

Determination of Melamine in Pet Food Inputs and Milk Products by Lateral Flow Immunoassay

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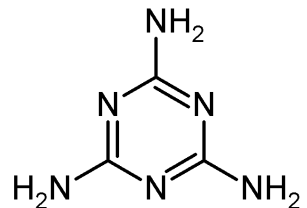


ABSTRACT

The inexpensive, nitrogen-rich compound, melamine (1,3,5-triazine-2,4,6-triamine) has been purposely added to feed and food ingredients and milk and milk products to yield inflated values for protein Kjeldahl analysis. The high toxicity of melamine adulterated food forced the feed, food and pet food industries to seek methods suitable for melamine screening. We have developed a 5 minute, qualitative lateral flow device for detection of melamine. This 5 minute competitive immunoassay requires a gold-labeled antibody to bind to an immobilized melamine-protein conjugate on the assay device and free melamine within the sample matrices. The shelf life is 6 months at room temperature or 18 months at 4°C. One-step sample preparation methods have been developed for dried and liquid samples. The detection threshold is 2.5 ppm among several matrices fortified with known concentrations of melamine examined to date: dried distillers grains, corn gluten, soybean and cottonseed meals. The detection limit in fortified milk and milk products is 0.5 ppm. One hundred percent correlation was observed between results obtained from the rapid assay and two reference methods, a commercially available ELISA and GC-MS for samples fortified over the range of 0-28 ppm. The developed test is an efficient screening tool for detection of melamine in adulterated food components.

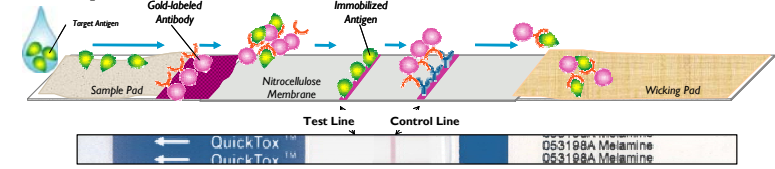
MELAMINE

- Spiked into feed and food to artificially increase nitrogen levels, as measured by the Kjeldahl reaction
- Falsely inflates estimated protein concentrations
- Forms crystals in the presence of cyanuric acid
- Concentrate in microtubules of kidneys, leads to kidney damage/failure
- Linked to pet death and infant kidney disease in China
- Industry need for rapid diagnostics at 2.5 ppm cutoff in feed commodities and milk products

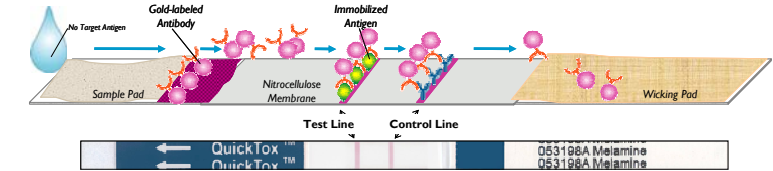


COMPETITIVE ASSAY

Positive Sample



Negative Sample



ASSAY PROCEDURE



Add 100 mL buffer to
50 g ground matrix



Shake 1 min., settle 1-
2 minutes



Add 500 μ L
supernatant to cup



Add Strip, 5 minutes,
read assay visually

Positive (2.5 ppm or greater)
Test Line lighter than Control Line



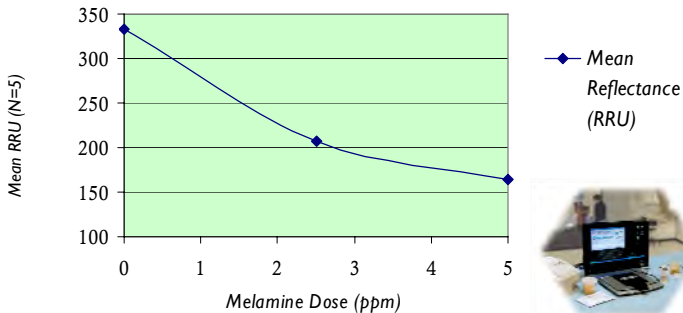
Negative (less than 2.5 ppm)
Test Line equal to or darker than Control Line



VALIDATION FOR VARIOUS GRAINS AT 2.5 PPM CUTOFF

Strip	C	T	Matrix	Dose (ppm)	Result
E 1			Corn Gluten Meal	0	Neg
C 2			Corn Gluten Meal	2.5	Pos
C 3			Corn Gluten Meal	50	Pos
~ 4			Soy Meal	0	Neg
~ 5			Soy Meal	2.5	Pos
L 6			Soy Meal	50	Pos
7			DDGS	0	Neg
8			DDGS	2.5	Pos
9			DDGS	50	Pos

TEST SIGNAL INTENSITY: MELAMINE DOSE RESPONSE CURVE



THE ASSAY SHOWS CONCORDANCE WITH ELISA AND GC-MS

Zero-ownership commercial samples

Ingredient	GC-MS (ppm)	ELISA (ppm)	QuickTox
Corn Gluten Feed	2.8	3.1	+
Corn Gluten Feed	3.2	2.8	+
Corn Gluten Feed	2.2	2.4	+
Corn Gluten Feed	3.2	2.9	+
Corn Gluten Feed	2.4	2.6	+
Corn Gluten Feed	2.6	2.5	+
Corn Gluten Feed	2.0	2.6	+
Ingredient	GC-MS (ppm)	ELISA (ppm)	QuickTox
Single Cell Protein	3.6	7.0	+
corn germ feed	ND	4.0	-
corn germ meal		<2.5	-
corn gluten meal		<2.5	-
corn gluten meal		<2.5	-
soybean meal		<2.5	-
soybean meal		<2.5	-
fishmeal		<2.5	-
cottonseed meal		<2.5	-
rapeseed meal		<2.5	-

Melamine spiked samples

Ingredient	spiked (ppm)	ELISA (ppm)	QuickTox
DDGS	0.0	0.0	-
DDGS	4.5	5.0	+
DDGS	28.0	26.6	+
corn gluten meal	0.0	0.0	-
corn gluten meal	3.0	4.2	+
corn gluten meal	15.0	9.9	+
soybean meal	0.0	0.0	-
soybean meal	3.5	4.1	+
soybean meal	22.0	16.0	+
cottonseed meal	0.0	0.0	-
cottonseed meal	2.5	2.5	+
cottonseed meal	19.0	16.0	+







MILK APPLICATIONS

0.25 ppm cutoff - Skimmed whole milk

Interpretation

If Test line is darker than Control line, assay is negative

If Test line is lighter than or equal to Control line intensity, assay is positive

Strip			Dose (ppm)
	C	T	
E D A			0.25
L B			2.5

CONCLUSIONS

- Melamine LFD satisfies the industry need for rapid, easy-to-use melamine screening in feed and food commodities.
- Melamine LFD is a useful tool in screening milk for melamine adulteration.
- Melamine LFD performance is comparable to instrument-based methodologies.

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