



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

030404

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

RIDASCREEN[®] FAST Peanut

manufactured 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 (December 16, 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 28, 2017

Date

METHOD AUTHORS

ORIGINAL VALIDATION: Ulrike Immer & Bernhard Reck
 MODIFICATION MARCH 2017: R-Biopharm

SUBMITTING COMPANY

R-Biopharm GmbH
 Dolivostr. 10
 D-64293 Darmstadt
 Germany

Current Sponsor

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

KIT NAME(S)

RIDASCREEN®FAST Peanut

CATALOG NUMBERS

R6202

INDEPENDENT LABORATORY

FDA Jamaica Regional Laboratory
 158-15 Liberty Ave
 Jamaica, NY 11433 USA
 Allergen Method Development
 Group, Food Research Division, BCS
 Health Canada
 Ross Avenue, Building 22
 PL 2203 D
 Ottawa Ontario, Canada

National Food Processors

Association
 1350 I Street, NW, Suite 300
 Washington, DC 20005-3305 USA

AOAC EXPERTS AND PEER REVIEWERS

J. M. Fremy¹, Samuel Rejeb², Kris Williams³, Sneha Bhandari^{4,5}
¹ French Food Safety Association
² Health Canada, Ottawa, Ontario, Canada
³ FDA/CFSA/DVA
⁴ Merieux Nutri Sciences, Crete, IL, USA
⁵ Modification March 2017

APPLICABILITY OF METHOD

Target analyte – Peanut

Matrices – cookies (1g), ice cream (1g), breakfast cereal (1g), milk chocolate (1g)

Performance claims - The performance characteristics of RIDASCREEN[®]FAST Peanut meet the specification as requested:

- 1) Time required for completion of an analysis of a pre-ground sample was less than 60 minutes.
- 2) Cut off value was < 2.5 mg/kg/ < 2.5 ppm.
- 3) Sensitivity and specificity of the test with blank samples and samples fortified at a level of approximately 5 ppm was 100 %, (no false negative and no false positive samples have been observed).
- 4) Accuracy measurement
 Samples fortified at approximately 5 ppm were found with a mean recovery of 94.3 %
- 5) The ELISA is not sensitive to temperature changes between 18 and 30 °C.
- 6) The ELISA was not sensitive to variation of incubation time between 3x 9 and 3x 11 minutes
- 7) The ELISA was not sensitive to small changes of reagent volumes between 90 and 110 µl
- 8) The test kit components are stable as indicated on the test kit labels.

ORIGINAL CERTIFICATION DATE

May 13, 2003

CERTIFICATION RENEWAL RECORD

Renewed Annually through December 2018

METHOD MODIFICATION RECORD

1. 2012
2. March 2017
3. December 2018 Level 1

SUMMARY OF MODIFICATION

1. Concentration of the Allergen Extraction changed from 20fold to 10fold
2. Change to a mercury-free preserving agent in the washing buffer (3)
3. Editorial changes to the insert for clarity.

Under this AOAC[®] Performance TestedSM License Number, 030404 this method is distributed by:
 NONE

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 NONE

PRINCIPLE OF THE METHOD (1)

The basis of the test is an antigen-antibody reaction. A peanut specific antibody developed in a rabbit is used for the detection of the analyte. A microtiter plate is coated with affinity chromatographic purified rabbit anti-peanut antibodies against raw and roasted peanut as capture antibodies. Peanut standard or samples were incubated for 10 minutes. After washing, the anti-peanut antibody enzyme conjugate is added for further 10 minutes. This conjugate binds to the peanut – antibody complex on plate (sandwich enzyme immunoassay). Any not bound enzyme conjugate is then removed by a washing step. Chromogen/substrate is added to the wells and incubated for 10 min. Bound enzyme converts the chromogen into a blue colored product. The addition of stop reagent inhibits the enzymatic process and causes a shift of the colored product to yellow. Measurement is performed photometrically at 450 nm (optional reference wavelength µ 600 nm). The resulting absorbance values are proportional to the concentration of peanut of an sample.

DISCUSSION OF THE VALIDATION STUDY (1)

The RIDASCREEN®FAST Peanut test kit investigated was proven being highly reproducible and accurate with respect to samples fortified between 3 and 15 ppm in an in-house study.

No false negative or false positive samples were found during the entire in house validation study. LOD calculation on the basis of 4 x 10 blank samples of all four matrices were calculated well below standard 2. Mean value + 3 x standard deviation of the 4 sets of blank samples was found at 0.7 ppm. Maximal LOD was calculated at 1.5 ppm. Spiked samples were analyzed at fortification levels of between 5 and 15 ppm. Mean recovery of 17 spiked samples tested repeatedly in independent runs was calculated at 97.2 %.

The test was insensitive against changes of the ambient temperature between 18 and 30 °C; against variation of incubation time +/-1 minute to the regular incubation time of 10 minutes and against variation of volume pipetted in each incubation step (100µl +/- 10 µl).

Cross reactivity was tested with a variety of 34 compounds including grains, vegetables, and nuts. Only lima beans, chicken peas and green peas were found cross reacting with the test, when applied as concentrated extracts (100%). After further dilution to 100 ppm (100 mg/ml), Lima beans were found below the cut off value, whereas the peas still were found within the measuring range. Cross reactivity of the latter compounds was calculated at < 0.005%.

An independent evaluation study was performed on the basis of 4 sets of 60 blank samples (240 blank samples) and 60 samples spiked at a 5 ppm level (240 spiked samples) with breakfast cereals, cookies, chocolate and ice cream. Three independent laboratories have analyzed 20 blank samples and 20 spiked samples of each matrix.

In first runs of the testing series 1 false negative result and 3 false positive results were obtained. However, since two of the independent laboratories have observed a deterioration of the test kit conjugate, part of the samples have been re-tested in agreement with AOAC-RI.

The manufacturer suspected a damage of the conjugate during transport (probably due to freezing part of the kits) and replaced the reagent with the same lot from the German stock, where no deterioration was observed.(* see statement attached)

By re-testing, one false positive and one false negative sample were found correctly according to the target values. Therefore, after the re-testing of one set of cookies the specificity of the RIDASCREEN®FAST Peanut test was re-calculated at 99.2 % (238 of 240 blank samples were found negative), the sensitivity of the test was calculated at 100 % (all of 240 spiked samples were found positive).

The false positive result of two blank samples (1 cookies and 1 breakfast cereal sample) could be caused by insufficient washing steps or by interference of a sample extract, which was not properly clarified by centrifugation or by a handling error like splashing of a positive sample; we recommend to retest these samples.

With in house studies, all negative samples were found well below the cut off (< 1.5) always.

Table 2a. LOD with breakfast cereals: Analysis of ten blank samples/run (1)

sample ID	absorbance	concentration (ppm)
Ce-1	0.129	< standard 2
Ce-2	0.081	< standard 2
Ce-3	0.096	< standard 2
Ce-4	0.112	< standard 2
Ce-5	0.095	< standard 2
Ce-6	0.100	< standard 2
Ce-7	0.109	< standard 2
Ce-8	0.119	< standard 2
Ce-9	0.122	< standard 2
Ce-10	0.113	< standard 2
Mean	0.108	0.29#
sd	0.0145	
Mean + 3 sd	0.151	0.61#
PN-118 Target: 6 ppm recovery (%)	0.461	5.4 90.0

#) extrapolated value,

Table 2b. LOD with cookies: Analysis of ten blank samples/run (1)

Sample ID	absorbance	concentration (ppm)
Co-1	0.119	< standard 2
Co-2	0.124	< standard 2
Co-3	0.15	< standard 2
Co-4	0.108	< standard 2
Co-5	0.104	< standard 2
Co-6	0.116	< standard 2
Co-7	0.117	< standard 2
Co-8	0.131	< standard 2
Co-9	0.133	< standard 2
Co-10	0.148	< standard 2
Mean	0.125	0.1#
sd	0.0155	
Mean + 3 sd	0.172	0.5#
PN-118 Target: 6 ppm recovery (%)	0.45	5.8 96.7

#) extrapolated value

Table 2c. LOD with ice cream: Analysis of ten blank samples/run (1)

Sample ID	absorbance	concentration (ppm)
Ice-1	0.202	< standard 2
Ice-2	0.145	< standard 2
Ice-3	0.141	< standard 2
Ice-4	0.179	< standard 2
Ice-5	0.191	< standard 2
Ice-6	0.162	< standard 2
Ice-7	0.161	< standard 2
Ice-8	0.209	< standard 2
Ice-9	0.170	< standard 2
Ice-10	0.168	< standard 2
Mean	0.173	0.55#
sd	0.0226	
Mean + 3 sd	0.241	1.5#
PN-118 Target: 6 ppm	0.463	5.5
recovery (%)		91.7

#) extrapolated value

Table 2d. LOD with chocolate: Analysis of ten blank samples/run (1)

Sample ID	absorbance	concentration (ppm)
Ch-1	0.155	< standard 2
Ch-2	0.142	< standard 2
Ch-3	0.124	< standard 2
Ch-4	0.144	< standard 2
Ch-5	0.149	< standard 2
Ch-6	0.140	< standard 2
Ch-7	0.132	< standard 2
Ch-8	0.158	< standard 2
Ch-9	0.156	< standard 2
Ch-10	0.129	< standard 2
Mean	0.143	0.09#
sd	0.118	
Mean + 3 sd	0.178	0.2#
PN-118 Target: 6 ppm	0.638	6.5
recovery (%)		108.3

#) extrapolated value

REFERENCES CITED

1. Immer, Ulrick, and Bernhard Reck, Evaluation of the RIDASCREEN® FAST Peanut, AOAC® Performance TestedSM certification number 030404.
2. AOAC Research Institute Validation Outline for RIDASCREEN® FAST Peanut, Approved – May 2003.
3. R-Biopharm, Evaluation of Requested exchange of thimerosal in RIDASCREEN® FAST Peanut R6202, AOAC® Performance TestedSM certification number 030404. Approved March 2017.