



The Environmental BioMonitoring Laboratory Provides Analytic Support to the Food Emergency Response Network Proficiency Testing Program

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Introduction

The Food Emergency Response Network (FERN) integrates the nation's food-testing laboratories at the local, state, and federal levels into a network that is able to respond to emergencies involving biological, chemical, or radiological contamination of food. The FERN plays a number of critical roles related to food security and food defense that include: early detection of threat agents in the nation's food supply, preparedness of national laboratories to respond to emergencies related to food, and a network of laboratories to restore confidence in the food supply following an emergency. EBML has been a member of the FERN since 2009. While EBML has not had to provide emergency services to the FERN, the lab has participated in proficiency testing and other studies on behalf of FERN.

Ricin Detection in Milk

As a member of the FERN, the Environmental Monitoring Biological Laboratory (EBML) participated in the proficiency testing of Ricin in a food matrix using FERN approved methods as well as EMBL's electrochemiluminescent (ECL) methods for detection of Ricin. While EMBL's method of detection via ECL is validated for environmental samples, this proved a great opportunity to assess EBML's method, instrumentation, and assays for detection of Ricin in a non-environmental matrix.

In this particular PT study, the FERN sent 8 samples for the detection of Ricin in raw unpasteurized milk. EBML was required to use FERN methods: "ELISA- 16 Detection of Ricin in Foods Using Enzyme-linked Immunosorbent Assays" and "LFD 21 Detection of Ricin in Foods Using Lateral Flow Devices" and report findings back to FERN within 3 business days.

Having only verified and validated environmental samples, it proved a great opportunity for EBML to assess its methods, instruments, and assays for Ricin detection in a food matrix. It was decided to analyze the milk samples on the Meso-Scale Discovery PR-2 1800.

Materials and Methods

Lateral Flow Device

FERN methods require that all samples be analyzed initially via Lateral Flow Device (LFD), using method LFD-21. LFDs are simple, cost effective, and have a high specificity of antibodies for the antigens that enable them to detect specific antigens. The LFD used for this study was Tetracore's Ricin BioThreat Alert Kit (Cat# TC-8008-025). A 1:10 dilution of each sample in PBS + 0.1% Tween20 + 5 grams/L of powdered skim milk (PBSTM) and was analyzed in duplicate via LFD. Any positive result was considered presumptive and then confirmed by ELISA.

ELISA

The Tetracore Ricin, pre-coated assay kits (Cat # TC-40004-001) were used for the study. Undiluted sample and a 1:10 dilution of sample in PBSTM were analyzed in duplicate by following the ELISA-16 FERN method. Procedures outlined in the commercially available ELISA were followed.

Electrochemiluminescence (ECL)

Undiluted sample, diluted sample 1:10 in PBSTM, and diluted samples 1:10 in PBS and 0.1% Triton (PBST) were analyzed on the Meso Scale Discovery PR -2 1800 with EBML assays. Meso Scale Discovery's technology uses proprietary MULTI-SPOT® microplates with electrodes integrated into the bottom of the plate. MSD assays use electrochemiluminescent labels for ultra-sensitive detection. These labels are non-radioactive, stable and offer a choice of convenient coupling chemistries. Electrochemiluminescent labels emit light when electrochemically stimulated. The detection process is initiated at electrodes located in the bottom of MSD's microplates. Only labels near the electrode are excited and detected. Multiple excitation cycles of each label amplify the signal to enhance light levels and improve sensitivity. Background signals are minimal because the stimulation mechanism (electricity) is decoupled from the signal (light).

Results and Data

Lateral Flow Device

The results for the Tetracore lateral flow devices were very weak. As this analysis technique does not possess the detection limits of traditional ELISA or ECL technologies, these results were somewhat expected. This technique resulted in positive calls for four of the eight PT samples (04, 05, 06, and 08).

ELISA

Results for the Tetracore ELISA pre-coated assay kits proved to be much stronger than the LFDs. Each sample and its diluted partner were analyzed in duplicate and the plate was read on a Beckman Coulter Multimode Detector DTX-880 for absorbance at 405nm. Per Tetracore instructions, the cut-off for the assay was equal to the mean of the negative control plus three times the standard deviation plus 0.15. This technique resulted in positive calls for six of the eight PT samples.

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Results and Data – cont.

Electrochemiluminescence (ECL)

The results obtained using the MSD ECL assays, proved to be even stronger than the ELISA kits. Each sample, a 1:10 diluted sample in PBSTM, and a 1:10 diluted sample in PBST were analyzed in triplicate and read on the MSD PR-2 1800. Standard EBML protocols for Ricin detection state that any signal to background value greater than 2.5 is concerned positive.

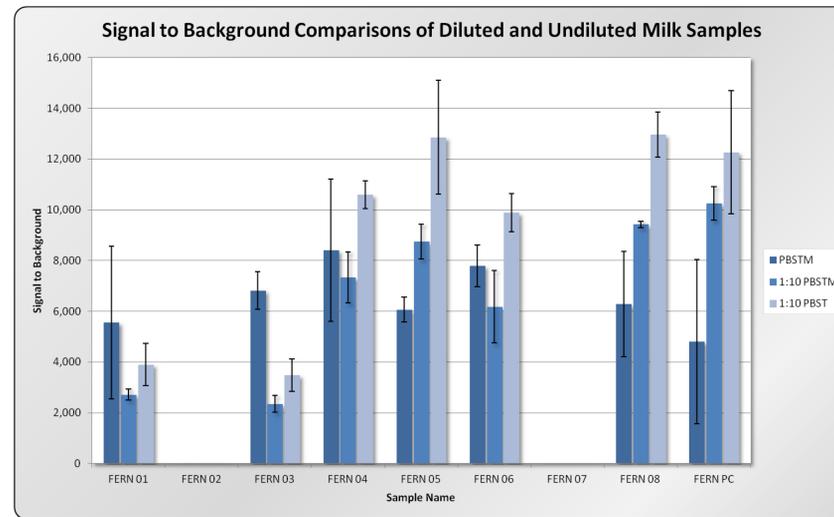


Figure 1. Signal to Background (negative control) for each FERN PT sample, undiluted, diluted 1:10 in PBSTM, and diluted 1:10 in PBST.

Conclusions

Of the 8 samples received by the laboratory, 6 were spiked with the Ricin toxin at three concentrations (1000ppb, 500ppb, and 100ppb). Using the ELISA and ECL protocols, the laboratory detected the toxin in all 6 spiked samples. Using the LFD protocol, the laboratory only detected the toxin in 4 of the 6 spiked samples. However, the 2 samples that were not detected were of the lowest spiked concentration of 100ppb.

Analysis by ECL for toxins in this particular food matrix proved to be very successful, and perhaps will allow EBML to supplement its ISO accreditation to include this and other food matrices.

Biological Agent Detection in Infant Formula

In 2009, the Environmental Monitoring Biological Laboratory (EBML) performed a study for FERN to determine the detection capabilities of the EBML in house PCR method for multiple biological agents in infant formula.

Materials and Methods

The EBML bead beating, DNA extraction, and PCR analysis were performed on undiluted samples of six infant formulas to determine if these methods are applicable for use with infant formula as the sample matrix. Each formula was spiked with a cocktail of four agents (*Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, and Orthopox virus), at a concentration of 1E5 CFU/mL or PFU/ml in order to analyze the sensitivity of known agents in various types of infant formula. Each formula sample and a process negative control (PNC) were analyzed using the DNA extraction method. Upon completion of extraction, the samples along with the PNC and the positive and negative controls were analyzed in triplicate by PCR. Sensitivity was defined as the ability of the spiked sample to produce a positive result.

Results and Data

All six infant formulas produced similar PCR results when analyzed using the EBML's current DNA extraction. None of the infant formula samples were inhibited by PCR so no further dilutions will be needed to analyze formula in the future. The spiking concentration of 1E5 CFU/ml or PFU/ml was detected in each of the formulas.

Results and Data – cont.

Table 1. C_t values for Multiple Biological targets in Infant Formula

<i>B. anthracis</i>			<i>F. tularensis</i>		
Infant Formula	AVG C _t	STDEV	Infant Formula	AVG C _t	STDEV
1	33.1	0.8	1	34.9	0.6
2	32.6	0.3	2	34.5	0.3
3	32.8	0.3	3	34.6	0.4
4	32.0	0.7	4	34.3	0.8
5	31.9	0.4	5	33.7	0.3
6	32.2	0.8	6	33.9	0.7

<i>Y. Pestis</i>			Orthopox virus		
Infant Formula	AVG C _t	STDEV	Infant Formula	AVG C _t	STDEV
1	24.9	0.1	1	30.6	0.6
2	24.5	0.4	2	29.4	0.4
3	24.7	1.0	3	29.0	0.7
4	24.1	1.3	4	28.8	1.0
5	23.8	0.9	5	29.4	0.3
6	24.0	1.2	6	29.1	0.6

Other EBML Collaborative Activities

FDA/FERN Training Course

In 2012, the Environmental Monitoring Biological Laboratory (EBML) traveled to Jamaica, NY to participate in the training course for FDA/FERN Screening Methodologies for the Detection of *Bacillus anthracis* and *Yersinia pestis*.

This course highlighted safe handling practices facility requirements for all FDA/FERN operation as well as various sample processing procedures used in the analysis of *B. anthracis* and *Y. pestis*.

For the detection and analysis aspect of the course, participants were instructed on both enrichment and direct plating techniques, followed by testing and interpreting results for the presumptive identification of *B. anthracis* and *Y. pestis* using cultural rapid screens.

Yersinia pestis Detection in Ground Beef

In 2012, the Environmental Monitoring Biological Laboratory (EBML) participated in the proficiency testing of *Y. pestis* in ground beef using PCR and ECL detection methods. After multiple laboratories reported unsuccessful attempts at detection, the FERN notified participating laboratories that it had identified an issue with the strain of *Y. pestis* inoculated in the ground beef. Ultimately, this proficiency test was aborted, and FERN has not as yet, announced another attempt.

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