



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
111901

The AOAC Research Institute hereby certifies the method known as:

Reveal[®] 3-D for Peanut

manufactured by

Neogen

620 Leshar Place

Lansing, MI 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.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date

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Expiration Date

December 31, 2024

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METHOD NAME Reveal® 3-D for Peanut	CATALOG NUMBER 901041L
INDEPENDENT LABORATORY Q Laboratories 1930 Radcliff Drive Cincinnati, OH 45204	APPLICABILITY OF METHOD Target Analyte – Peanut. Matrixes – Clean-in-place (CIP) rinses, including water, peroxyacetic acid/hydrogen peroxide-based sanitizers (up to 1% v/v solution), and quaternary ammonium-based sanitizers (up to 25% v/v solution); environmental swabs from stainless steel and plastic surfaces Performance claims Probability of Detection (POD) 1. Water. —1.0 at 2.8 ppm, 0.9 at 1.0 ppm. 2. Peroxyacetic acid/hydrogen peroxide sanitizer. —1.0 at 2.2 ppm, 0.9 at 0.8 ppm. 3. Quaternary ammonium sanitizer. —1.0 at 4.2 ppm, 0.97 at 2.2 ppm. 4. Swab from wet stainless steel. —1.0 at 3.0 µg/cm ² , 0.77 at 2.0 µg/cm ² . 5. Swab from dry stainless steel. —1.0 at 4.0 µg/cm ² , 0.67 at 1.4 µg/cm ² . 6. Swab from wet plastic. —1.0 at 3.0 µg/cm ² , 0.33 at 1.2 µg/cm ² . 7. Swab from dry plastic. —1.0 at 3.0 µg/cm ² , 0.27 at 1.2 µg/cm ² .
ORIGINAL CERTIFICATION DATE November 19, 2019	CERTIFICATION RENEWAL RECORD Renewed annually through December 2024.
METHOD MODIFICATION RECORD 1. December 2020 Level 1 2. November 2021 Level 1	SUMMARY OF MODIFICATION 1. Editorial to add PTM Certification Mark to insert. 2. Editorial changes for clarity.
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PRINCIPLE OF THE METHOD (1)

Reveal 3-D for Peanut is a lateral flow, immunochromatographic test for detection of peanut proteins. Sample is introduced to the test device where peanut protein (antigen) binds with colloidal gold-conjugated anti-peanut antibodies. The resulting complex flows along a membrane where it encounters a test line coated with anti-peanut antibodies. As the complexed gold particles accumulate, a visible red line is formed. An overload line is included to indicate conditions of antigen excess in the test sample. The overload line is an immobilized mixture of peanut proteins. In the absence (or presence of trace amounts) of peanut in a sample, the anti-peanut gold conjugate binds to the immobilized proteins and forms a visible line. When large amounts of peanut are present in a sample, the antibody gold conjugate will bind the peanut proteins and is inhibited from binding to the overload line. The overload line is carefully balanced to indicate highly positive samples that may cause a hook effect (false-negative result at high concentration) at the test line. Finally, additional reagents are included in the test device to generate a control line, indicating that the test is functioning properly.

DISCUSSION OF THE VALIDATION STUDY (1)

Results of the validation study reported here demonstrate that the Reveal 3-D for Peanut lateral flow test is a sensitive and specific method for determination of peanut residue in CIP rinses and in swabs from environmental surfaces. For CIP rinses, including water, peroxyacetic acid/hydrogen peroxide (1% working strength), and quaternary ammonium (25% working strength) sanitizers, considering all trials, POD was 1 in the range of 2–4 ppm peanut. For surface swabs from stainless steel and plastic, POD was 1 at 3–4 ppm. The independent laboratory study with CIP rinse and wet and dry stainless steel surfaces were consistent with those of the internal trials.

Specificity testing with 29 common food commodities showed the assay to be very specific, with no clear evidence of cross-reactivity. Walnut was shown to interfere with the assay's ability to detect peanut at 10 ppm, although the interference can be avoided with 1:10 dilution of the sample prior to assay. So long as the CIP rinse or environmental surface swab contains <10% walnut, test kit users can be confident that there will be no interference. Residual quaternary ammonium sanitizer did not interfere with the ability of the assay to detect low levels of peanut in swabs from a stainless steel surface. Results of robustness testing showed that the assay can withstand modest variation in multiple operating parameters simultaneously. Results of stability and consistency testing trials support expiration dating for the Reveal 3-D for Peanut test kit of at least 23 months.

Table 3. Results of CIP rinse testing for the peanut lateral flow assay (1)

Sample	Target Spike Level (ppm)	Actual Spike Level (ppm) ^a	No. Test Portions	No. Positive	POD (95% CI) ^b
Water	0	0	30	0	0 (0, 0.11)
	0.5	0.5	30	18	0.60 (0.42, 0.75)
	1.5	1.0	30	27	0.90 (0.74, 0.97)
	3	2.8	30	30	1 (0.89, 1)
	5	4.3	30	30	1 (0.89, 1)
	10	11.6	30	30	1 (0.89, 1)
Peroxyacetic acid/ hydrogen peroxide (1% working strength)	0	0	30	0	0 (0, 0.11)
	0.5	0.4	30	17	0.57 (0.39, 0.73)
	1.5	0.8	30	27	0.90 (0.74, 0.97)
	3	2.2	30	30	1 (0.89, 1)
	5	6.7	30	30	1 (0.89, 1)
	10	25.6 ^c	30	30	1 (0.89, 1)
Quaternary ammonium (25% working strength)	0	0	30	0	0 (0, 0.11)
	1	0.7	30	12	0.40 (0.25, 0.58)
	3	2.2	30	29	0.97 (0.83, 1)
	5	4.2	30	30	1 (0.89, 1)
	10	10.1	30	30	1 (0.89, 1)
Quaternary ammonium ^d (25% working)	0	0	30	0	0 (0, 0.11)
	1-2	0.9	30	8	0.27 (0.14, 0.44)
	3	3.1	30	23	0.77 (0.59, 0.88)
	5	4.5	30	30	1 (0.89, 1)
	10	10.9	30	30	1 (0.89, 1)

^a Determined using the Veratox for Peanut Allergen quantitative assay.

^b Probability of detection with 95% confidence interval.

^c Spike was known to be incorrect.

^d Trial performed by independent laboratory.

Table 4. Results of environmental surface testing for the peanut lateral flow assay (1)

Sample	Target Spike Level (µg/100 cm ²)	Actual Spike Level (µg/100 cm ²) ^a	No. Test Portions	No. Positive	POD (95% CI) ^b
Stainless steel - wet	0	0	5	0	0 (0, 0.43)
	1	0.95	30	7	0.23 (0.12, 0.41)
	2	2.0	30	23	0.77 (0.59, 0.88)
	3	3.0	30	30	1 (0.89, 1)
	5	4.8	5	5	1 (0.57, 1)
Stainless steel - wet ^c	0	0	5	0	0 (0, 0.43)
	1	1.0	30	19	0.63 (0.46, 0.78)
	2	2.0	30	30	1 (0.89, 1)
	5	5.0	5	5	1 (0.57, 1)
Stainless steel - dry	0	0	5	0	0 (0, 0.43)
	1	1.4	30	20	0.67 (0.49, 0.81)
	2	1.9	30	27	0.90 (0.74, 0.97)
	3	3.0	30	29	0.97 (0.83, 1)
	5	4.5	5	5	1 (0.57, 1)
Stainless steel - dry ^c	0	0	5	0	0 (0, 0.43)
	1	1.0	30	12	0.40 (0.25, 0.58)
	2	2.0	30	30	1 (0.89, 1)
	5	5.0	5	5	1 (0.57, 1)
Plastic - wet	0	0	5	0	0 (0, 0.43)
	1	1.2	30	10	0.33 (0.19, 0.51)
	2	2.0	30	29	0.97 (0.83, 1)
	3	3.0	30	30	1 (0.89, 1)
	0	0	5	0	0 (0, 0.43)
Plastic - dry	1	1.2	30	8	0.27 (0.14, 0.44)
	2	2.0	30	28	0.93 (0.79, 0.98)
	3	3.0	30	30	1 (0.89, 1)
	5	5.0	5	5	1 (0.57, 1)

^a Determined using the Veratox for Peanut Allergen quantitative assay.

^b Probability of detection with 95% confidence interval.

^c Trial performed by independent laboratory.

REFERENCES CITED

1. Le, Q.N., Vance, A., Bakir, N., Almy, D., Slenk, E., Roman, B., Klass, N., Bastin, B., and Donofrio, R., Validation of the Reveal® 3-D for Peanut Lateral Flow Test, AOAC *Performance Tested Methods*SM certification number 111901.