Caution: Some reagents in the kit contain 1 g/L concentrations of sodium azide. Check local regulations prior to disposal. Disposal of these reagents into sinks with copper or lead plumbing should be followed immediately with large quantities of water to prevent potential hazards.

See Table 2007.06 for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

The VIDAS Staph enterotoxin II (SET 2) test is an enzyme-linked fluorescent immunoassay (ELFA) used on the automated VIDAS instrument for the specific detection of Staphylococcal enterotoxins (SET) A, B, C1, C2, C3, D, and E. The solid-phase-receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and predispensed in the sealed reagent strips.

The instrument performs all of the assay steps automatically. The user places the sample extract into the reagent strip. Then the sample is cycled in and out of the SPR for a specific length of time. SET present in the sample will bind to the anti-SET monoclonal antibodies, which are coated on the interior of the SPR. Unbound sample components are washed away. Alkaline phosphatase-labeled antibodies are cycled in and out of the SPR and will bind to any SET captured on the SPR wall. Further wash steps remove unbound conjugate.

During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The bound enzyme conjugate catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliflaron), the fluorescence of which is measured at 450 nm.

SET are among the most common causes of food poisoning. These heat-stable toxins have been found in meat, poultry, canned mushrooms, dairy products, eggs, mayonnaise, and other foods.

B. Reagents

Items are available as the VIDAS SET 2 test kit from bioMérieux, Inc. (595 Anglum Rd, Hazelwood, MO 63042, USA). Each kit contains sufficient materials and reagents for 30 tests. Store the SET 2 kit at 2–8°C until use.

(a) Anti-SET-coated SPRs.

(b) Reagent strips.—Containing prewash solution, wash buffer, alkaline phosphatase-labeled anti-SET antibodies, and 4-methyl-umbelliferyl phosphate.

(c) SET standard.—Contains purified SET A with 1 g/L sodium azide and protein stabilizers.

(d) SET positive control solution.—Contains purified SET A with 1 g/L sodium azide and protein stabilizers.

(e) SET negative control solution.—Contains TRIS buffered saline (150 mmol/L) Tween pH 7.6 with 1 g/L sodium azide.

(f) SET 2 concentrated extraction buffer.—Contains 2.5 mol/L TRIS, 10 g/L Tween, 10 g/L MIT, pH 8.0. Diluted extraction buffer can be stored up to 3 months at 2–8°C.

(g) Master lot entry (MLE) card.—To calibrate the test.

(h) Package insert.

(i) NaOH.—1 N.

(j) HCl.—5 N.

C. Apparatus

(a) VIDAS or mini-VIDAS automated immunoassay system.—Available from bioMérieux.

(b) Balance.—Weight range and tolerance of the balance should be ±0.1 g.

(c) Pipet with disposable tips.—Calibrated to dispense 500 μL.

(d) Blender and blender jars.

(e) Centrifuge and centrifugation tubes (50 mL).

(f) Syringes.—10 mL.

(g) Absorbent cotton.

(h) pH paper.—Strip paper with 3 color bands and a precision at least equal to 0.5 pH unit.

D. VIDAS SET 2 Procedure

Extraction and concentration protocols are food-type specific and are described in the method. The concentrated extracts are tested immediately after preparation. The VIDAS or mini-VIDAS must be readied by entering the master lot data and calibrating the instrument. A 500 μL aliquot of sample is then added to the reagent strip. Standards and controls are analyzed in the same run. The strips and SPRs are inserted into the instrument and the assay is initiated according to the operator’s manual.

When the assay is completed, the results are analyzed automatically by the instrument and a test value is generated. This test value is compared to a threshold and each result is interpreted as positive or negative and a printed report is generated.

E. VIDAS SET 2 Assay

(a) For complete instructions, see the VIDAS or mini-VIDAS operator’s manual.

(b) Enter factory master calibration curve data into the instrument using the MLE card.

(c) Remove the kit reagents and materials from refrigerated storage and allow them to come to room temperature for at least 30 min.

(d) Use one VIDAS SET 2 reagent strip and one VIDAS SET 2 SPR for each sample, control, or standard to be tested. Reseal the storage pouch after removing the required number of SPRs.

(e) Enter the test code by typing or selecting “SET 2” on the instrument. If the standard is to be tested, identify the standard by “S1” and test in duplicate. If the positive control is to be tested, identify it by “C1.” If the negative control is to be tested, identify it by “C2.”

Note: The standard must be tested upon receipt of a new lot of reagents and then every 14 days. The relative fluorescence value (RFV) of the standard must fall within the set range provided with the kit.
Table 2007.06. Interlaboratory study results for the detection of Staphylococcal enterotoxins in selected foods by the VIDAS SET 2 assay

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Toxin type</th>
<th>Toxin level, ng/g</th>
<th>Replicates/lab</th>
<th>Total tested</th>
<th>Total positive</th>
<th>Detection rate(a), %</th>
<th>95% Confidence interval</th>
<th>LOD/g(b)</th>
<th>Specificity(c)</th>
<th>95% Confidence interval</th>
<th>False positive(d), %</th>
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</thead>
<tbody>
<tr>
<td>Cooked chicken</td>
<td>A</td>
<td>0.5</td>
<td>6</td>
<td>114</td>
<td>111</td>
<td>97.4</td>
<td>92.50–99.45</td>
<td>0.25</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>6</td>
<td>114</td>
<td>112</td>
<td>98.2</td>
<td>93.81–99.79</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>6</td>
<td>114</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>97.41–100</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Ham</td>
<td>B</td>
<td>0.5</td>
<td>6</td>
<td>108</td>
<td>106</td>
<td>98.1</td>
<td>93.47–99.78</td>
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<td>—</td>
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<td></td>
<td></td>
<td>0.25</td>
<td>6</td>
<td>108</td>
<td>107</td>
<td>99</td>
<td>94.95–99.98</td>
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<td>—</td>
<td>—</td>
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<td></td>
<td></td>
<td>0</td>
<td>6</td>
<td>108</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>97.41–100</td>
<td>0</td>
<td>—</td>
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<tr>
<td>Potato salad</td>
<td>C1</td>
<td>0.5</td>
<td>6</td>
<td>108</td>
<td>108</td>
<td>100</td>
<td>97.26–100</td>
<td>0.25</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>6</td>
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<td>107</td>
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<td>6</td>
<td>108</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>97.26–100</td>
<td>0</td>
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<tr>
<td>Pasteurized liquid whole milk</td>
<td>D</td>
<td>0.5</td>
<td>6</td>
<td>102</td>
<td>101</td>
<td>99</td>
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<tr>
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<td></td>
<td>0.25</td>
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<td></td>
<td></td>
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<td>6</td>
<td>102</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>97.26–100</td>
<td>0</td>
<td>—</td>
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<tr>
<td>Canned mushrooms</td>
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<td>6</td>
<td>114</td>
<td>114</td>
<td>100</td>
<td>97.41–100</td>
<td>0.25</td>
<td>—</td>
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<td></td>
<td></td>
<td>0.25</td>
<td>6</td>
<td>114</td>
<td>114</td>
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<td>97.41–100</td>
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<tr>
<td></td>
<td></td>
<td>0</td>
<td>6</td>
<td>114</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>97.41–100</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

\(a\) Detection rate was defined as 100 \times the total number positive test portions divided by the total number of test portions.

\(b\) LOD/g = limit of detection of the assay per gram; for milk, the units are LOD/mL.

\(c\) Specificity rate was defined as 100 \times the total number of analyzed negative test portions divided by the total number of known negative test portions.

\(d\) Incidence rate was defined as 100 \times the total number positive test portions divided by the total number of test portions.

\(e\) — = Not determined.
Thoroughly mix the standard, controls, and samples before use.

(f) Pipet 500 µL of sample, standard, or control into the sample well of the reagent strip.

Note: Test samples and controls singly and standard in duplicate.

(g) Insert the SPRs and reagent strips into the instrument. Verify that the color labels with the assay code on the SPRs and reagent strips match.

(h) Initiate the assay as directed in the operator’s manual. The instrument performs all assay steps and data interpretation automatically and the assay will be completed within ca 70 min.

(i) After the assay is completed, remove the SPRs and reagent strips from the instrument and dispose of properly.

1) General extraction protocol.

(a) To 25 g food, add 25 mL reconstituted extraction buffer.

(b) Blend at high speed to obtain a homogeneous suspension.

(c) Let stand for 15 min at 18–25°C.

(d) Centrifuge the blended sample for 15 min at 3000–5000 × g at 18–25°C. Pump the supernatant liquid through moistened absorbent cotton placed in a syringe, using the plunger.

(e) Check the filtrate pH and adjust to between 7.5 and 8.0 if necessary, using 1 N NaOH.

2) Liquid food protocol.

(a) Dilute the concentrated food product as indicated by the manufacturer. For ready-to-drink products, go to step (b).

(b) In the case of a precipitate, centrifuge the diluted product for 15 min at 3000–5000 × g at 18–25°C and pump the supernatant liquid through moistened absorbent cotton placed in a syringe, using the plunger.

(c) Check the filtrate pH and adjust to between 7.5 and 8.0 if necessary, using 1 N NaOH.

3) Canned food protocol.

(a) Blend at high speed the whole canned food or a representative aliquot to obtain a homogeneous suspension.

(b) To 25 g suspension, add 25 mL reconstituted extraction buffer.

(c) Proceed as described in General Extraction Protocol from step b.

4) Raw meat products, seafood, and delicatessen meats protocol.

This protocol includes an acidification step, which precipitates some proteins that may interfere in the test. The SET remain soluble at this pH. After centrifugation, readjustment of the extract to neutral pH is necessary for an optimum antigen-antibody reaction.

(a) To 25 g food, add 25 mL distilled water.

(b) Blend at high speed to obtain a homogeneous suspension. If the suspension is too dense, add an additional 25 mL distilled water and reblend or restomach.

(c) Adjust pH to 4.0 with 5 N HCl.

(d) Let stand for 15 to 30 min at 18–25°C.

(e) Centrifuge the suspension for 15 min at 3000–5000 × g at 18–25°C.

(f) Using the plunger, pump the supernatant liquid through moistened absorbent cotton placed in a syringe.

(g) Check the filtrate pH and adjust to between 7.5 and 8.0 with 1 N NaOH.

(h) If a precipitate forms after pH adjustment, centrifuge an aliquot for 15 min at 3000–5000 × g at 18–25°C.

5) Dairy product protocol without TCA precipitation.—This protocol includes an acidification step, which precipitates milk proteins that may interfere in the test. The SET remain soluble at this pH. After centrifugation, readjustment of the extract to neutral pH is necessary for an optimum antigen-antibody reaction.

(a) For liquid products, adjust pH of 25 mL sample to between 3.5 and 4.0 with 5 N HCl. Proceed to step f.

(b) For solid products, add 40 mL distilled water prewarmed to 38 ± 2°C to 25 g food.

(c) Blend at high speed to obtain a homogeneous suspension.

(d) Let stand for 30 min at 18–25°C.

(e) Check the pH and adjust to between 3.5 and 4.0 using 5 N HCl.

(f) Centrifuge the suspension for 15 min at 3000–5000 × g at 18–25°C.

(g) Recover the supernatant liquid and adjust the pH to between 7.5 and 8.0 with 1 N NaOH.

(h) Centrifuge the suspension for 15 min at 3000–5000 × g at 18–25°C and filter if necessary. Recover the filtrate.

F. Assay Results

The test value is calculated by the instrument and is equal to the sample RFV/standard RFV. A “negative” result has a test value less than the threshold (0.13) and indicates that the sample does not contain SET or contains SET at a concentration below the detection limit. A “positive” result has a test value equal to or greater than the threshold (≥0.13) and indicates that the sample is contaminated with SET. If the background reading is above a predetermined cutoff, then the result is reported as invalid.