Collaborative Study of the Charm ROSA Safe Level Aflatoxin M1 Quantitative (SLAFMQ) Lateral Flow Test for Raw Bovine Milk

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Introduction:

A 21 laboratory collaborative study tested raw bovine milk containing aflatoxin M1 using the Charm ROSA Safe Level Aflatoxin M1 Quantitative (SLAFMQ) lateral flow method. A ROSA reader quantitatively interpreted test strips with ppt readings. Readings lower than or equal to 400 ppt were negative, and readings greater than 400 ppt were interpreted positive. Initial positive samples were subsequently assayed two times. If both retests were greater than 400 ppt, the sample was called positive/actionable at 500 ppt (1,2).

Method:

Aflatoxin M1 standard (Sigma # 49319-U) used to prepare raw milk at 300, 350, 400, 450, 500 and 550 ppt.
Duplicate samples were blind coded, shipped on ice and tested within 1 week. Data were reported to a third party unaffiliated with sample preparer, Charm Sciences Inc

Laboratories performed the SLAFMQ method as follows in Figure 1

Figure1: Summary of SLAFMQ method

1. Place strip into ROSA incubator at 56ºC.

2. Dilute raw milk into cold dilution buffer, mix and pipet 300 µL into strip. Seal Strip and close incubator lid which starts an 8 min timer.

3. After 8 minutes, place strip into ROSA reader for 5 sec analysis. The reader displays the determined part per trillion concentration. Values greater than 400 ppt are positive and need retesting in duplicate. Two more duplicate results greater than 400 ppt indicate an actionable sample.

Figure 2. Initial data points at each concentration and Dose Response using 400 Control Point

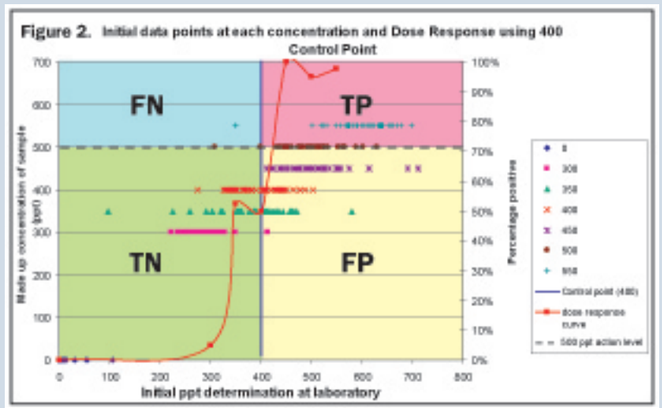

Figure 2 Legend: Laboratories quantified results are plotted on x-axis versus prepared concentration on y-axis. Graph is divided into 4 quadrants by the blue vertical 400 ppt reader control point line and by the dotted horizontal 500 ppt action level line. Data points are true positive (TP) if they appear in red area, true negative (TN) if they appear in green area, false negative (FN) if they appear in blue area, and false violative-containing aflatoxin less than the action level (FP) if they appear in yellow area. The red curve plots the initial result percent positive (# positive/#tested x 100) on secondary y-axis versus concentration.

Results:

The initial first sample results are plotted in Figure 2.
The combined laboratory results of the SLAFMQ method, including positive retesting, and the initial first test result are presented in Table 1. The 90% positive level with 95% confidence is calculated from probit analysis (3).
The statistical parameters of the initial test SLAFMQ quantitative reading are presented in Table 2 (4).

HPLC analyses of study samples were performed by five laboratories with one of those laboratories analyzing the samples twice (5, 6). A 38% false negative rate was found since 9 of 24 samples at 500 or 550 ppt aflatoxin M1 were reported to contain less than the 500 ppt action level. There were no false violative results in the final reported HPLC determinations.

Table 1: Dose Responses of SLAFMQ Method and Initial Test with 90% Positive Concentrations with 95% Confidence

Added Concentration (ppt)	# Samples Tested	SLAFMQ Method		Initial Test	
		# positive	% positive	# positive	% positive
0	41*	0	0%	0	0%
300	42	0	0%	2	5%
350	42	9	21%	22	52%
400	42	6	14%	21	50%
450	42	39	93%	42	100%
500	42	39	93%	40	95%
550	41*	40	98%	41	100%
90% Positive Concentration with 95% Confidence (one tail)		503 ppt		470 ppt	
Pearson Chi Square		25.7		20.2	

*Outliers at 0 and 550 removed

Table 2: Charm SL Aflatoxin M1 Quantitative Test (SLAFMQ) statistical parameters showing mean, repeatability, reproducibility, and Horrat statistics

Added conc. (ppt)	Mean ($\bar{x}$) of SLAFMQ Determinations	Intra-laboratory Repeatability Statistics				Inter-laboratory Reproducibility Statistics			
		StDev$_r$	CV_r% (RSV$_r$)	Repeatability r = 2.8 (StDev$_r$)	Horrat$_r$ Value (RSV$_r$/PRSV$_R$)	StDev$_R$	CV_R% (RSV$_R$)	Reproducibility R = 2.8 (StDev$_R$)	Horrat$_R$ Value (RSV$_R$/PRSV$_R$)
0	8	6	119%	16	1.25	13	282%	36	2.73
300	291	31	11%	86	0.20	41	14%	115	0.26
350	388	61	16%	170	0.30	78	20%	218	0.39
400	394	40	10%	112	0.20	50	13%	139	0.25
450	505	48	9%	133	0.19	63	12%	176	0.25
500	495	62	13%	173	0.25	62	13%	173	0.25
550	596	36	6%	101	0.12	45	8%	126	0.15

Discussion:

The Charm SL Aflatoxin M1 Quantitative (SLAFMQ) assay for raw milk in a 21 laboratory collaborative study detected US and Codex action levels with a 90% positive concentration with 95% confidence at 503 ppt and a 4.8% false negative rate. The initial test results prior to retesting have a 470 ppt 90/95 detection level with 2% false negative rate, which meet Interstate Milk Shipments single laboratory method parameters (7). False violative results were reduced to half from the initial assay result using a confirmation procedure that required samples with initial positive results to be retested twice, and for both retests to be positive.

The calculated repeatability (r) and reproducibility (R) for the SLAFMQ method were lower than published values for HPLC methods at comparable concentrations (8). These statistical parameters represent the 95% variation range expected from identical sample determinations within a laboratory (r) and between different laboratories (R). Lower r and R values and very low Horrat values, Horrat$r$ < 0.3 and Horrat$R$ < 0.5, indicate the SLAFMQ method has precision equivalent or better to HPLC for quantitation of aflatoxin M1 levels in milk. The SLAFMQ method had greater confidence at detecting actionable samples, at 500 and 550 ppt, than HPLC methods performed on the same samples.

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