

# VIDAS Staphylococcal Enterotoxin II (SET2)

## Pre-collaborative Study Report: AOAC Performance Tested Method<sup>SM</sup>

Ann M. Schultz, Wendy A. McMahon, Victoria A. Aleo, Silliker, Inc., and Ronald L. Johnson, bioMérieux, Inc.  
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### Abstract

The VIDAS Staphylococcal Enterotoxin II (SET2) assay detects staphylococcal enterotoxins A, B, C (C1, C2, C3), D and E in extracts of a variety of foods. Fifteen foods were spiked with enterotoxin, extracted and tested in the VIDAS system. Dairy foods were validated with and without a trichloroacetic acid (TCA) precipitation step to concentrate the extract. The VIDAS SET2 method demonstrated a limit of detection of 0.25 ng toxin per gram of food in all food/toxin type combinations tested. Ice cream required a TCA precipitation step to reach this limit of detection. The overall sensitivity for VIDAS SET2 was 100% when TCA precipitation was included for the extraction of dairy products. Overall sensitivity for VIDAS SET2 was 98.0% when TCA precipitation was not included in the extraction of dairy products. Three false negatives were observed at the 0.25 ng/g level in ice cream without TCA precipitation. The specificity in this study was 100%, with no false positives. After a simple extraction, the SET 2 test is fully automated and results are obtained within 80 minutes allowing for the rapid detection of staphylococcal enterotoxins in foods.

### Materials and Methods

**Analytes:** Enterotoxin types A, B, C<sub>2</sub>, D, and E were obtained from Toxin Technology, Sarasota, FL. Enterotoxins were hydrated and diluted with phosphate buffered saline (PBS; 0.01M Butterfield's phosphate buffer, 0.85M NaCl) with bovine serum albumin (BSA; 0.1%; 1 g/L) and sodium azide (0.05%; 0.5 g/L), and stored at -20°C until needed.

**Food Products:** Test products were obtained from local grocers for spiking and testing. Fifteen foods were evaluated - frozen prepared lasagna, chocolate éclair, canned mushrooms (post retort spiking), powdered egg, roast beef, cooked chicken, ham, cheddar cheese, raw milk, yogurt, Italian salami, smoked salmon, potato salad, ice cream, and non-instant nonfat dry milk.

**Preparation of Inoculated Foods:** Four enterotoxin levels were evaluated for this study: 2.00 ng/g, 1.00 ng/g, 0.25 ng/g, and 0 ng/g. Five replicates at each enterotoxin level were evaluated. Each product was spiked with enterotoxin according to table below.

Food Matrix	Toxin Type	SET II Package Insert Protocol
Frozen, Prepared Lasagna	D	General extraction
Chocolate éclair	C <sub>2</sub>	General extraction
Canned Mushrooms	D	Canned food
Powdered Egg	E	Dehydrated food
Roast Beef	A	Raw meat products, seafood, and deli meats
Cooked Chicken	C <sub>2</sub>	Raw meat products, seafood, and deli meats
Ham	B	Raw meat products, seafood, and deli meats
Cheddar Cheese	A	Dairy products Dairy products with TCA concentration
Raw Milk	E	Dairy products Dairy products with TCA concentration
Yogurt	B	Dairy products Dairy products with TCA concentration
Italian Salami	A	Raw meat products, seafood, and deli meats
Smoked Salmon	B	Raw meat products, seafood, and deli meats
Potato Salad	C <sub>2</sub>	General extraction
Ice Cream	D	Dairy products Dairy products with TCA concentration
Nonfat Dry Milk	E	Dehydrated food followed by Dairy Products Dehydrated food followed by Dairy Products with TCA concentration

TCA = Trichloroacetic Acid

Toxin was hydrated and diluted with PBS-BSA-Azide and twenty-five (25) gram portions were individually spiked with appropriate amount of diluted toxin. Samples were extracted immediately after spiking with toxin.

**Analysis of Foods:** Samples were analyzed using the VIDAS SET 2 method only. A reference protocol was not included due to the lower sensitivity of the microslide gel double diffusion (Bennett, R.W., *Bacteriological Analytical Manual (BAM) Online*, 2001, Chapter 13A) method. Twenty-five (25) grams of sample was directly prepared for analysis using the VIDAS SET 2 assay. Analysis of product was conducted as described in VIDAS SET 2 package insert. Protocols specified in the package insert for each food group were followed and are listed below. Dairy products (cheddar cheese, raw milk, yogurt, ice cream, nonfat dry milk) were analyzed by both the dairy product protocol and the Trichloroacetic acid (TCA) concentration protocol.

### VIDAS SET 2 Protocols

**General Extraction:** Add 25 mL reconstituted extraction buffer to 25 g of food. Blend to obtain a homogeneous suspension. Let stand for 15 minutes at 18-25°C. Centrifuge the blended sample in the extraction solutions for 15 minutes at 3000-5000 g at 18-25°C. Pump the supernatant through moistened absorbent cotton placed in a syringe, using the plunger. Check the filtrate pH and adjust to between 7.5 and 8.0, if necessary, using 1N NaOH. Recover 500 µL of the filtrate.

**Liquid Food:** Dilute the concentrated food product as indicated by the manufacture. Check the filtrate pH and adjust to between 7.5 and 8.0, if necessary, using 1N NaOH. In case of precipitate, centrifuge and filter the suspension as described in the general extraction protocol. Recover 500 µL of the filtrate.

**Dehydrated Food:** Hydrate the food product with an equivalent volume of distilled water or according to the manufacturer's instructions. Leave the re-hydrated sample for one hour at room temperature. Weigh 25 g of re-hydrated food and add 25 mL of reconstituted extraction buffer. Proceed as described in the General Extraction Protocol.

**Canned Food:** Blend the whole canned food or a representative aliquot to obtain a homogenous suspension. Add 25 mL of reconstituted extraction buffer to 25 g of food. Proceed as described in the General Extraction Protocol.

**Raw Meat Products, Seafood, and Delicatessen Meats:** Add 25 mL of distilled water to 25 g of food. Blend to obtain a homogeneous suspension. If the suspension is too dense, add an additional 25 mL of distilled water and re-blend. Recover the whole extract. Check the pH and adjust it to 4.0 using 5N HCl. Let stand for 15 to 30 minutes at 18-25°C. Centrifuge the blended sample in the extraction solution for 15 minutes at 3000-500 g at 18-25°C. Pump the supernatant through moistened absorbent cotton placed in a syringe, using the plunger. Check the filtrate pH and adjust it to between 7.5 and 8.0 if necessary, with 1N NaOH. In case of a precipitate, centrifuge an aliquot as described previously. Recover 500 µL of the filtrate.

**Dairy Products without Concentration:** Add 40 mL distilled water pre-warmed at 38 ± 2°C to 25 g food. Blend to obtain a homogeneous suspension. Let stand for 30 minutes at 18-25°C. Check pH and adjust it to between 3.5 and 4.0 using 5N HCl. Centrifuge this suspension for 15 minutes at 3000-5000 g at 18-25°C. Recover the supernatant and adjust the pH to between 7.5 and 8.0 using 1N NaOH. Centrifuge for 15 minutes at 18-25°C at 3000-5000 g and filter if necessary

**For Liquid Dairy Products** (i.e. milk): adjust the pH of 25 mL (or 25 g) of product to between 3.5 and 4.0 using 5N HCl. Proceed then as previously described in the protocol for Dairy Products without Concentration.

### Dairy Products with Trichloroacetic Acid (TCA)

**Concentration:** Proceed as described in the dairy product protocol until the end of the first centrifugation. Recover the supernatant and measure its volume (V). Add to the supernatant V, a volume Y of a TCA solution (at 90% in water) to obtain a 5% final concentration: Y = V x 5/100. Homogenize and precipitate the proteins for 30 minutes at 18-25°C. Centrifuge for 30 minutes at 3500-5000 g at 18-25°C. Carefully discard the supernatant. Dissolve the protein pellet in a volume of TRIS 0.3M pH 8.0 corresponding to 1/10 of the starting volume V of the supernatant. Adjust the pH to between 7.5 and 8.0 using 4N NaOH. At this pH, the milky solution becomes clear. If the solution shows particles in suspension, centrifuge again for 15 minutes at 3500-5000 g at 18-25°C. Recover 500 µL of the filtrate.

**VIDAS Instrument:** Add 500 µL of the filtrate to the sample well of the VIDAS SET 2 reagent strip. Initiate assay by placing strip in the VIDAS instrument for fully automated the SET 2 test is fully automated rapid detection. Follow package insert instructions.

**Data Analysis:** VIDAS SET2 assay results were analyzed for the detection of SET at four toxin levels: 2.00, 1.00, 0.25, and 0 ng per gram. Results with a test value greater or equal to 0.13 indicate the sample contains staphylococcal enterotoxin. Results with a test value less than 0.13 indicate the sample does not contain staphylococcal enterotoxin or contains staphylococcal enterotoxin at a concentration below the detection limit.

### Results and Discussion

A summary of results is presented in Table 1. Of the 300 spiked samples, 297 were determined to be positive by the VIDAS method, yielding 99.0% sensitivity overall for spiked samples. In the 100 control food samples, none were determined to be positive by the VIDAS method, for a specificity of 100% in this study. The false negative rate was 1% overall (3 false negative results out of 300 spiked samples) and there were no false positive results. All three false negative results occurred in ice cream without TCA concentration at 0.25 ng/g spiking level. There was little difference in the method sensitivity with and without TCA concentration. Sensitivity for the fifteen matrices overall was 100% (225/225) when TCA concentration was used with the dairy extracts and, when TCA concentration was not used, the sensitivity was 98.0% (222/225).

### Recommendation

It is recommended that a collaborative study be performed to evaluate the VIDAS SET II method for Official Methods of Analysis approval.

Table 1. Summary of results for samples analyzed for Staphylococcal enterotoxins by VIDAS SET 2.

Food Type	Toxin Level (ng/g)	VIDAS SET 2 Results for 5 Replicate Samples		
		Average RFV*	Average Test Value*	# Positive
Prepared Lasagna	0	23	0.00	0
	0.25	1044	0.25	5
	1.00	3823	0.92	5
	2.00	6255	1.51	5
Chocolate Eclair	0	39	0.00	0
	0.25	948	0.23	5
	1.00	3644	0.88	5
	2.00	5815	1.40	5
Canned Mushrooms (post retort spiking)	0	23	0.00	0
	0.25	985	0.24	5
	1.00	3878	0.93	5
	2.00	6197	1.50	5
Powdered Egg	0	20	0.00	0
	0.25	2427	0.56	5
	1.00	6979	1.63	5
	2.00	8962	2.08	5
Roast Beef	0	32	0.00	0
	0.25	1344	0.32	5
	1.00	4942	1.19	5
	2.00	7207	1.74	5
Cooked Chicken	0	27	0.00	0
	0.25	735	0.17	5
	1.00	3045	0.73	5
	2.00	5099	1.23	5
Ham	0	24	0.00	0
	0.25	358	0.34	5
	1.00	9987	2.41	5
	2.00	10892	2.63	5
Cheddar Cheese without TCA Concentration	0	22	0.00	0
	0.25	1225	0.29	5
	1.00	3995	0.96	5
	2.00	6714	1.62	5
Cheddar Cheese with TCA Concentration	0	26	0.00	0
	0.25	2780	0.63	5
	1.00	8280	1.88	5
	2.00	9831	2.26	5
Raw Milk without TCA Concentration	0	30	0.00	0
	0.25	1859	0.42	5
	1.00	5863	1.29	5
	2.00	6176	1.41	5
Raw Milk with TCA Concentration	0	25	0.00	0
	0.25	4786	1.09	5
	1.00	7853	1.75	5
	2.00	11213	2.58	5
Yogurt without TCA Concentration	0	23	0.00	0
	0.25	3500	0.84	5
	1.00	9923	2.23	5
	2.00	10678	2.58	5
Yogurt with TCA Concentration	0	20	0.00	0
	0.25	10724	2.44	5
	1.00	10702	2.43	5
	2.00	8789	1.99	5
Italian Salami	0	26	0.00	0
	0.25	960	0.22	5
	1.00	3112	0.71	5
	2.00	4599	1.11	5
Smoked Salmon	0	33	0.00	0
	0.25	1941	0.45	5
	1.00	6028	1.41	5
	2.00	8122	1.80	5
Potato Salad	0	25	0.00	0
	0.25	1051	0.24	5
	1.00	3830	0.88	5
	2.00	6751	1.54	5
Ice Cream without TCA Concentration	0	24	0.00	0
	0.25	5714	0.13	2
	1.00	1615	0.38	5
	2.00	3353	0.76	5
Ice Cream with TCA Concentration	0	18	0.00	0
	0.25	1287	0.29	5
	1.00	2204	0.51	5
	2.00	4000	0.93	5
NFDM without TCA Concentration	0	24	0.00	0
	0.25	1519	0.35	5
	1.00	5660	1.30	5
	2.00	6566	1.52	5
NFDM with TCA Concentration	0	28	0.00	0
	0.25	6367	1.46	5
	1.00	11103	2.54	5
	2.00	11920	2.67	5

\* Mean value of 5 replicates