



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
100904

The AOAC Research Institute hereby certifies the method known as:

VitaFast® Pantothenic Acid Microbiological Microtiter Plate Test for the Determination of Pantothenic Acid

manufactured by
Institut für Produktqualität GmbH
Wagner-Régeny-Str. 8
12489 Berlin
Germany

distributed by
R-Biopharm AG
An der neuen Bergstraße 17
64297 Darmstadt
Germany

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads 'Scott Coates'.

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date	December 2, 2022
Expiration Date	December 31, 2023

AUTHORS Jessica Kerr and Kurt Johnson	SUBMITTING COMPANY R-Biopharm Inc. 7950 Old US 27 South Marshall, MI 49068	Current Sponsor R-Biopharm AG An der neuen Bergstraße 17 64297 Darmstadt Germany
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METHOD NAME VitaFast® Pantothenic Acid Microbiological Microtiter Plate Test for the Determination of Pantothenic Acid	CATALOG NUMBER P1005
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INDEPENDENT LABORATORY University of Guelph Laboratory Services Division 95 Stone Road West Guelph, ON N1G 2Z4 Canada	AOAC EXPERTS AND PEER REVIEWERS Sneh Bhandari ^{1,3} , Michael Rychlik ² ¹ Silliker Laboratories, Illinois, USA ² Technische Universität München, GERMANY ³ Modification March 2017 (9)
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APPLICABILITY OF METHOD
Target analyte – B vitamin Pantothenic acid

Matrixes – (1 g) – Cereals, processed meats, multivitamin pills, powders, beverages like fruit juice & milk

Performance claims – The performance characteristics of VitaFast® Pantothenic Acid kit meet the following specifications:

- 1) Time required for completion of the sample extraction was 2 hours and less than 48 hours for the test implementation.
- 2) The test kit components are stable as indicated on the test kit labels (shelf life is 12 months).
- 3) Analytical Sensitivity was found at LOD 0.0035mg / 100 g as measured by 10 blank samples from 10 different lots. LOQ was set at 0.04 mg Ca-Pantothenate / 100 g sample, which corresponds to standard 1 of the curve.
- 4) Accuracy was investigated by analysis of reference materials from proficiency programs, internal reference materials, and also by commercial product analysis and spike recovery studies. In general recovery was within acceptable limits.
- 5) The VitaFast test kit was shown to have a high degree of precision, with inter-assay variances below 10 % for all matrixes.
- 6) The VitaFast plate test is not sensitive to temperature changes between 36 °C and 38 °C, incubation time between 24 and 28 hours, or assay medium volumes between 145 and 155 µl.

ORIGINAL CERTIFICATION DATE October 28, 2009	CERTIFICATION RENEWAL RECORD Renewed annually through December 2023.
METHOD MODIFICATION RECORD 1. March 2017 Level 2	SUMMARY OF MODIFICATION 1. Manufacturing location change to Wagner-Régeny-Str., Berlin.
Under this AOAC <i>Performance Tested Methods</i> SM License Number, 100904 this method is distributed by: R-Biopharm AG	Under this AOAC <i>Performance Tested Methods</i> SM License Number, 100904 this method is distributed as: VitaFast® Pantothenic Acid Microbiological Microtiter Plate Test for the Determination of Pantothenic Acid

PRINCIPLE OF THE METHOD (1)

Pantothenic acid is extracted from the sample and the extract is diluted. The diluted extract and the pantothenic acid assay - medium are pipetted into the wells of a microtiter plate which are coated with *Lactobacillus plantarum*. The growth of *L. plantarum* is dependent on the supply of pantothenic acid. Following the addition of pantothenic acid as a standard or as a compound of the sample, the bacteria grow until the vitamin is consumed. The incubation is done in the dark at 37 °C (98.6 °F) for 20 - 28 h. The intensity of metabolism or growth in relation to the extracted pantothenic acid is measured as turbidity and compared to a standard curve. The measurement is done using a microtiter plate reader at 610 - 630 nm (alternatively at 540 - 550 nm).

DISCUSSION OF THE VALIDATION STUDY (1)

The VitaFast Pantothenic Acid test kit is calibrated according to a standard curve of five standard concentrations, using 4-parameter fitting software. The curve shown in figure 1 is typical. Variation within the curve is consistently minor, at a level of variance below 10 %. Stability is also demonstrated over the entire shelf life of the product, and regular quality tests ensure this is true for all lots produced.

In the independent laboratory study, the accuracy and repeatability of the VitaFast method is well-proven in the analysis of the reference material from NIST. There is however, a discrepancy between the result obtained by VitaFast and the value which was declared on the package label, whereas method 945.74 produced a result that agreed with the label claim. Results of the AOAC extract on the VitaFast plate were also lower than the label claim of the sample. This indicates the presence of an inhibitory substance in the sample. When inhibition is suspected, a spike recovery study may be carried out to confirm whether this is the case. Results of the internal validations show excellent recovery from several cereal matrixes, indicating that the inhibition observed in the independent study is a rare occurrence and not typical of the cereal matrix.

Table 6 Intra-assay variance of food samples (1)

Sample description	Concentration indicated on label (mg / 100g)	Mean result of dilutions in mg / 100 mg (n=3)	Coefficient of variation in %
Infant formula	1.76	2.1	4.1
Multi vitamin sweet (bonbon)	26.6	33.6	2.0
Cereals	5.55	5.5	1.5
Multi vitamin pills	400	465	1.4
Ham sausage 1	6.95	7.82	1.5
Ham sausage 2	6.95	6.89	2.2
Dextrose powder	6	9.2	4.6
RM – Vit001 intern			
Milk powder	3.8	4.4	5.4
RM – Vit002 intern			
Fruit juice	0.9	1.07	2.5
Energy drink	2	1.83	1.0
Multivitamin juice drink	3	3.48	0.8

Table 13: Data summary for pantothenic acid test in infant formula and cereal using AOAC official method 945.74, and VitaFast P1005. (1)

Sample (1)	Infant Formula (µg/g) (2)		Cereal (µg/g) (3)		Cereal (µg/g) ¹⁾ (4)	
	AOAC	VitaFast	AOAC	VitaFast	AOAC Extraction	VitaFast Extraction
1	26.02	47.30	32.88	11.70	10.90	9.90
2	30.86	65.20	37.84	12.80	12.25	8.85
3	47.08	40.50	31.75	13.40	11.65	9.75
4	56.37	79.20	30.64	11.40	11.60	8.10
5	50.72	41.25	40.50	13.90	15.90	9.65
6	37.53	37.35	27.63	13.40	14.50	9.40
7	72.87	56.30	40.56	14.70	15.45	9.80
8	50.51	48.30	27.47	13.60	13.05	9.10
Mean	46.50	51.93	33.66	13.11	13.16	9.32
Standard Deviation	14.98	14.30	5.34	1.11	1.898	0.612
95% Confidence (±)	10.38	9.91	3.70	0.77	1.315	0.424
Label Value ²⁾	48.7±7.3		35 µg/g			
RDA ³⁾	7 mg					

¹⁾A test added to test the AOAC extraction by VitaFast kit.

²⁾Direct from label (infant formula) or calculated from label on box and its RDA (cereal).

³⁾RDA, recommended daily allowance.

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