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CERTIFICATION

AOAC Research Institute
Performance Tested Methods[SM]

Certificate No.
122201

The AOAC Research Institute hereby certifies the method known as:

Reveal® 3-D for Gluten
manufactured by

Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912

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

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METHOD NAME	CATALOG NUMBER
Reveal® 3-D for Gluten	8505

INDEPENDENT LABORATORY	APPLICABILITY OF METHOD
Q Laboratories 1930 Radcliff Dr. Cincinnati, OH 45204	**Analytes – Wheat Gluten** **Matrixes – Quaternary ammonium-based clean-in-place rinse, swabs from stainless steel surfaces.** **Performance claims – Probability of detection (POD): In 25% working strength clean-in-place rinse, 0.63 (19/30 positive) at 1.4 mg/kg gluten, (30/30 positive) at 2.8 mg/kg gluten; in swabs (wet) from a stainless steel surface, 0.80 (24/30 positive) at 2.3 µg/100 cm² gluten, 1.0 (5/5 positive) at 4.7 µg/100 cm² gluten; in swabs (dry) from a stainless steel surface, 0.83 (25/30 positive) at 2.8 µg/cm² gluten, 1.0 (5/5 positive) at 4.7 µg/cm² gluten.**

ORIGINAL CERTIFICATION DATE	CERTIFICATION RENEWAL RECORD
December 2, 2022	**Renewed annually through December 2024.**

METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
NONE	**NONE**

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NONE |

PRINCIPLE OF THE METHOD (1)

Reveal 3-D for Gluten is an immunochromatographic assay using proprietary monoclonal antibodies specific for the prolamin fraction of gluten. Sample extract is introduced to the test device where it reacts with antibodies conjugated to colored particles. The complex flows across a membrane where it encounters a test line of capture antibody. If gluten is present in the sample, the gluten-detector antibody complex will be captured at the test line and the resulting congregation of particles will result in development of a visible line. The test device also contains an overload line to indicate conditions of antigen excess in the test sample. The overload line is comprised of immobilized wheat gliadin. In the absence, or presence of trace amounts, of gluten in the sample, the labeled detector antibody binds to gluten at the overload line and forms a visible line. When large amounts of gluten are present in the sample, the gluten-detector antibody complex will not bind to the overload line. The overload line is carefully balanced to indicate highly positive samples that may cause a hook effect (false-negative result due to antigen excess) at the test line. Lastly, the test includes independent reagents to form a control line that should always be present when the test is properly performed.

DISCUSSION OF THE VALIDATION STUDY (1)

Results of the selectivity/interference, matrix, assay robustness, and stability/lot-to-lot consistency studies reported here demonstrate that the Reveal 3-D for gluten test is an accurate and reliable method for qualitative detection of gluten in select CIP rinse and environmental samples.

In CIP rinse, POD was 1.0 (95% CI of 0.89, 1.0) at a gluten concentration of 2.8 mg/kg and 0.63 (0.46, 0.78) at 1.4 mg/kg. With swabs from both wet and dry stainless steel surfaces, POD was 1.0 (0.57, 1.0) at a level of 4.7 µg/100 cm² gluten in both method developer and independent laboratory trials. Fractional results were obtained at levels of 1.9-2.8 mg/kg in the various trials, with POD values ranging from 0.37 to 0.83.

In selectivity testing, all nine target matrixes were detected. Of 39 non-target matrixes, four produced positive results when tested without dilution: almond flour, sesame flour, yellow pea flour and cornstarch. For yellow pea flour, there is evidence that the product was contaminated with gluten. Thirty replicate Reveal 3-D tests were conducted with yellow pea flour and all produced positive results. When spiked with 9.3 mg/kg gluten, Reveal 3-D device test lines were more intense than those produced with unspiked product. Additionally, when tested with the OMA **2012.01** method, unspiked product yielded a result of 6 mg/kg gluten. When spiked with 9.3 mg/kg gluten, a result of 16 mg/kg gluten was obtained. For almond flour and sesame flour, negative results were obtained with 1:10 dilution of the matrixes. Cornstarch and cornmeal produced negative results with the Reveal 3-D test when tested using Gluten Extraction Buffer supplied with the kit instead of 3-D Gluten Food Buffer, demonstrating that these commodities would not be expected to cause false positive results when testing environmental swab or CIP rinse samples.

In interference testing of the same 39 food commodities, all produced positive results when spiked with gluten at a level of 9.3 mg/kg with two exceptions, guar gum and xanthan gum. It can be concluded that these matrixes produce interference with the Reveal 3-D assay and prevent normal detection of gluten, and require dilution (1:10 for xanthan gum, 1:100 for guar gum) in order to produce accurate results. One additional matrix, chestnut flour, produced a positive result but was tested with 1:5 dilution due to known matrix interference. Samples with less than 10% xanthan gum, 1% guar gum, or 20% chestnut flour do not show interference.

Table 1. Results of food commodity selectivity and interference testing for the Reveal 3-D for Gluten lateral flow assay (1)

Commodity	Reveal 3-D for Gluten Assay Results	
	Without Added Gluten	With 9.3 mg/kg Gluten Spike[a]
Barley	Positive	NA[b]
Bulgur wheat	Positive	NA
Common wheat	Positive	NA
Durum wheat	Positive	NA
Einkorn wheat	Positive	NA
Emmer wheat	Positive	NA
Khorasan wheat	Positive	NA
Spelt wheat	Positive	NA
Rye	Positive	NA
Almond flour, 1:10	Negative[c]	Positive
Amaranth flour	Negative	Positive
Arrowroot	Negative	Positive
Beef (ground)	Negative	Positive
Black bean flour	Negative	Positive
Brown rice flour	Negative	Positive
Buckwheat flour	Negative	Positive
Chestnut flour, 1:5	Negative	Positive[d]
Coconut flour	Negative	Positive
Coffee	Negative	Positive
Cornstarch – 3-D Gluten Food Buffer	Positive[e]	Positive
Cornmeal – 3-D Gluten Food Buffer	Positive[e]	Positive
Cornstarch – Gluten Extraction Buffer	Negative	Positive
Cornmeal – Gluten Extraction Buffer	Negative	Positive
Dried cranberries	Negative	Positive
Egg powder	Negative	Positive
Fava bean flour	Negative	Positive
Flax seed flour/meal	Negative	Positive
Garfava flour	Negative	Positive
Green pea flour	Negative	Positive
Guar gum	Negative	Negative[f]
Hazelnut flour	Negative	Positive
Lentil flour (red)	Negative	Positive
Lima bean flour	Negative	Positive
Milk powder	Negative	Positive
Millet flour	Negative	Positive
Oat flour	Negative	Positive
Oregano leaves	Negative	Positive
Potato flour/starch	Negative	Positive
Quinoa flour	Negative	Positive
Romano bean flour	Negative	Positive
Sesame flour, 1:10	Negative[c]	Positive
Sorghum flour	Negative	Positive
Soy flour	Negative	Positive
Sweet rice flour	Negative	Positive
Tapioca flour	Negative	Positive
Tea	Negative	Positive
Teff	Negative	Positive
White bean flour	Negative	Positive
White rice flour	Negative	Positive
Xanthan gum	Negative	Negative[g]
Yellow pea flour	Positive[h]	Positive

[a]Extract of wheat flour.
[b]Not applicable.
[c]Tested at 1:10 dilution due to known cross-reactivity.
[d]Tested at 1:5 dilution due to known matrix interference.
[e]Negative with 1:100 dilution.
[f]Positive with 10 ppm gluten spike when matrix diluted 1:100. Indicates matrix interference.
[g]Positive with 10 ppm gluten spike when matrix diluted 1:10. Indicates matrix interference.
[h]Evidence of contamination with gluten (see Discussion).

Table 2. Results of CIP rinse interference testing for the Reveal 3-D for Gluten lateral flow assay – 25% working strength quaternary ammonium compound (1)		
Gluten Spike (mg/kg)	No. Positive/30	POD (95% CI)[a]
0	0	0 (0, 0.11)
0.93	5	0.17 (0.07, 0.34)
1.4	19	0.63 (0.46, 0.78)
2.8	30	1 (0.89, 1)
4.7	30	1 (0.89, 1)
9.3	30	1 (0.89, 1)

[a]Probability of detection with 95% confidence interval.

Table 3. Results of stainless steel surface testing for the Reveal 3-D for Gluten lateral flow assay				
Surface Type	Gluten Spike ($\mu g/100\ cm^2$)	No. Replicates	No. Positive	POD (95% CI)[a]
	0	5	0	0 (0, 0.43)
Stainless steel – wet	1.4	30	12	0.40 (0.25, 0.58)
	2.3	30	24	0.80 (0.63, 0.90)
	4.7	5	5	1 (0.57, 1)
	0	5	0	0 (0, 0.43)
Stainless steel - wet[b]	0.93	30	6	0.20 (0.10, 0.37)
	1.9	30	13	0.43 (0.27, 0.61)
	4.7	5	5	1 (0.57, 1)
	0	5	0	0 (0, 0.43)
	1.4	30	9	0.30 (0.17, 0.48)
Stainless steel – dry	2.3	30	14	0.47 (0.30, 0.64)
	2.8	30	25	0.83 (0.66, 0.93)
	4.7	5	5	1 (0.57, 1)
	0	5	0	0 (0, 0.43)
Stainless steel - dry[b]	0.93	30	0	0 (0, 0.11)
	1.9	30	11	0.37 (0.22, 0.55)
	4.7	5	5	1 (0.57, 1)

[a]Probability of detection with 95% confidence interval.
[b]Trial performed by independent laboratory.

REFERENCES CITED

1. Le, Q.N., Beck, N., Schwingel, Z., Roman, B., Mozola, M., Sperry, A., Almy, D., and Donofrio, R., Validation of the Reveal® 3-D for Gluten Assay for Detection of Gluten in Clean-in-Place Rinses and Stainless Steel Environmental Surfaces, AOAC *Performance Tested Methods*SM certification number 122201. Approved December 2, 2022.