DECIDE WITH CONFIDENCE

FOOD SAFETY

VIDAS® Staph enterotoxin II (SET2)
Ultra Performance Summary

Food safety professionals have a variety of test kits to choose from when looking to fill a need in their laboratory. One of the main criteria used in making their decision is an evaluation of the certifications the test kit has received. Validation studies provide a user with confidence in the performance of the test kit. This document summarizes the bioMérieux VIDAS® Staph enterotoxin II (SET2) test method validation studies. The VIDAS SET2 method was certified by the AOAC Research Institute *Performance Tested Method*℠ (PTM) program after the completion of a single laboratory study and an independent laboratory study. These studies included an evaluation of the following parameters: inclusivity, exclusivity, robustness, lot-to-lot/stability and method comparison. The VIDAS SET2 method was also adopted as an AOAC *Official Method of Analysis* (OMA), First Action status after the completion of a multi-laboratory method comparison study. Additionally, the VIDAS SET2 method was evaluated by a European reference laboratory and was recognized as an official method of analysis in Europe. All of the validation studies demonstrated that the VIDAS SET2 method was statistically equivalent to the corresponding reference method for the matrices tested with 95% confidence.

BIOMÉRIEUX INDUSTRY

AOAC Performance Tested Method: PTM#070404

In September 2011, the VIDAS SET2 Assay was granted *Performance Tested Method*℠ (PTM) status by the AOAC Research Institute for the detection of staphylococcal enterotoxins in a variety of foods. The VIDAS SET2 method was validated according to harmonized PTM and *Official Method of Analysis* guidelines (2002). Results of the validation study demonstrated the ability of the VIDAS SET2 method to: 1) support a 12-month shelf life and quality of the test kit; 2) perform appropriately after varied protocol parameters including sample volume used in the assay (300, 400, 450, 500, 550, 600 µL, extraction pH (6, 6.5, 7, 7.5, 8, 8.5), time reagents held at room temperature (after refrigeration) prior to performing VIDAS SET2 method (0, 30, 60, 120 min); and 3) demonstrate no statistically significant differences when compared to a reference method for 15 claimed matrices using a 25g test portion size: frozen lasagna, chocolate éclair, canned mushrooms (post-retort spiking), powdered eggs, roast beef, cooked chicken, ham, unpasteurized milk, cheddar cheese and yogurt (internal); Italian salami, smoked salmon, potato salad, ice cream and nonfat dry milk (non-instant) (contract). The Independent Study evaluated smoked salmon.

AOAC Official Method: Certificate No: 2007.06

In 2007, the VIDAS SET2 Assay was granted First Action AOAC *Official Methods of Analysis*℠ (OMA) status by the AOAC Research Institute for the detection of staphylococcal enterotoxins after the completion of the AOAC PTM evaluation and a collaboratively studied evaluation of cooked chicken, ham, potato salad, pasteurized liquid whole milk, and canned mushrooms. Nineteen laboratories received six replicate test portions from each of the three contamination levels. Following the appropriate extraction protocol, each test portion was analyzed by the VIDAS SET2 method. Performance parameters calculated included detection rate, specificity, and percent false positive.

European Validation Study

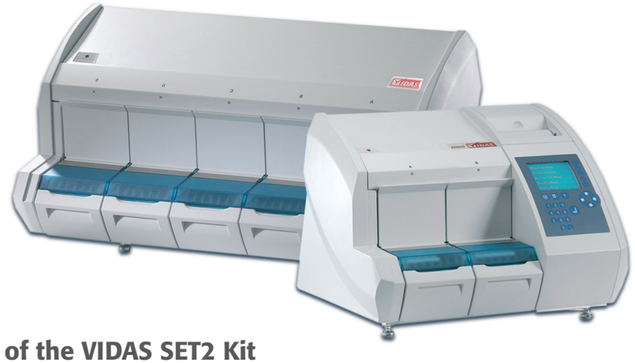
An independent laboratory evaluated the ability of the VIDAS SET2 method to detect 46 different *Staphylococcus aureus* isolated for the presence of enterotoxins as well as a method comparison study comparing the VIDAS SET2 to the CRL confirmatory method on 62 milk and milk product samples. A VIDAS SET2 interlaboratory study (21 laboratories) was performed using freeze-dried cheese samples contaminated with known concentrations of enterotoxins.

PERFORMANCE DOCUMENT VIDAS® Staph enterotoxin II (SET2)

Table of Contents

VIDAS® Staph enterotoxin II (SET2)

Catalog Number - REF 30 705



Contents of the VIDAS SET2 Kit

- 30 SET2 Strips
- 30 SET2 SPR®s
- Standard SET2
- SET2 Positive Control
- Negative Control
- SET2 Concentrated Extraction Buffer
- 1 MLE Card (Master Lot Entry)
- 1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib

Principle of the Assay

VIDAS Staph enterotoxin II is an enzyme-linked fluorescent immunoassay (ELFA) for use on the VIDAS family instrument (see the Operator's Manual) for the specific detection of staphylococcal enterotoxins. The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device. The interior of the SPR is coated with anti staphylococcal enterotoxin antibodies. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. Part of the food extract is dispensed into the reagent strip. The antigens present will bind to the anti-enterotoxin 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 Staphylococcal enterotoxins which are themselves bound to the antibodies 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- umbelliferone), the fluorescence of which is measured at 450 nm. At the end of the assay, the results are automatically analyzed by the instrument which calculates a test value each sample. This value is compared to internal references (thresholds) and each result is interpreted (positive, negative).

PERFORMANCE DOCUMENT VIDAS® Staph enterotoxin II (SET2)

Figure 1. Flow diagrams showing the VIDAS SET2 standard protocols

VIDAS SET2 General Extraction Protocol

To 25 g of food, add 25 ml of reconstituted extraction buffer diluted as indicated previously, in a Stomacher- type bag.

↓

Blend at high speed for 3 minutes to obtain a homogeneous suspension.

↓

Let stand for 15 minutes at 18-25°C.

↓

Centrifuge the blended sample in the extraction solution for 15 minutes at 3000-5000 g at 18-25°C.

↓

Pump the supernatant through a 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, using 1N NaOH.

↓

Recover 500 µL of the filtrate and place in the sample well of a VIDAS SET2 Reagent strip before initiating the assay.

Performance Tested Method℠ (PTM)

The AOAC *Performance Tested Methods℠* (PTM) program was formed in 1992 and is a method certification program for proprietary methods. Methods certified as *Performance Tested℠* were found to perform according to the manufacturer's documented claims and are used throughout the global market place and within the regulatory arena. The PTM program offers certification as an endpoint for method evaluation or as an entry to method validation for programs requiring increased confidence and method reproducibility information. Validation study protocols are written according to AOAC Microbiology Guidelines (2002 or 2012) and include the following technical requirements: inclusivity/exclusivity, method developer method comparison, independent laboratory method comparison, robustness, product consistency, product stability and instrument variation (where applicable) studies. More information can be found at www.aoac.org.

Official Methods of Analysis℠ (OMA)

The Official Methods of Analysis℠ (OMA) program is offered by AOAC INTERNATIONAL and evaluates chemistry, microbiology, and molecular biology methods. It also evaluates traditional bench-top methods, instrumental methods, and proprietary, commercial, and/or alternative methods. Validation study protocols are written according to AOAC Microbiology Guidelines (2012) and include the following technical requirements: inclusivity/exclusivity, method developer method comparison and collaborative method comparison studies. More information can be found at www.aoac.org.

Table A. Validation Study Technical Requirements

Study Type	AOAC PTM	AOAC OMA	AFNOR
Method Developer	●	●	
Independent	●		●
Collaborative		●	●

AOAC PTM Validation Study

PTM Certification#: 070404

PTM Certified: September 2011

PTM Matrix Extension Certified: January 2013

Guideline document: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Qualitative and Quantitative Food Microbiological Official Methods of Analysis (2002)

Method Comparison (Tables B and C): The method comparison study was performed at both the method developer (internal) and independent laboratories. Food matrices were spiked with individual toxin serotypes at three levels: 2.00 ng/g, 1.00 ng/g and 0.25 ng/g. Each toxin serotype was tested in three of the fifteen foods. Twenty-five gram portions were individually spiked with the appropriate amount of diluted toxin and samples were extracted immediately after spiking. Five replicate samples at each level were examined by the VIDAS SET2 method as well as five unspiked controls.

Combining the data from both laboratories, of the 300 spiked samples, 297 were determined to be positive by the VIDAS method, yielding 99.0% overall detection for spiked samples. The percentage of spiked samples detected was 100% at the 2.00 ng/g and 1.00 ng/g levels and 97.0% at the 0.25 ng/g level. In the 100 uninoculated 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 precipitation at 0.25 ng/g spiking level. There were slight differences in the percentage of spiked samples detected with and without TCA precipitation. For the fifteen matrices, detection of spiked samples overall was 100% (225/225) when TCA precipitation was used with the dairy extracts and, when TCA precipitation was not used, the detection was 98.0% (222/225).

The limit of detection was determined to be 0.25 ng/g for all toxin serotypes and foods, except ice cream, which had a limit of detection of 1.0 ng/g in this study. TCA concentration of ice cream at an initial spiking level of 0.25 ng toxin per gram of food extract allowed positive detection of this sample.

Lot-to-lot: Three lots of VIDAS SET2 reagents were compared using purified toxins A, B, C2, D and E each at 0.5 ng/ml, 0.25 ng/ml and 0.10 ng/ml. Unspiked foods were included as negative controls. The variability between lots was not considered significant.

Stability: Stability of the VIDAS SET2 kit was tested in real-time experiments. Kits were held at normal storage temperature (5 ± 3°C) for up to 12 months and tested at various intervals. Test samples included purified toxins, kit controls and a negative food sample. The variability between the kits over time was not considered significant.

Ruggedness: Three test parameters were varied for ruggedness testing, including sample volume, test kit temperature equilibration and extract pH. Purified toxins (0.1 ng/ml or 0.25 ng/ml) and negative food samples were tested with the sample volume (300 to 600 µl) and temperature equilibration (0 to 240 minutes at room temperature) variations. To demonstrate the effect of final extract pH on the VIDAS results, three foods were each spiked with toxins A – E or left unspiked. After extraction, the extracts were adjusted to various pH values and tested on the VIDAS system. There were no differences seen in the number of positive results after with each of the variations in the protocol.

Inter-assay Reproducibility: Toxins A, B, C2, D and E diluted in PBS BSA and standard S1 (toxin A) were tested in duplicate in 46 assays over 8 weeks (2 assays per day and 3 days per week for the first 7 weeks, then 2 days per week for the last week). This yielded 92 data points for each toxin. Two production lots of VIDAS SET2 reagents were tested. Test values were calculated using the value of the standard S1 tested every 14 days. The variability between assays was not considered significant.

Inter-instrument Reproducibility: Toxins A, B, C2, D and E diluted in PBS BSA, three negative food matrices and control C2 were tested singly and standard S1 in duplicate on five different VIDAS instruments. Test values were determined using the average value of standard S1. One lot of VIDAS reagents was used for this study. The variability between instruments was not considered significant.

Official Methods of Analysis (OMA) Validation Study

Certificate No: 2007.06

Certification date: 2006

Guideline document: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Qualitative and Quantitative Food Microbiological Official Methods of Analysis (2002)

Collaborative Study: Pasteurized Milk (Table D): A multilaboratory study was conducted to determine the limit of detection (LOD) of Staphylococcal enterotoxins (SET) in 5 foods. Cooked chicken, ham, potato salad, pasteurized liquid whole milk, and canned mushrooms were each spiked with a different enterotoxin (A, B, C1, D, or E), and tested at 0.25 and 0.5 ng/g SET levels to determine the LOD of the assay for those foods in a collaborative study. Unspiked controls were also included. A total of 19 laboratories representing government and industry participated. In this study, 1674 test portions were analyzed, of which 1638 were used in the statistical analysis. Of the 1638 test portions used in the statistical analysis, 1104 were spiked test portions, of which 1073 were positive by the VIDAS Staph enterotoxin II (SET 2) method. The detection rates at the 0.25 ng/mL level were: cooked chicken, 98.2%; ham, 99.0%; potato salad, 99.1%; liquid whole milk, 85.2%; and canned mushrooms, 100%. The detection rates at the 0.5 ng/mL level were cooked chicken, 97.4%; ham, 98.1%; potato salad, 100%; liquid whole milk, 99.0%; and canned mushrooms, 100%. The data indicate that the SET 2 method is capable of detecting SET at 0.25 ng/g in cooked chicken, ham, potato salad, and canned mushrooms and at 0.5 ng/g in pasteurized liquid whole milk.

Table B. AOAC PTM Method Comparison Study Results - Internal

INTERNAL LABORATORY DATA	Toxin	Extraction Protocol	Level (ng/g)	Total Tested	Total Positive	Detection rate, %	LOD/g	Specificity	False positive, %
Roast beef	A	Meat & seafood	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Cheddar cheese	A	Dairy with TCA	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Cheddar cheese	A	Dairy without TCA	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Ham	B	Meat & seafood	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Yogurt	B	Dairy with TCA	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Yogurt	B	Dairy without TCA	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Chocolate éclair	C	General	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Cooked chicken	C	Meat & seafood	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Frozen lasagna	D	General	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Canned mushrooms	D	Canned food	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Powdered eggs	E	Dehydrated protocol followed by Dairy	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Unpasteurized milk	E	Dairy with TCA	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Unpasteurized milk	E	Dairy without TCA	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0

Detection rate = total number of positive test portions/total number of test portions x 100
LOD/g = limit of detection of the assay per gram/mL
Specificity = total number of negative test portions/total number of know negative test portions x 100
False positive = 100-sensitivity

Table C. AOAC PTM Method Comparison Study Results – Independent

INTERNAL LABORATORY DATA	Toxin	Extraction Protocol	Level (ng/g)	Total Tested	Total Positive	Detection rate, %	LOD/g	Specificity	False positive, %
Meat & seafood	A	Meat & seafood	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Meat & seafood	B	Meat & seafood	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
General	C	General	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Dairy with TCA	D	Dairy with TCA	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Dairy without TCA	D	Dairy without TCA	2	5	5	100	1.0		
			1	5	5	100			
			0.25	2	2	40			
			0	0	0	0		100	0
Dehydrated followed by Dairy with TCA	E	Dehydrated followed by Dairy with TCA	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0
Dehydrated followed by Dairy without TCA	E	Dehydrated followed by Dairy without TCA	2	5	5	100	0.25		
			1	5	5	100			
			0.25	5	5	100			
			0	0	0	0		100	0

Detection rate = total number of positive test portions/total number of test portions x 100
LOD/g = limit of detection of the assay per gram/mL
Specificity = total number of negative test portions/total number of known negative test portions x 100
False positive = 100-sensitivity

Table D. AOAC OMA Collaborative Study Data Summary

INTERNAL LABORATORY DATA	Toxin	Extraction Protocol	Level (ng/g)	Total Tested	Total Positive	Detection rate, %	LOD/g	Specificity	False positive, %
Cooked chicken	A	Meat & seafood	0.5	114	111	97.4			
			0.25	114	112	98.2			
			0	114	0			100	0
Ham	B	Meat & seafood	0.5	108	106	98.1			
			0.25	108	107	99			
			0	108	0			100	0
Potato Salad	C1	General	0.5	108	108	100	0.25		
			0.25	108	107	99.1			
			0	108	0			100	0
Pasteurized liquid whole milk	D	Dairy without TCA	0.5	102	101	99	0.25		
			0.25	102	87	85.2			
			0	102	0			100	0
Canned mushrooms	E	Canned food	0.5	114	114	100	1.0		
			0.25	114	114	100			
			0	114	0			100	0

Detection rate = total number of positive test portions/total number of test portions x 100
LOD/g = limit of detection of the assay per gram/mL
Specificity = total number of negative test portions/total number of know negative test portions x 100
False positive = 100-sensitivity

European Validation Study

Single Laboratory Study: Intralaboratory validation according to the EN ISO 16 140 Standard of the VIDAS SET2 detection kit for use in official controls of staphylococcal enterotoxins in milk products, Journal of Applied Microbiology 102 (2007) 1261–1272

Interlaboratory Study: Interlaboratory Validation of the VIDAS SET2 Kit for Detection of Staphylococcal Enterotoxins in Milk Products, Hennekinne et al.: JOURNAL OF AOAC INTERNATIONAL Vol. 90, No. 3, 2007

Guideline document: ISO 16140, Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods (2003)

Reference Method: Community Reference Laboratory (CRL) Confirmatory Method

Single Laboratory Study

Inclusivity (Table E): The inclusivity studies demonstrated that the VIDAS SET2 method 97.8% agreement between VIDAS SET2 and PCR method when analyzing 46 different *Staphylococcus aureus* isolates from food poisoning outbreaks.

Table E. Inclusivity Results

		PCR Assay	
		+	-
VIDAS SET2	+	32	1
	-	0	13

Method Comparison (Table F): The purpose of these tests was to evaluate the performance of the VIDAS SET2 method with respect to the CRL Confirmatory Method, on test portions naturally contaminated with milk and milk products. The study evaluated 62 test portions: cheese – 59, cake – 1, milk – 2. The results demonstrated perfect agreement (no positive or negative deviations) between the VIDAS SET2 and CRL method.

Table F. Method Comparison Results

		CRL Confirmatory Method	
		+	-
VIDAS SET2	+	20	0
	-	0	42

Inter-laboratory Study (Table G): An interlaboratory study was organized to evaluate freeze-dried, low-fat cheese contaminated with known toxin concentrations. Six freeze-dried samples (2 uninoculated, 2 inoculated at a low level, 0.1 ng/g and 2 inoculated at a high level, 0.25 ng/g) and 3 ready-to-use concentrated extracts were analyzed, in duplicate, by 21 laboratories following the VIDAS SET2 method. Due to laboratory errors, results from the freeze-dried samples were used from 18 laboratories and results from the concentrated extracts from 19 laboratories. Testing showed 100% detection using the VIDAS SET2 method.

Table G. VIDAS SET2 Results From Inter-laboratory Study

	Freeze-dried samples		Ready-to-use concentrated extracts	
	Total Tested	Total Positive	Total Tested	Total Positive
Uninoculated	108	108	19	19
Low	108	108	19	19
High	108	108	19	19

Glossary of Terms

Collaborative Study – A validation study performed by multiple laboratories to estimate critical VIDAS SET2 method performance parameters. See also inter-laboratory study.

Detection rate – Total number of positive test portions/total number of test portions x 100.

False positive – 100-sensitivity

Inter-laboratory Study – A validation study performed by multiple laboratories to estimate critical VIDAS SET2 method performance parameters. See also collaborative study.

Limit of detection (LOD) – The lowest level tested yielding positive results for all replicates.

Negative agreement – Results for both the VIDAS SET2 method and the reference method are negative.

Negative deviation – Result for the VIDAS SET2 method is negative and the result for the reference method is positive.

Positive agreement – Results for both the VIDAS SET2 method and the reference method are positive.

Positive deviation – Result for the VIDAS SET2 method is positive and the result for the reference method is negative.

Specificity – The total number of negative test portions/total number of known negative test portions x 100.

Test portion – A specified quantity of the sample that is taken for analysis by the method.

PERFORMANCE DOCUMENT VIDAS® Staph enterotoxin II (SET2)

For more information, please contact your local
bioMérieux Account Representative.
www.bioMerieux-usa.com/phage