



Laboratory Guidebook Notice of Change

Chapter new, **revised**, or archived:

Title: Preliminary and Confirmatory Testing of FSIS Regulated Products for Staphylococcal Enterotoxins (SET)

Effective Date: 04/01/18

Description and purpose of change(s):

The current SET detection and ID ELISA kits are no longer being produced. A replacement kit, RIDASCREEN® SET Total, was validated and added for the detection of SET in FSIS regulated products. The SET Toxin ID section has been taken out because a suitable toxin identification test was not found. The VIDAS® SET2 has been changed from the confirmation process to the screening process. The RIDASCREEN® SET Total assay will be used to confirm all samples that are positive on VIDAS® SET2.

The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use. Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled “FSIS Guidance for Evaluating Test Kit Performance” available on the FSIS website.

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39.1 Introduction

39.1.1 General

These methods are for screening and confirmation of FSIS regulated products. Any products that are identified as being potentially tampered with should be further analyzed.

The tests are intended to identify Staphylococcal enterotoxins A, B, C, D and E in FSIS regulated products. Although the tests are antibody-based tests, the technologies and specific antibodies differ between tests. VIDAS[®] SET2 is an Enzyme-Linked Fluorescent Assay (ELFA) and the RIDASCREEN[®] SET Total are Enzyme-Linked Immunosorbent Assays (ELISA). The VIDAS[®] SET2 and the RIDASCREEN[®] SET Total tests are applicable to ready-to-eat (RTE) products, pasteurized egg products, and raw meat products.

Unless otherwise stated all measurements cited in this method have a tolerance range of $\pm 2\%$.

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39.1.2 Limits of Detection

Matrices validated included egg, fermented sausage, and hotdog in Buffered Peptone Water (BPW) spiked with SEA, SEB, SEC and SED.

Sensitivity is < 1 ng SET Toxin/gram or ml of sample with VIDAS® SET2.
Sensitivity is <1 ng SET Toxin/gram or ml of sample with RIDASCREEN® SET Total.

39.2 Safety Precautions

When working with samples suspected of containing staphylococcal toxins, laboratory personnel are to wear proper laboratory attire such as laboratory coats, gloves, and protective eyewear. All sample manipulations should be carried out under Biosafety Level 2 requirements. The use of a biological safety cabinet is recommended. Only trained personnel should perform these tests. Work surfaces should be decontaminated prior to and after use with a 0.5% solution of sodium hypochlorite made fresh daily or a 2% solution of sodium hypochlorite which may be used up to 30 days following preparation. Contaminated, non-disposable test materials should be placed in a decontamination solution until autoclaved. Disposable material should be placed in closed containers/biohazard bags and autoclaved at least 60 min, 121 °C, 15 psi, slow exhaust before disposal.

39.3 Quality Control Procedures

39.3.1 VIDAS® SET2 Method Controls

Use a total of up to 5 controls per run.

- a. Use provided kit positive control labeled C1 in one reagent strip.
- b. Use provided kit negative control labeled C2 in one reagent strip.
- c. Use BPW as an additional negative control in one reagent strip.
- d. If necessary run S1 twice using 2 reagent strips (required every 14 days).

39.3.2 RIDASCREEN® SET Total Method Controls

Use a total of 3 controls per run.

- a. Use provided kit positive control 100 µl (undiluted).
- b. Use provided kit negative control 100 µl (undiluted).
- c. Use BPW as an additional negative control.

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39.4 Equipment, Materials, Media, Reagents, and Test Kits

39.4.1 Equipment

- a. Incubator, 35-37°C
- b. Polypropylene tubes, (approx. 10mL or larger if needed)
- c. Micropipettes capable of dispensing 100 µl
- d. Dispensing bottle, plastic (500mL)
- e. Automated microplate washer (optional)
- f. Mechanical or repeat pipettor capable of dispensing 200 µl
- g. Pipette capable of dispensing 500 µl
- h. Blending/mixing equipment
- i. Sterile Stomacher™ bags (size should accommodate 1:10 diluted sample) or Whirl-Pak™ bags (or equivalent)
- j. Balance, sensitivity of 0.1 g
- k. Disposable polypropylene columns Screening Columns
- l. Microplate Reader (450/620 ± 10 nm)

39.4.2 Reagents

- a. Refer to kit instructions for other reagents needed for the assay.
- b. 0.5 % sodium hypochlorite solution.
- c. Buffered peptone water (BPW)
- d. 1N NaOH

39.4.3 Test Kits

- a. BioMerieux VIDAS® Staph enterotoxin (SET2) kit
- b. RIDASCREEN® SET Total (Art. No.: R4105)

39.5 Procedures

39.5.1 Preparation and Tests

Prepare samples as specified in “MLG 4: Isolation and Identification of *Salmonella* for Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges”. The sample portion can be reduced as

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long as the appropriate matrix to media ratio is maintained. Aseptically transfer an adequate volume for the following procedures:

Follow additional sample preparation procedures described in the kit instructions for the VIDAS[®] SET2 and RIDASCREEN SET TOTAL[®] assays.

- After sample preparation, let the sample sit for 15 minutes at 18-25°C.
- Centrifuge at 3000-5000g at 18-25°C for 15 minutes.
- Filter the supernatant using a screening column
- Check the filtrate and adjust the pH to 7.5-8.0 using 1 N NaOH (if necessary).

Each test run includes the test samples along with control samples described in Section 39.3.

Proceed with the VIDAS[®] SET2 according to kit instructions.

If a sample or samples cannot be ruled out by the VIDAS[®] SET2 (see result interpretations below) the sample(s) will be tested using the RIDASCREEN[®] SET Total.

- Run samples with the RIDASCREEN[®] SET Total according to kit instructions. **The substrate/chromogen incubation time to achieve a positive control value of at least 1.0 will vary and may take up to 25 minutes.**

39.5.2 Result Interpretations

39.5.2.1 VIDAS[®] SET2

All readings are performed automatically and the results are interpreted by the VIDAS software.

For a valid test, the Negative control shall be ≤ 0.13 , the Positive control shall be ≥ 0.13 and the standards shall be within the instrument RFV (Relative Fluorescence Value). If this is not the case, recalibrate (see manufacturer instructions).

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A sample is negative when the test is valid and the sample value is less than 0.13.

A sample is positive when the test is valid and the sample is ≥ 0.13 .

39.5.2.2 RIDASCREEN® SET Total

Measure the absorbance at $450/620 \pm 10$ nm with a microplate reader.

The Negative Control must be <0.1 and the Positive control must be >1.0 for the run to be valid.

A sample is POSITIVE when the test is valid and the absorbance of the sample is greater than or equal to the cut-off value. The cut-off value is calculated by adding 0.15 to the OD-value of the negative control.

A sample is considered NEGATIVE when the absorbance of the sample is lower than the cut-off value.

39.5.3 Test Results and Interpretation

If the VIDAS® result is negative, no further analysis is necessary. If the VIDAS® result is positive, the analyzing laboratory is required to perform the RIDASCREEN® SET Total. The three possible combinations of test results are described in Table 1. The presence of staphylococcal enterotoxin detected in food products shall be reported to the Federal Select Agent Program managed by the Center for Disease Control and Protection (CDC):

<https://www.selectagents.gov/form4.html> by completing APHIS/CDC Form 4

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Table 1: SET Result Interpretation

TEST	TEST RESULT	INTERPRETATION
VIDAS [®] SET2	NEGATIVE	Negative
VIDAS [®] SET2	POSITIVE	Negative
RIDASCREEN [®] SET Total	NEGATIVE	
VIDAS [®] SET2	POSITIVE	Confirmed Positive For SET
RIDASCREEN [®] SET Total	POSITIVE	

39.6 Selected References

AOAC Official Method 993.06.

AOAC Official Method 2007.06.

Centers for Disease Control and Prevention and National Institutes of Health (CDC/NIH). 2007. BioSafety in Microbiological and Biomedical Laboratories, 5th ed. or current. <http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm>

RIDASCREEN[®] SET Total (Art. No.: R4105) Methods Manual, current version.

VIDAS[®] Staph enterotoxin II (SET2) REF 30 705, current version. Bailey, J. S., J. Y. Chiu, N. A. Cox, and R. W. Johnston. 1988. Improved selective procedure for detection of salmonellae from poultry and sausage products. J. Food Prot. **51**:391-396.