



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

100903

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

VitaFast[®] Folic Acid Microbiological Microtiter Plate Test for the Determination of Folic Acid

manufactured by
ifp 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[®] *Performance Tested MethodsSM* Program, and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance TestedSM* certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (November 12, 2018 – December 31, 2019). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

November 12, 2018

Date

METHOD AUTHORS

ORIGINAL VALIDATION: Jessica Kerr and Kurt Johnson

MODIFICATION FEBRUARY 2017: Wolfgang Weber

SUBMITTING COMPANYR-Biopharm Inc.
7950 Old US 27 South
Marshall, MI 49068**Current Sponsor**R-Biopharm AG
An der neuen Bergstraße 17
64297 Darmstadt
Germany**KIT NAME(S)**

VitaFast® Vitamin Folic Acid Microbiological Microtiter Plate Test for the Determination of Folic Acid

CATALOG NUMBERS

P1001

INDEPENDENT LABORATORYUniversity of Guelph Laboratory Services Division
95 Stone Road West
Guelph, ON N1G 2Z4
Canada**AOAC EXPERTS AND PEER REVIEWERS**Sneh Bhandari^{1,3}, Michael Rychlik²
¹ Silliker Laboratories, Homewood, IL, USA
² Technische Universität München, Germany
³ Modifications: February 2017**APPLICABILITY OF METHOD**

Target analyte – B vitamin folic acid

Matrices – (1 g) - Cereals, infant formula, processed meats, multivitamin pills, powders, beverages like fruit juice & milk

Performance claims - The performance characteristics of VitaFast® Folic acid 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 0.018 µg / 100 g (ml) as measured by 190 blank samples from 10 different lots. LOQ was set at 0.16 µg Folic acid / 100 g sample, which corresponds to standard 1 of the curve.
- 4) Accuracy was investigated by analysis of reference materials from five proficiency programs, 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 matrices.
- 6) The VitaFast plate test is not sensitive to temperature changes between 36 and 38 °C, incubation time between 44 and 52 hours, or assay medium volumes between 145 and 155 µl.

ORIGINAL CERTIFICATION DATE

October 28, 2009

CERTIFICATION RENEWAL RECORD

Renewed Annually through December 2019

METHOD MODIFICATION RECORD

1. February 2017 Level 2

SUMMARY OF MODIFICATION

1. Location change (10)

Under this AOAC® *Performance Tested*SM License Number, 100903 this method is distributed by:
R-Biopharm AGUnder this AOAC® *Performance Tested*SM License Number, 100903 this method is distributed as:
VitaFast® Vitamin B2 (Riboflavin) Microbiological Microtiter Plate Test for the Determination of Vitamin B2 Riboflavin**PRINCIPLE OF THE METHOD (1)**

Folic acid is extracted from the sample and the extract is diluted. The diluted extract and the folic acid assay - medium are pipetted into the wells of a microtiter plate which are coated with *Lactobacillus rhamnosus*. The growth of *L. rhamnosus* is dependent on the supply of folic acid. Following the addition of folic 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 44 - 48 h. The intensity of metabolism or growth in relation to the extracted folic 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® Folic 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. Lot-to-lot tests show a high degree of repeatability across lots. Not only was the result for the AACC reference material consistent across the four lots with a CV of less than 2 %, but CVs for the raw absorbance data of the standards across the four lots indicate little variation in the calibration of individual tests. This demonstrates the excellent uniformity of the kits.

Accuracy was established in the internal study using recognized and reliable reference materials, as well as spike recovery data and analysis of various food products available on the market.

It was shown that small variations in test implementation did not significantly affect the performance of the test kit. The assay was sufficiently rugged across varying incubation times and temperatures, and reagent volumes which may be introduced non-purposefully by the operating technician. These ruggedness studies show that the test kit will still reliably produce high quality results under minor fluctuations in conditions. The Test kit components showed excellent stability over a period of 12 months without any loss of analytical capacity.

Furthermore the test was not influenced by small changes above and below the environmental and operating parameters specified in the leaflet such as temperature, incubation time and volume.

In the independent laboratory study, VitaFast produced a mean result for the NIST reference material approximately 7% higher than the upper limit of uncertainty, whereas the AOAC reference method 944.12 produced a result of almost 100% the declared value. Although the VitaFast method did produce a result slightly above the specification of the sample, still demonstrates reasonable accuracy and excellent precision, as shown by the RSD.

Furthermore, VitaFast produced a result for the cereal sample very close to the label claim (104% recovery). AOAC method 944.12 also agreed closely with the label claim for the cereal product, yielding a result of 93% recovery.

Table 7 Intra-assay variance of food samples (triplicate analysis per sample dilution) (1)

Sample description (conc. indicated on label in µg / 100 g (ml))	Dilution factor	Mean result in µg / 100 g (ml)	Mean result of dilutions in µg / 100 g (ml)	Coefficient of variation in %
Cereal flakes with fruits (261)	2000	254.6	275.7	7.6
	1500	296.6		
	1000	276.0		
Diet potato soup (100)	600	117.4	123.2	6.5
	400	132.4		
	200	119.9		
Tomato soup (200)	1200	108.0	111.8	9.6
	800	103.4		
	400	123.9		
Infant formula (15)	100	29.1	28.2	4.1
	60	28.6		
	40	26.9		
Semolina with milk (62)	100	101	104	6.9
	150	96		
	200	104		
	300	113		
Hospital milk drink (60)	100	81	80	1.8
	200	79		
Baby formula (140)	200	91	90	1.6
	400	89		

Table 8. Intra-assay variance of food samples (1)

Sample description	Conc. indicated on label in µg / 100 g	Mean result of dilutions in µg / 100 g	Coefficient of variation in %
Dextrose powder RM – Vit001 internal Reference material	200	204 (n=4) 211 (n=3) 205 (n=4) 204 (n=4)	6.8 8.4 6.2 6.6
Ham sausage	200	415 (n=3)	2.1
Cake	150	119 (n=4)	6.0
Cereal (tested by four different groups – internal training)	500	617 (n=6) 557 (n=6) 665 (n=6) 642 (n=6)	7.5
Pineapple tangerine drink	30	28 (n=2) 29 (n=6)	1.7
Multivitamin juice	30	40 (n=2) 41 (n=6)	1.2
Multivitamin nectar	30	33 (n=2) 34 (n=2) 35 (n=4)	2.8

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