**Vidas Staphylococcal Enterotoxin II (SET2)**

**Pre-collaborative Study Report: AOAC Performance Tested Method**

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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 Vidasys system. Dairy foods were validated with and without a trichloroacetic acid (TCA) precipitation step to concentrate the extract. The Vidasys SET2 method demonstrated a limit of detection of 0.25 ng toxin per gram of food in all foodtype combination tests. Ice cream required a TCA precipitation step to reach this limit of detection. The overall sensitivity for SET2 was 100% when TCA precipitation was included for the extraction of dairy products. Overall sensitivity for Vidasys SET2 was 90% 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.

**Toxin Analysis**

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 Vidasys SET2 method only. A reference protocol was not included due to the lower sensitivity of the microtiter 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 Vidasys SET2 assay. Analysis of product was conducted as described in Vidasys SET2 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.

**Vidasys SET2 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 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 0.1N NaOH. Recover 500 µL of the filtrate.

**Liquid Food**: Dilute the concentrated food product as indicated by the manufacturer. 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 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 dehydrated 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 homogeneous 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 filtrate pH and adjust it to 4.0 using 0.5N HCl. Let stand for 15 to 30 minutes at 18-25°C. Centrifuge the sample in the extraction solution 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 it to between 7.5 and 8.0, if necessary, with 0.1N NaOH. Recover 500 µL of the filtrate.

**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 Vidasys method, yielding 99.0% sensitivity overall for spiked samples. In the 100 control food samples, none were determined to be positive by the Vidasys 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 precipitation at a 0.25 ng/g spiking level. There was little difference in the detection sensitivity with and without TCA precipitation. Sensitivity for the five replicates overall was 100% (225/225) when TCA precipitation was used on the dairy extracts, and when TCA precipitation was not used, the sensitivity was 98.0% (222/225).

**Table 1. Summary of results for samples analyzed for Staphylococcal enterotoxins by Vidasys SET2**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Mean Value of 5 Replicates</th>
<th>Range</th>
<th>Mean Value of 5 Replicates</th>
<th>Range</th>
<th>Mean Value of 5 Replicates</th>
<th>Range</th>
</tr>
</thead>
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* Mean value of 5 replicates