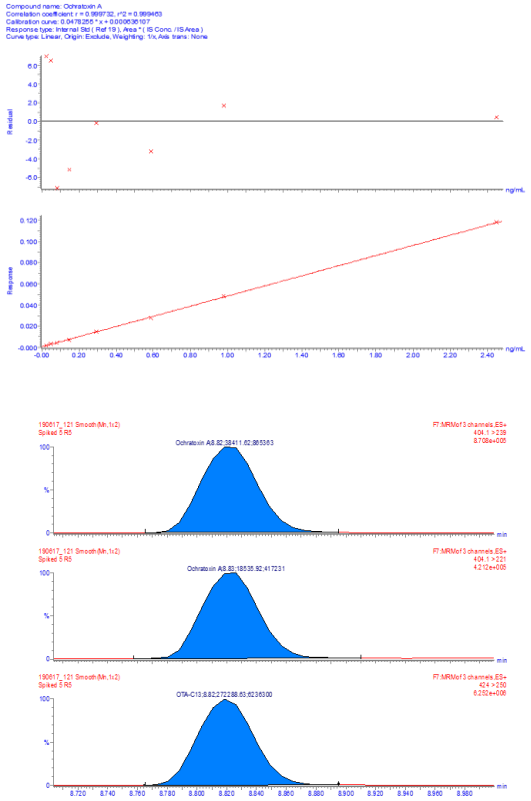
MS/MS conditions – multi-toxin method

Acquity UPLC® UPLC I-Class system

Xevo™ TQ-XS

Parameter	Setting
Ionisation mode	$ESI^{+/-}$
Capillary voltage kV	+0.5/-0.3
Source offset V	30
Source temperature ^{o}C	150
Desolvation temperature ^{o}C	500
Desolvation gas flow L/h	800
Cone gas flow L/h	150
Collision gas flow (mL/min) argon	0.15



2 transitions were monitored for each analyte & 1 transition was used for the respective ^{13}C-labelled isomer

Characteristics of Mass Spectrometry

- Instrument can be used for other analysis besides mycotoxins (such as pesticides)
- Mass Spec can detect molecules that do not fluoresce or absorb
- Good for multiple mycotoxin analysis
- Good for confirmation of mycotoxins

- Must have a laboratory environment
- Triple Quad (MS/MS) gives best results (lower limits of detection and confirmation)
- Best for well trained scientists
- Need to accurately measure and store mycotoxins in order to get accurate results
- Need to use matrix matched calibration standards or internal calibrators to adjust for matrix affects

Myco6in1+ References

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- 3. Park, J et al, Distribution Analysis of twelve Mycotoxins in Corn and Corn-Derived Products by LC-MS/MS to Evaluate the Carry-Over Ratio During Wet-Milling, Toxins, 2018; 10, 319.

- 4. Soleimany, F., Jinap, S., Rahmani, A., Khatib A.,Simultaneous detection of 12 mycotoxins in cereals using RP-HPLC-PDA-FLD with PHRED and a post-column derivatization system, Food Additives and Contaminants Part A Chem Anal Control Expo Risk Assess, 2011 Apr 28(4):494-501.

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- 6. Kim, D.H., Jang, H.S., Choi, G.H., Kim, H.J., Kim, H.J., Kim, H.L., Cho, H.J., Lee C., Occurrence of Mycotoxins in Korean Grains and Their Simultaneous Analysis, Korean J. Food Sci. Technol., 2013; Vol. 45, No 1, pp. 111-119.

- 7. Solfrizzo, M., Gambacorta, L., Lattanzio V.M.T., Powers, S.,Visconti, A., Simultaneous LC–MS/MS determination of aflatoxin M1, ochratoxin A, deoxynivalenol, de-epoxydeoxynivalenol, α and β-zearalenols and fumonisin B1 in urine as a multi-biomarker method to assess exposure to mycotoxins, Anal. Bioanal. Chem., 2011; 401:2831-2841.

- 8. Vaclavikova, M., MacMahon, S., Zhang, K., Begley, T. Application of single immunoaffinity clean-up for simultaneous determination of regulated mycotoxins in cereals and nuts, Talanta, 2013; 117: 345-351.

- 9. Kim, D.H., et al, Simultaneous Determination of Multi-Mycotoxins in Cereal Grains Collected from South Korea by LC/MS/MS, Toxins, 2017; 9, 106 : 1-13.

In addition to testing for mycotoxins in incoming ingredients and finished feed, exposure of dairy cattle to aflatoxin can be determined by testing for aflatoxin M_1 in their milk.

Monitoring exposure of dairy cows to aflatoxins

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- Aflatoxin M_1 is the metabolic byproduct created when a dairy animal ingests feed that contains aflatoxin B_1.
- Aflatoxin M_1 is a Group 2B carcinogen.
- Aflatoxin M_1 is regulated worldwide in raw and processed milk:

Contaminated Feed Rations…

Hydroxylation of AFB_1/AFB_2

Secreted as Less Toxic AFM_1/AFM_2 in Milk

AFM_1 Predominant Residue: Heat Stable, Survives Processing

AFM_1: Classified as a Group 2B Carcinogen by the IARC

Got [AFM_1 Free] Milk ?

World AFM_1 Thresholds

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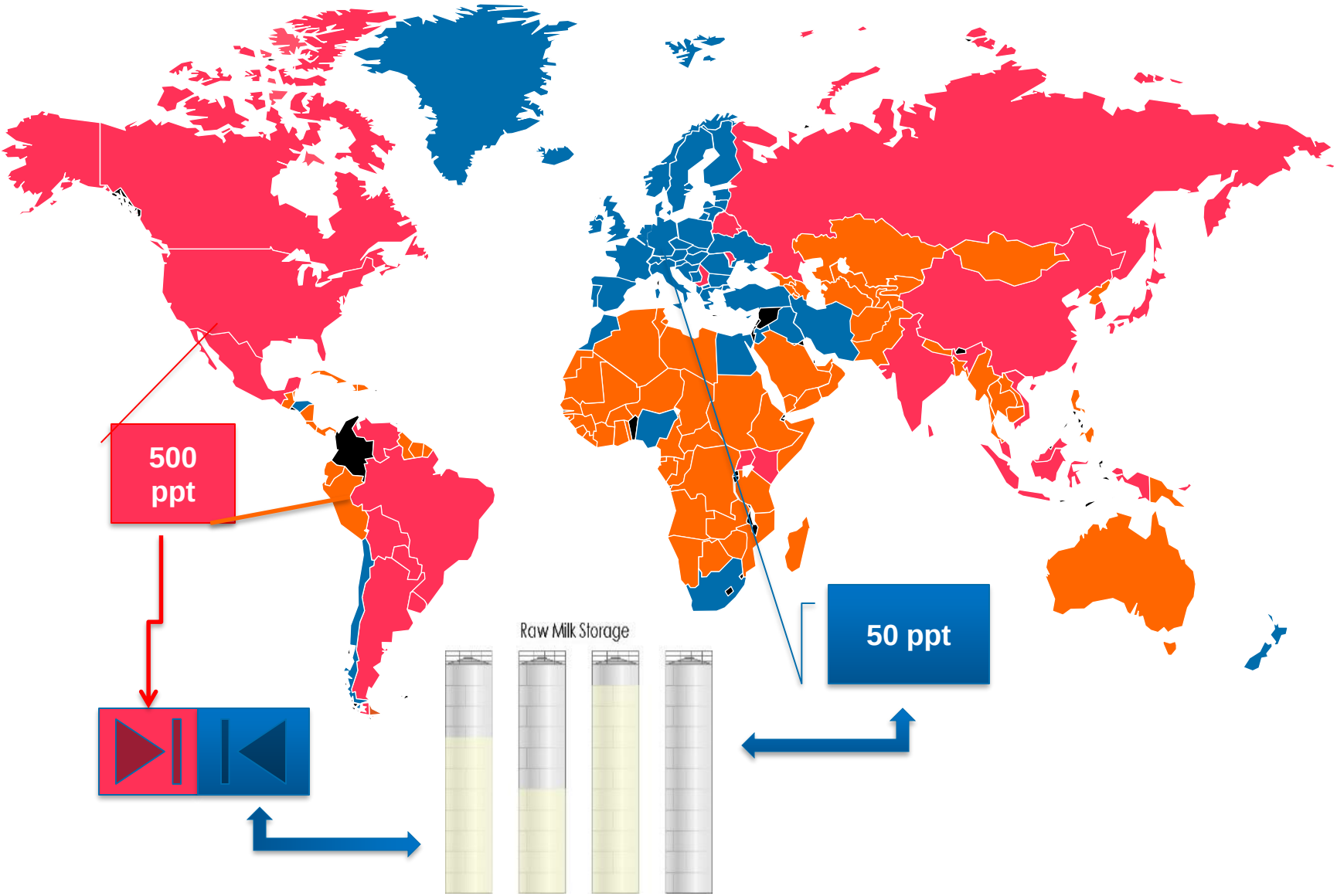

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BIOMARKER

Metabolite resulting from transformation
of a molecule to which an individual has been exposed and
which can be identified in body fluids or excreta
of the receiving organism.

Dairy Herd Improvement

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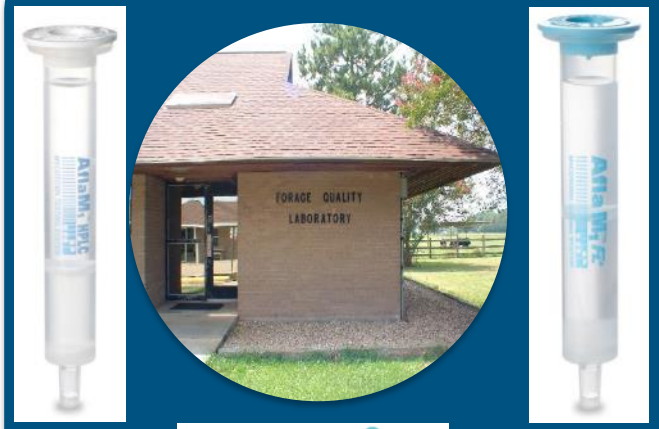
AFLA B_1/B_2 in Maize, Cottonseed, CGM, Distillers Grains, Hominy Feed, Peanut Meal, Canola, Silage, Copra, Citrus Pulp, Soybean Meal, Wheat Bran, Rice

Decreased Milk Production, Ataxia, Elevated SCCs', Feed Refusal & Reduced Reproductive Efficiency



Afla M_1-V™
QUANTITATIVE STRIP TESTS

Opportunity for Aflatoxin M_1

AFB_1 → AFM_1: 1 to 6% Carry-Over to Milk, 12 - 24 hours from first ingestion

Aflatoxin M_1 as a Biomarker for aflatoxin B_1 exposure

- Aflatoxin M_1 appears in milk at the first milking (1hr) after cows are fed aflatoxin B_1 contaminated feed
- After feeding aflatoxin B_1 ceases, aflatoxin M_1 decreases in the milk for up to 7 days
- Testing for aflatoxin M_1 in milk can determine if cows have been exposed to aflatoxin B_1 in their feed

Monitoring exposure of cows to aflatoxins

- Biomarkers can not only be indicators of mycotoxin exposure, but also excellent and invaluable indicators of the effectiveness of binders added to feed.

- The methodologies for the determination of biomarkers are especially valuable if they can be used in the field in a non-invasive, fast and simple way.

Rapid and Simple

Add 200µl Cold Milk

Vortex 3 times for 5 seconds each time to mix

Add strip and incubate 40ºC for 10 minutes

Read on Vertu

Limit of Detection 25ppt (0.025ppb)
Assay range 0.025-0.75ppb

Rapid Milk AFM_1 Testing

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Lateral Flow

- Dairy Farm
- Transfer Stations
- Receiving Stations
- Milk Depots
- Milk Collection Centers

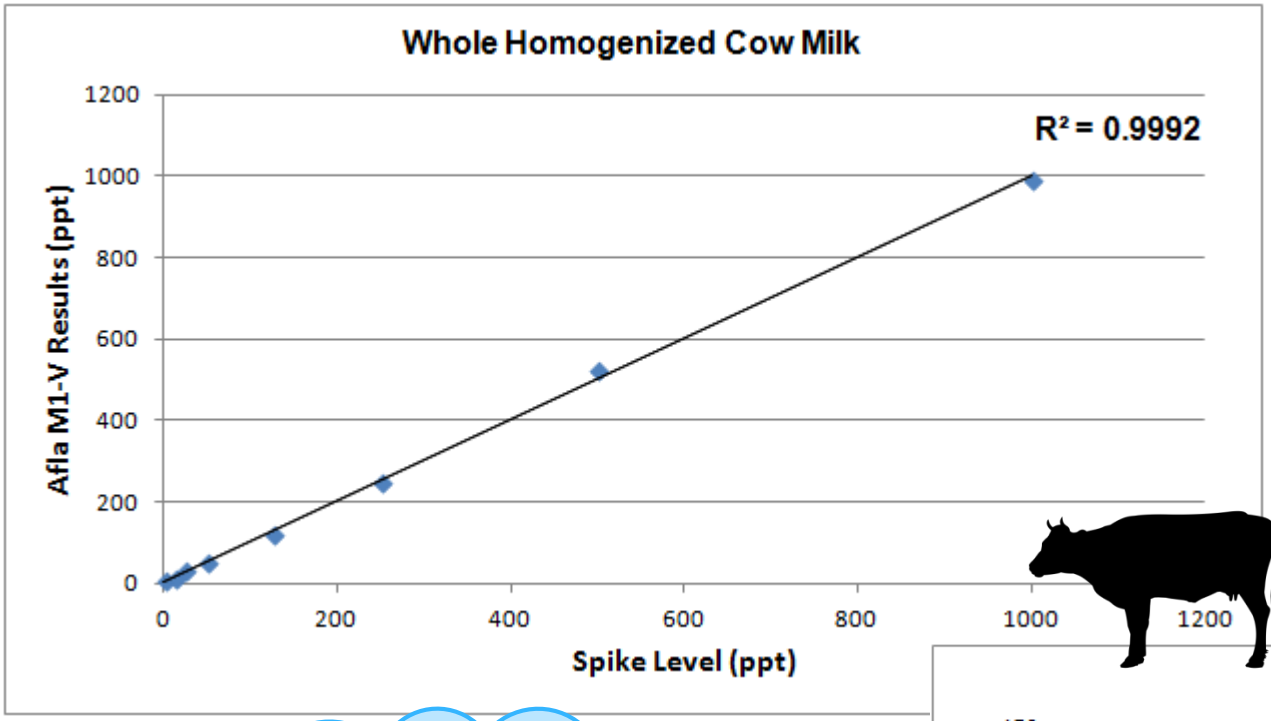
Lateral Flow

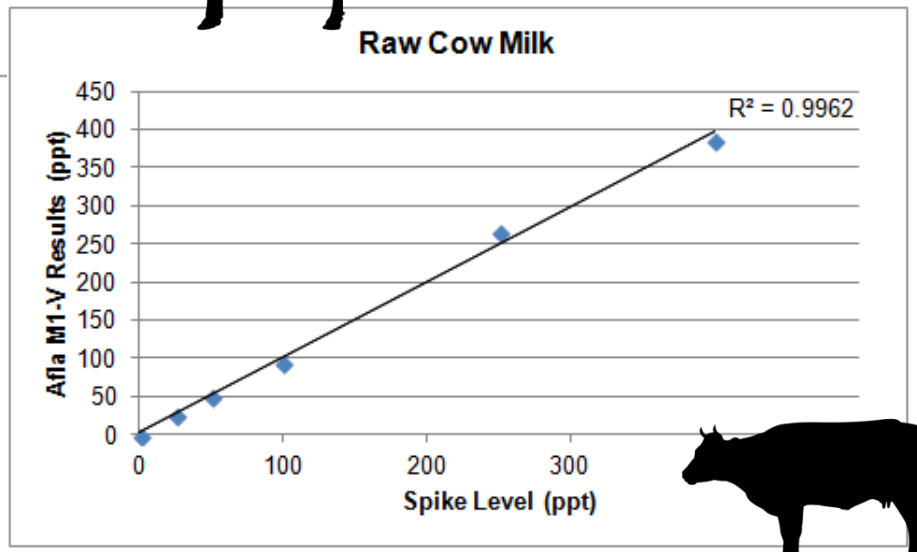
- Milk Receiving Bay – Processing Plant
- Milk Testing Lab – Processing Plant

Lateral Flow, ELISA, Fluorometric-IAC, LC-IAC

- Central Milk Testing/ Producer Payment Lab
- Import Inspection Lab
- Regulatory / Public Health Lab
- Dairy Extension/ Research Lab
- Corporate Industry Lab, Contract Testing Lab
- Bovine Veterinary Producer Support Lab

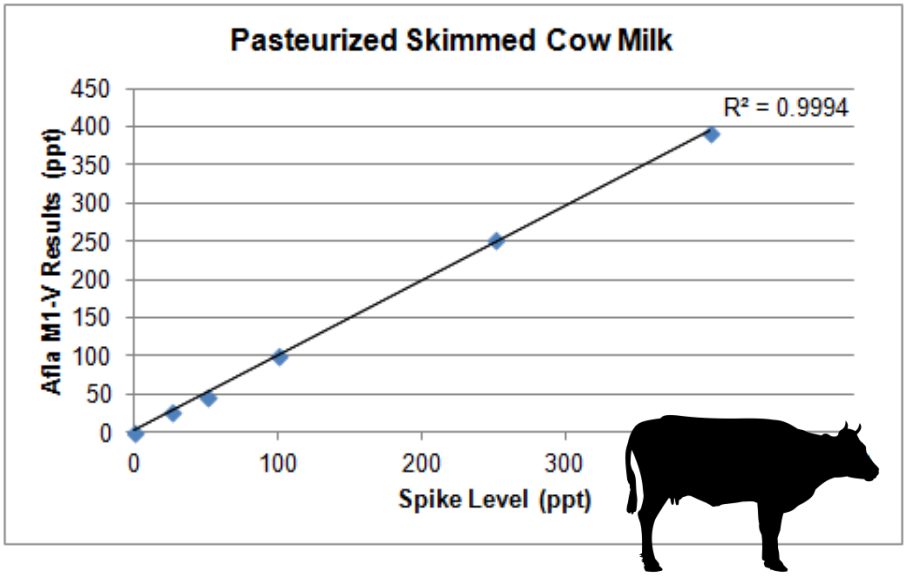
Test Performance – Linearity & Range

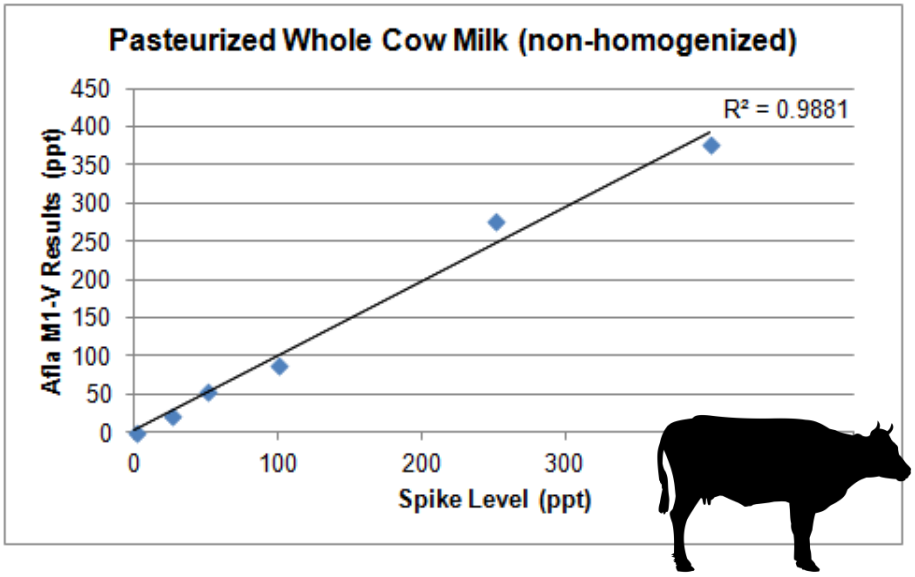


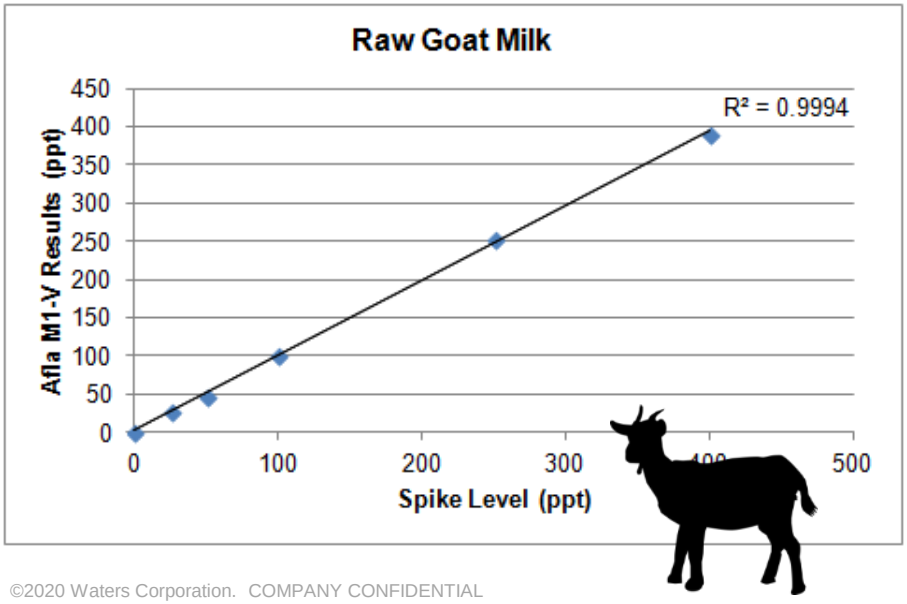


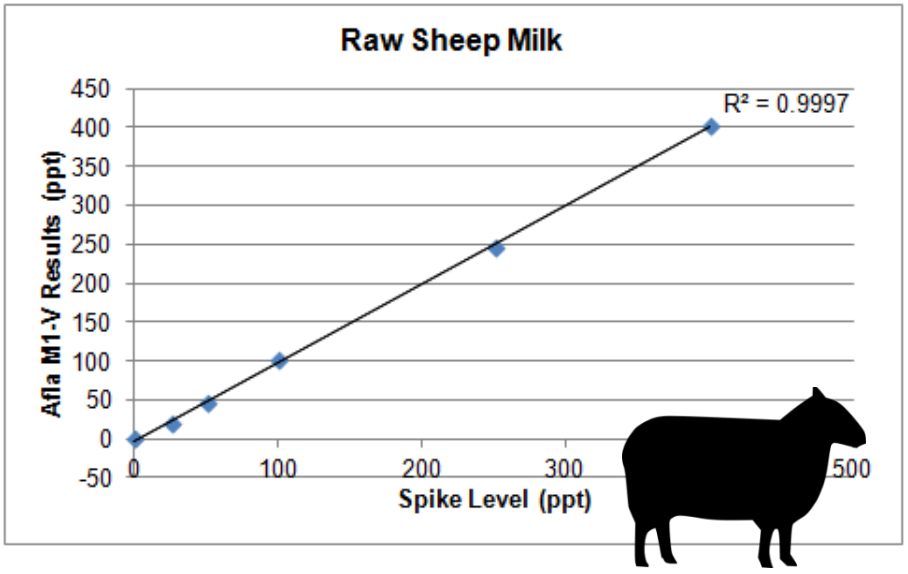
Range covers most worldwide regulations for Afla M_1 levels in **one** rapid test

Test Performance – Linearity & Range

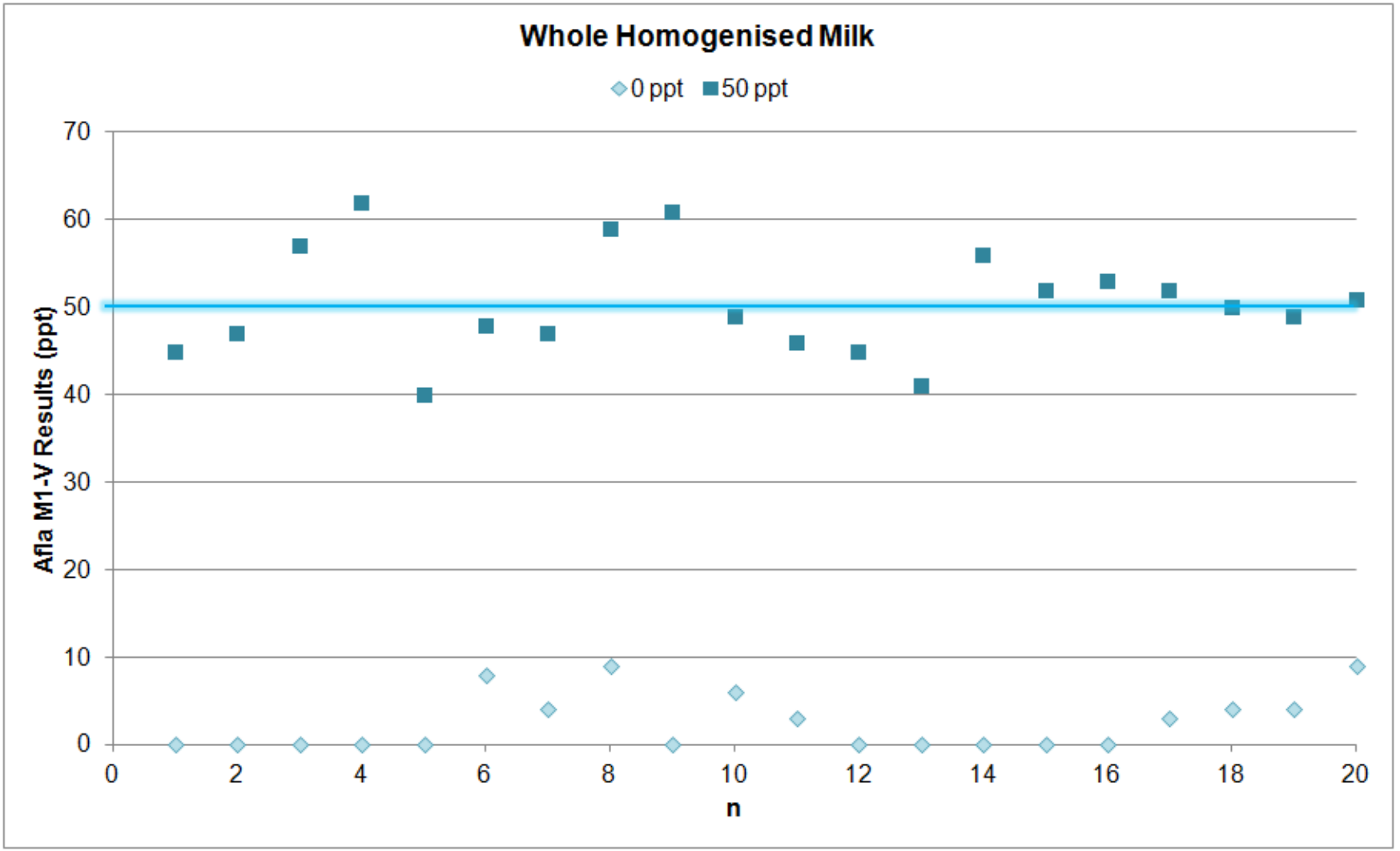








Test Performance – Precision and Repeatability



Afla M_1-V kit part # 176004148

Afla M_1-V BEQ Part # 176004172 (110v) 176004173 (220V)

Conclusions

- The combined use of methodologies for multiple simultaneous analyzes of mycotoxins in feed ingredients and analysis of biomarkers in fluids of animals that consume the feed, may in the immediate future improve the control of problems arising from exposure to mycotoxins.

- Would you like information on multiply mycotoxin testing in corn or feed or aflatoxin M_1 testing in milk?

- Please contact me here in person or after the meeting at nancy_zabe_collette@waters.com

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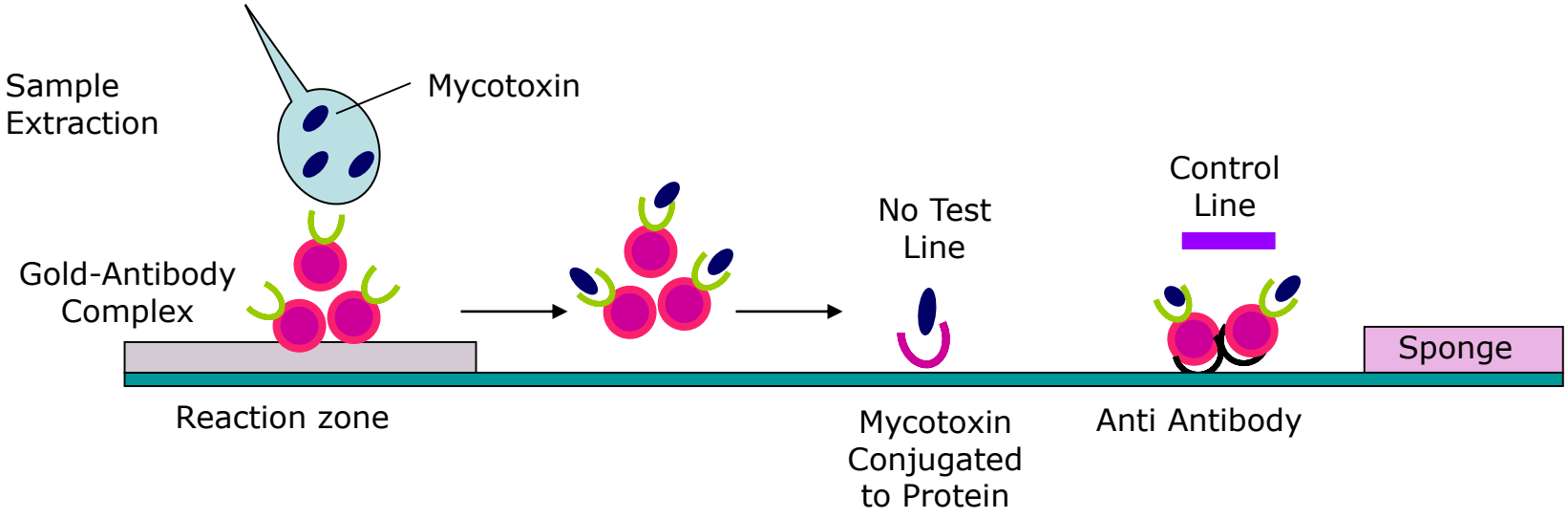
Any Questions?

Thank You

When mycotoxin contamination is positive



When sample is mycotoxin free (negative)