CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

101702

The AOAC Research Institute hereby certifies the method known as:

RIDA®QUICK Gliadin

manufactured by

R-Biopharm AG
An der neuen Bergstraβe 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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date	December 3, 2022
Expiration Date	December 31, 2023

2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 Fax: +1-301-924-7089
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

AUTHORS
Markus Lacorn and Thomas Weiss

SUBMITTING COMPANY
R-Biopharm AG
An der neuen Bergstraβe 17
64297 Darmstadt, Germany

METHOD NAME
RIDA®QUICK Gliadin

CATALOG NUMBERS
R7003, R7004, R7005

INDEPENDENT LABORATORY
Q Laboratories, Inc.
1400 Harrison Avenue
Cincinnati, OH 45214
USA

AOAC EXPERTS AND PEER REVIEWERS
Terry Koerner[1], Joe Boison[2], Mary Trucksess[3]
[1] Health Canada, Ontario, CANADA
[2] Canadian Food Inspection Agency, Saskatchewan, CANADA
[3] Consultant, Virginia, USA

APPLICABILITY OF METHOD
Target analyte – Gliadin

Matrixes – (surfaces 10 x 10 cm) stainless steel, sealed ceramic, plastic, silicone rubber, and clean-in-place waters (50 µL with detergent and 250 µL without detergent)

Performance claims – The RIDA®QUICK Gliadin detects gluten with an $LOD_{95\%}$ of 1.6 – 3.0 µg/100 cm^2 gluten depending on the surface. The minimum detectable gluten concentration in cleansing reagents containing CIP waters is between 50 and 100 ng/mL while CIP waters with no reagents allows gluten detection at about 10 ng/mL. No cross-reacting substance has been identified by the manufacturer. Parallel measurements in various matrixes using the quantitative RIDASCREEN® Gliadin (AOAC OMA 2012.01) and the RIDA®QUICK Gliadin showed accurate detection of the claimed analytes by the dip-stick format. There is no high-dose hook-effect for wheat, rye, and barley.

Study followed the AOAC INTERNATIONAL Guidelines found in Appendix N

ORIGINAL CERTIFICATION DATE
October 23, 2017

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2023.

METHOD MODIFICATION RECORD
NONE

SUMMARY OF MODIFICATION
NONE

Under this AOAC *Performance Tested Methods℠* License Number, 101702 this method is distributed by:
NONE

Under this AOAC *Performance Tested Methods℠* License Number, 101702 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
The dip-stick consists of different zones. Prolamins in the sample solution will be "chromatographed" above the 'maximum line' and react with the R5-antibody coupled to a red latex microsphere. The 'maximum line' indicates the user the maximal liquid level of the sample solution.
The 'result window' contains a small band of immobilized R5 antibody when T = test band (red) is positive and a second line C = Control band (blue) the reaction was valid. Results are read visually only. Generally, the higher the analyte level in the sample the stronger the red color of the test band will be (until a maximum of color is reached).

DISCUSSION OF THE VALIDATION STUDY (1)
The immuno-chromatographic dip-stick RIDA®QUICK Gliadin investigated in this validation study was demonstrated to be applicable for the detection of traces of gliadin on surfaces and in CIP waters. The in-house validation included a target and non-target compound study, a matrix study with four different surfaces, different cleansing reagents, a lot-to-lot comparability and stability testing, and ruggedness testing.
The claimed target prolamins (gliadin, secalin, and hordein) were shown to react in a comparable manner as the "reference" WGPAT gliadin preparation. The 68 non-target compounds (oats, pseudocereals, vegetables, seeds, nuts, fruits, spices, and alternative protein sources such as egg, mlik and soy) were checked to be gluten-free (<5 mg gluten/kg) with the R5 sandwich ELISA (AOAC OMA Final Action 2012.01). The subsequent analysis with the dip-stick revealed no positive results which proved the selectivity of the method.
Stainless-steel, plastic, sealed ceramic and silicone rubber surfaces contaminated with WGPAT gliadin showed corresponding gluten concentrations at which all results were positive at 4.0 µg/100 cm^2, 4.0 µg/100 cm^2, 4.0 µg/100 cm^2, 2.0 µg/100 cm^2, respectively. Using a 4-parameter curve fitting, $LOD_{95\%}$ concentrations of 3.0 µg/100 cm^2, 1.6 µg/100 cm^2, 2.8 µg/100 cm^2, and 1.6 µg/100 cm^2, were estimated respectively.
Three chemically different cleansing reagents were spiked with gluten and revealed 100% positive results at or above 50 to 100 ng/ml gluten. Spiking of clean water with gluten resulted in 100% positive read-outs at or above 9.1 ng/ml gluten.

DISCUSSION OF THE VALIDATION STUDY Continued (1)

A thorough ruggedness testing included the analysis of variation of ethanol extraction time (30 s and 30 s plus 10 min), incubation temperature of extraction and dip-stick analysis (16°C, 23°C, 30°C), incubation time of the dip-stick (4 min, 5 min, 6 min), and Lot-to-Lot comparison (shelf life and samples). Except incubation times, no parameter was found to influence the result in a way that could be critical under practical conditions. The longer the incubation time the higher the probability of detection for a given concentration was. Therefore, the test kit insert clearly recommends to incubating the dip-stick for exactly 5 min. Nevertheless, this effect is only visible at very low concentrations and not at the legal threshold. As stated by the manufacturer, the shelf life is 18 months at minimum and all tested lots were comparable even when using spiked samples after ethanol or Cocktail extraction.

An independent laboratory validation study using contaminated stainless steel surfaces and spiked CIP solutions proved that the manufacturer's claims are correct.

Table 4. Results for testing a gliadin-contaminated stainless steel surface with an area of 10 x 10 cm for each swabbing experiment; 20 replicates per amount; WGPAT gliadin preparation was used for contamination

µg/100 cm² gliadin	result for each repeat					POD
0.00	-	-	-	-	-	0.00
	-	-	-	-	-	
	-	-	-	-	-	
	-	-	-	-	-	
0.25	-	-	-	-	+	0.25
	-	+	-	+	-	
	+	-	-	-	-	
	-	-	-	-	+	
0.5	+	+	-	-	-	0.25
	+	-	+	+	-	
	-	-	-	-	-	
	-	-	-	-	-	
1.00	+	+	+	+	+	0.80
	+	+	+	+	+	
	+	+	+	+	-	
	-	-	-	+	+	
2.00	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
4.00	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	

Table 5. Results for testing a gliadin-contaminated plastic surface with an area of 10 x 10 cm for each swabbing experiment; 20 replicates per amount; WGPAT gliadin preparation was used for contamination

µg/100 cm² gliadin	result for each repeat					POD
0.00	-	-	-	-	-	0.05
	-	-	-	+	-	
	-	-	-	-	-	
	-	-	-	-	-	
0.25	-	-	-	+	+	0.50
	+	+	+	+	-	
	-	+	-	-	-	
	+	+	-	+	-	
0.5	-	-	+	+	+	0.85
	+	+	+	+	+	
	+	+	+	+	+	
	-	+	+	+	+	
1.00	+	+	-	+	+	0.95
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
2.00	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
4.00	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	

Table 6. Results for testing a gliadin-contaminated silicone rubber surface with an area of 10 x 10 cm for each swabbing experiment; 20 replicates per amount; WGPAT gliadin preparation was used for contamination

µg/100 cm² gliadin	result for each repeat					POD
0.00	-	-	-	-	-	0.00
	-	-	-	-	-	
	-	-	-	-	-	
	-	-	-	-	-	
0.25	-	-	-	-	-	0.25
	-	+	+	-	+	
	-	-	+	-	+	
	-	-	-	-	-	
0.5	-	+	+	+	-	0.80
	+	-	+	+	+	
	+	+	+	+	+	
	+	+	+	+	-	
1.00	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
2.00	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
4.00	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	

Table 7. Results for testing a gliadin-contaminated sealed ceramic surface with an area of 10 x 10 cm for each swabbing experiment; 20 replicates per amount; WGPAT gliadin preparation was used for contamination

µg/100 cm² gliadin	result for each repeat					POD
0.00	-	-	-	-	-	0.05
	-	-	-	-	-	
	-	+	-	-	-	
	-	-	-	-	-	
0.25	-	-	-	-	+	0.35
	-	+	-	-	+	
	+	-	+	+	+	
	-	-	-	-	-	
0.5	+	+	+	-	+	0.65
	+	+	-	+	+	
	+	+	+	+	+	
	-	-	-	-	-	
1.00	+	+	+	+	+	0.90
	+	+	+	+	+	
	+	+	+	+	+	
	-	-	+	+	+	
2.00	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
4.00	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	

Table 8. Results for testing of a gluten-contaminated CIP water (1% Micro Quat Classic); 20 replicates per concentration; a Sigma gluten preparation was used for spiking

ng/ml gluten	result for each repeat					POD
0	-	-	-	-	-	0.00
	-	-	-	-	-	
	-	-	-	-	-	
	-	-	-	-	-	
25	+	+	+	+	+	0.70
	+	+	+	+	+	
	+	-	+	+	+	
	-	-	-	-	-	
50	+	+	+	+	+	0.90
	+	+	+	+	+	
	+	+	-	-	+	
	+	+	+	+	+	
100	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
200	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	

Table 9. Results for testing of a gluten-contaminated CIP water (10% Acifoam VF10); 20 replicates per concentration; a Sigma gluten preparation was used for spiking

ng/ml gluten	result for each repeat					POD
0	-	-	-	-	-	0.00
	-	-	-	-	-	
	-	-	-	-	-	
	-	-	-	-	-	
25	-	-	+	-	+	0.25
	-	-	-	+	+	
	-	-	-	-	-	
	-	+	-	-	-	
50	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
100	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
200	+	+	+	+	+	0.90
	+	-	+	+	+	
	+	+	+	-	+	
	+	+	+	+	+	

Table 10. Results for testing of a gluten-contaminated CIP water (1.8% Divosan Extra VT55); 20 replicates per concentration; a Sigma gluten preparation was used for spiking

ng/ml gluten	result for each repeat					POD
0	-	-	-	-	-	0.00
	-	-	-	-	-	
	-	-	-	-	-	
	-	-	-	-	-	
25	-	-	-	+	+	0.65
	+	+	+	+	-	
	-	+	-	+	-	
	+	+	+	+	+	
50	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
100	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
200	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	

Table 11. Results for testing of a gluten-contaminated CIP water (no cleansing reagent); 20 replicates per concentration; a Sigma gluten preparation was used for spiking

ng/ml gluten	result for each repeat					POD
0	-	-	-	-	-	0.00
	-	-	-	-	-	
	-	-	-	-	-	
	-	-	-	-	-	
4.5	-	-	-	+	-	0.05
	-	-	-	-	-	
	-	-	-	-	-	
	-	-	-	-	-	
9.1	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
18.2	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	
36.4	+	+	+	+	+	1.00
	+	+	+	+	+	
	+	+	+	+	+	
	+	+	+	+	+	

REFERENCES CITED

1. Lacorn, M. and Weiss, T., Evaluation of the R-Biopharm RIDA®QUICK Gliadin, AOAC *Performance Tested Methods℠* certification number 101702.
2. Codex Alimentarius Commission. Codex Standard 118-1979 (rev. 2008), Foods for special dietary use for persons intolerant to gluten. Codex Alimentarius. FAO/WHO, Rome, 2008.
3. Thompson, T., 2003. Oats and the gluten-free diet. J. Am. Diet. Assoc. 103: 376-379.
4. Van Eckert, R., Berghofer, E., Ciclitira, P. J., Chrido, F., Denery-Papini, S., Ellis, H. J., Ferranti, P., Goodwin, P., Immer, U., Mamone, G., Méndez, E., Mothes, T., Novalin, S., Osman, A., Rumbo, M., Stern, M., Thorell, L., Whim, A. and Wieser, H., 2006. Towards a new gliadin reference material – isolation and characterization. J. Cereal Sci. 43: 331-341.
5. AOAC International. Appendix N: ISPAM Guidelines for validation of qualitative binary chemistry methods. AOAC Official Methods of Analysis. Gaithersburg. MD, 2013.
6. Koerner, T., Abbott, M., Godefroy, S.B., Popping, B., Yeung, J.M., Diaz-Amigo, C., Roberts, J., Taylor, S.L., Baumert, J.L., Ulberth, F., Wehling, P., and Koehler, P., 2013. Validation procedures for quantitative gluten ELISA methods: AOAC allergen community guidance and best practices. J. AOAC Internat. 96: 1033-1040.
7. Lacorn, M., Scherf, K.A., Uhlig, S., and Weiss, T., 2016. Determination of Gluten in processed and non-processed corn products by qualitative R5 Immunochromatographic Dip-Stick: Collaborative Study, First Action 2015.16. J. AOAC Int. (accepted)
8. Scherf, K.A., Uhlig,. S, Simon,. K, Frost, K., Koehler, P., Weiss, T., and Lacorn, M., 2016. Validation of a qualitative R5 dip-stick for gluten detection with a new mathematical-statistical approach. Qual. Assur. Safety Crops Foods. DOI 10.3920/QAS2015.0818.
9. AOAC Research Institute Performance Tested MethodsSM Program validation outline protocol: Independent Laboratory Validation Protocol for the R-Biopharm RIDA®QUICK Gliadin (March 2017)
10. Official Methods of Analysis of AOAC INTERNATIONAL (2013) Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods, AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org/app_n.pdf (Accessed May 2017)
11. Wehling, P., LaBudde, R., Brunelle, S., Nelson, M. Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods. Journal of AOAC International, Vol. 94, No. 1, 2011.
12. My Curve Fit, Online Curve Fitting [Beta]. https://www.mycurvefit.com/ (Accessed May 2017)