



Reveal® 3-D for Gluten

VALIDATION REPORT

Revision 2, June 2023



Reveal[®] 3-D

for Gluten

Summary

Reveal[®] 3-D for Gluten (Item number 8505) is uniquely designed with three lines of detection and can be used virtually anywhere to screen environmental swabs, clean-in-place (CIP) rinses, and food samples for the presence of wheat, barley or rye. The test employs the principles of lateral flow chromatography immunoassay and is a highly sensitive and specific test designed to screen for very low parts per million (ppm) levels of gluten.

This validation report details the findings of the experimental evaluation undertaken to determine the test parameters and establish the performance characteristics for the suitability of testing surfaces, CIP rinses and foods (foods in conjunction with #8503 3-D Gluten Food Buffer).

In an AOAC Research Institute Performance Tested Method (PTM) study, the Reveal for 3-D Gluten was found to be an effective method for the qualitative determination of the presence of gluten from wheat in environmental swabs from stainless steel and CIP rinse. The details of this validation are published in the AOAC Journal.

Limit of detection (LOD): The LOD of the Reveal 3-D for Gluten test in a matrix was found to be 5 ppm gluten. Swabbing experiments detected 5 µg/100 cm² gluten on stainless steel, plastic and a non-stick surface.

Selectivity: Testing shows that Reveal for 3-D Gluten shows positive results in wheat, barley and rye sources, as well as in purified gliadin.

Cross-reactivity: Cross-reactivity to almond was observed in the Reveal 3-D Gluten test. No other cross-reactors were observed in select gluten-free commodities.

Ruggedness: To determine ruggedness of the test, three operators tested two different lots of devices over two days. It was concluded that the ruggedness data produced validated the limit of detection published in the assay kit insert.

Robustness: Devices were shown to report accurately despite variation in several sample parameters, including extraction time, run time, and sample size.



Materials and Methods

All tests were conducted on standard quality control (QC) approved lots of Reveal 3-D for Gluten assays. All assays were performed in accordance with the test kit insert.

Scoring of the Lines

Throughout the report, the line intensity of the control, test, and overload line was scored by comparing the device to a reference card. This card is used for Neogen purposes only. The scale is measured between 0 (no line intensity) – 5 (the highest line intensity).

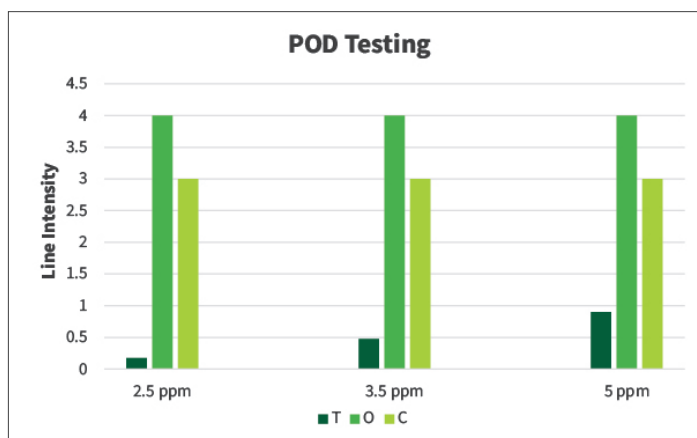
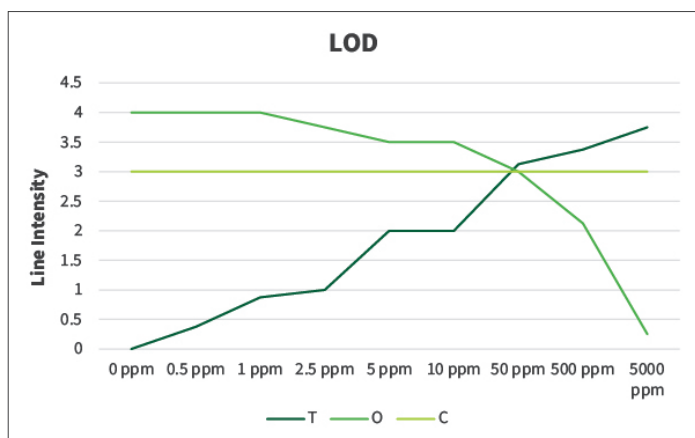
Gliadin

This assay reports results based on ppm gluten. However, some studies in this validation report were based on ppm gliadin. As mentioned in the summary, gliadin is a prolamin of gluten that makes up about 50% of gluten by weight. Any studies that measure gliadin will be labeled as such. Any result recorded in ppm gliadin can be converted to ppm gluten by multiplying the gliadin value by 2.

LOD

Surface Recovery

Surfaces commonly found in industry; stainless steel, plastic, and non-stick (Teflon), were artificially contaminated with known levels of gluten. Gluten protein solutions equivalent to 1, 5, 10, and 20 $\mu\text{g}/100\text{ cm}^2$ were deposited on the surface and left to dry. The surface was swabbed and extracted following the test kit insert instructions. Extracted sample was run on the device (n=3) and the line intensity of the test, overload and control lines were recorded.



Gluten Surface Recovery: LOD

	0 $\mu\text{g}/100\text{ cm}^2$	1 $\mu\text{g}/100\text{ cm}^2$	5 $\mu\text{g}/100\text{ cm}^2$	10 $\mu\text{g}/100\text{ cm}^2$	20 $\mu\text{g}/100\text{ cm}^2$
% Positive Results					
Stainless Steel	0	0	100	100	100
Teflon	0	0	100	100	100
Plastic	0	0	100	100	100

Results

The mean of three squares was calculated for the test line, overload line and control line for each sample. On stainless steel and a plastic surface, a mean visual score of >1 was observed at 5 $\mu\text{g}/100\text{ cm}^2$. On the non-stick surface a mean visual score of 0.8 at 5 $\mu\text{g}/100\text{ cm}^2$ was observed. The data demonstrate that all three surfaces give 100% positive results at 5 $\mu\text{g}/100\text{ cm}^2$ and greater.

LOD

To determine the absolute LOD and behavior of the assay, a qualified gluten allergen solution was diluted in 60% ethanol and extracted in buffer to give a representative range of ppm levels and tested on the Reveal 3-D for Gluten devices.

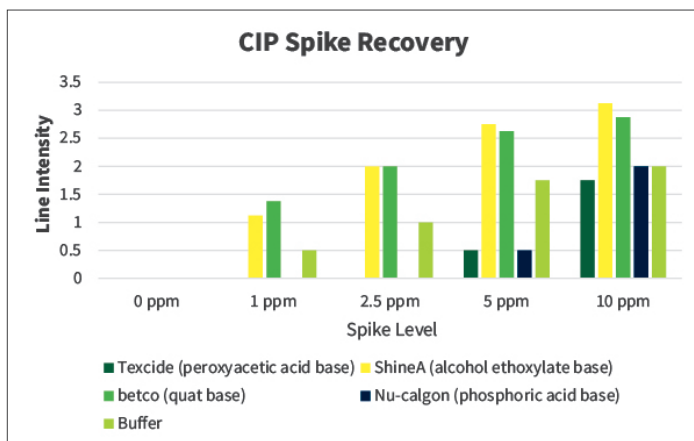
The probability of detection (POD) was determined through spiking buffer and diluting to concentrations ranging from 0 to 5 ppm gluten (n=20).

Results

The POD is 100% at 5 ppm gluten in buffer.

CIP Rinse Testing

Four working strength cleaners common to the food and beverage industry were tested to determine device response and recovery with the Reveal 3-D for Gluten assay. Gliadin extract was spiked into the cleaners. Results in the chart below are measured in ppm gliadin (see note on gliadin in Materials and Methods).



Results

3 of the 4 cleaners tested positive at 2.5 ppm gliadin (5 ppm gluten). The phosphoric acid-based cleaner interferes with the assay and did not test positive until 5 ppm gliadin (10 ppm gluten).

Commodity Testing

A variety of gluten-containing food commodities across multiple processing conditions were also evaluated on the assay.

Food Commodity Testing

Sample	Dilution Level	Results
Whole Wheat Pasta	1:100	Positive
Crispy Onions	1:100	Positive
Cereal	1:100	Positive
Pretzels	1:1000	Positive
Wheat Crackers	1:1000	Positive
Chocolate Chip Cookies	1:100	Positive
Canned Pasta	1:10	Positive
Whole Grain Waffles	1:100	Positive
Meatballs	1:100	Positive
Pizza Rolls	1:1000	Positive
Corn Dogs	1:1000	Positive
Chocolate Chip Cookie Dough Ice Cream	1:100	Positive

Results

The assay detected gluten present in multiple foods with various types of processing.

Spike and Recovery

Various gluten-free food commodities were spiked with gluten. Product samples were extracted in accordance with the Food Extraction protocol and using Item Number 8503 3-D Gluten Food Buffer. They were then diluted, tested, and confirmed on Veratox[®] for Gliadin R5. Results below are measured in ppm gluten.

Gluten-free Food Commodity Spike and Recovery

Sample	Result
Bread 5 ppm	Positive
Cheerios 5 ppm	Positive
Cookie 5 ppm	Positive
Rice 5 ppm	Positive
Soy Flour 5 ppm	Negative
Soy Flour 20 ppm	Positive/Fractional
Soy Flour 40 ppm	Positive

Results

Neat soy flour showed some interference at low levels of gluten. This was likely due to matrix interference from high levels of soy. The interference goes away at 20 ppm gluten.

Selectivity

The effectiveness of the test was measured against wheat, rye and barley, as well as purified gliadin. Gluten protein is found in wheat, barley and rye. Gliadin samples were prepared using purified gliadin extract while commercial wheat, rye and barley flours were obtained. Samples were prepared and extracted via kit method and diluted in kit running buffer to the listed levels. Concentration of gluten in flours was determined based on mass of sample

Selectivity to Different Gluten Sources

Gliadin (ppm)	Result	Barley (ppm gluten)	Result
0.5	Positive/ Fractional	0.5	Positive/ Fractional
1	Positive	1	Positive/ Fractional
2.5	Positive	2.5	Positive
5	Positive	5	Positive
10	Positive	10	Positive
50	Positive	50	Positive
500	Positive	500	Positive
5000	Positive	5000	Positive

Wheat (ppm)	Result	Rye (ppm gluten)	Result
0.5	Positive/ Fractional	0.5	Positive/ Fractional
1	Positive/ Fractional	1	Positive/ Fractional
2.5	Positive/ Fractional	2.5	Positive
5	Positive	5	Positive
10	Positive	10	Positive
50	Positive	50	Positive
500	Positive	500	Positive
5000	Positive	5000	Positive

Results

The kit showed 100% positive results for all samples at 5 ppm gluten (2.5 ppm gliadin for the gliadin samples). Fractional positives were observed as low as 0.5 ppm gliadin/1 ppm gluten.

Cross-reactivity

A panel of 46 potential cross-reactors were tested and tabulated in the figure below. The Reveal 3-D for Gluten test cross-reacts with almond and sesame. Corn also demonstrated cross-reactivity using the food method only, no cross-reactivity was observed when testing environmental samples. Sample dilutions may assist with removing a false positive signal, please consult with your Neogen representative. All other cross-reactors test negative at 100%.

Chestnut flour does not cross-react with gluten. Chestnut flour is known to inhibit detection of gluten in this assay. A decreased response was observed in chestnut flour up to a 1:50 dilution. The effect of chestnut flour can be matrix-dependent, please contact a Neogen representative for further details.

Cross-reactivity Data

Sample (Neat)	Result
Almond	Positive
Beef	Negative
Black-eyed Pea	Negative
Black Bean	Negative
Brazil Nut	Negative
Brown Rice Flour	Negative
Cashew	Negative
Cinnamon	Negative
Cocoa	Negative
Coconut Flour	Negative
Corn	Negative (Pos for Food Method)
Chestnut Flour	Negative
Chick Pea Flour	Negative
Egg Powder	Negative
Flaxseed	Negative
Great Northern Bean	Negative
Green Pea	Negative
Hazelnut	Negative
Kidney Bean	Negative
Lentil	Negative
Lima Bean	Negative
Macadamia Nut	Negative
Milk	Negative
Navy Bean	Negative

Sample (Neat)	Result
Non-fat Dried Milk	Negative
Oat	Negative
Parsley	Negative
Peppercorn	Negative
Pine Nut	Negative
Pinto Bean	Negative
Pistachio	Negative
Poppy Seed	Negative
Pork	Negative
Pumpkin	Negative
Quinoa Flour	Negative
Rice Flour	Negative
Rosemary	Negative
Sesame	Positive
Sorghum Flour	Negative
Soy Flour	Negative
Sunflower Seed	Negative
Tapioca Flour	Negative
Tea	Negative
Turkey	Negative
UHT Almond Milk	Negative
Wine	Negative
Yeast	Negative

Ruggedness: Inter- and Intra-assay Variability

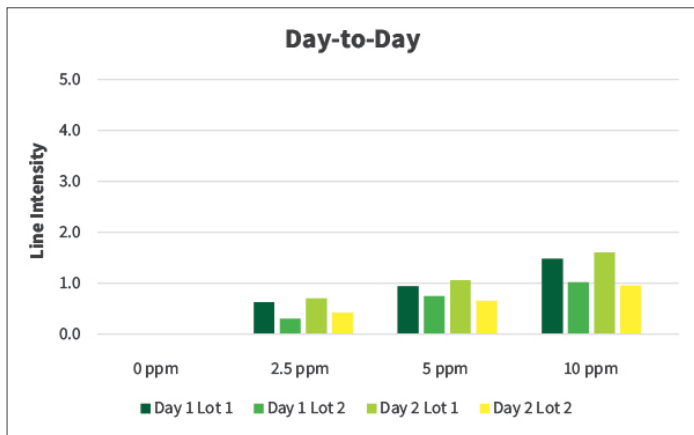
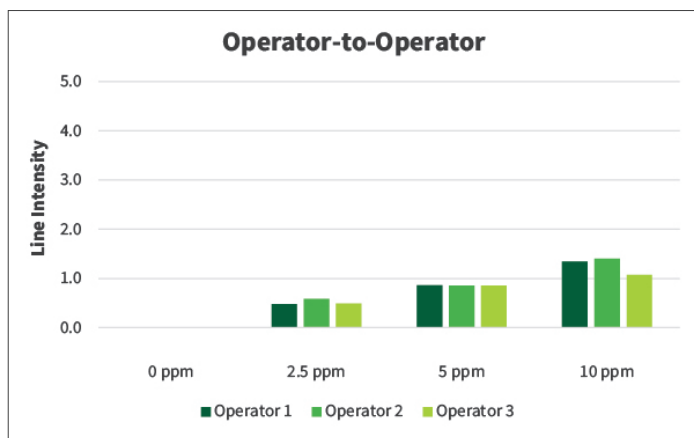
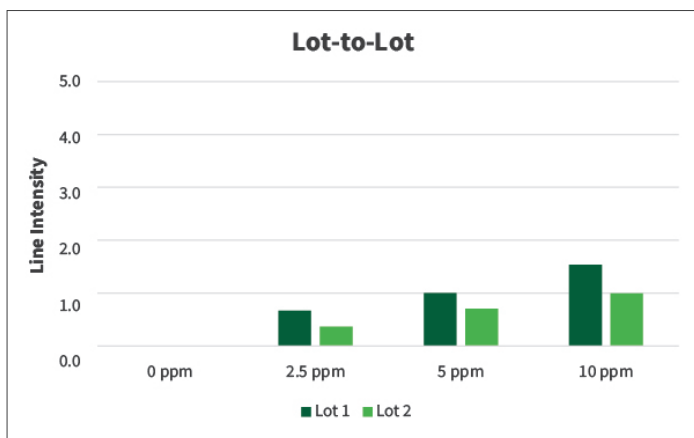
Inter-assay and intra-assay variability were evaluated by testing standards at 0, 2.5, 5, and 10 ppm gluten. with three different operators throughout two days using two different batches of devices, and five devices per level. All standards were presented to each operator randomized and blind.

Results

The data demonstrate all gluten-positive samples are detected properly across multiple lots, users and days.

Lot-to-lot, day-to-day and operator-to-operator variability in line intensity is minimal. Slight variation in the visual scoring of the line is attributed to operator subjectivity.

Ruggedness and LOD testing (see LOD section above) demonstrate that the 100% probability of detection for gluten is 5 ppm or less.



Robustness

To determine the level of robustness of Reveal 3-D for Gluten test, various parameters were intentionally altered relative to the standard method conditions. These were performed alongside the standard running conditions and tested at 0 and 5 ppm.

Device Parameters and Results

Condition	Variation	0 ppm (score)	5 ppm (score)
Extraction Time	30 seconds	Neg (0)	Pos (1)
	60 seconds*	Neg (0)	Pos (2)
	90 seconds	Neg (0)	Pos (2)
Incubation Time	4 min	Neg (0)	Pos (1)
	5 min*	Neg (0)	Pos (1)
	6 min	Neg (0)	Pos (2)
Sample Volume	0.2 mL	Neg (0)	Pos (2)
	0.25 mL*	Neg (0)	Pos (2)
	0.30 mL	Neg (0)	Pos (2)

*Standard testing conditions

Results

All devices reported correctly across all variations to the standard procedure, indicating a highly robust device.

Conclusion

Reveal 3-D for Gluten is a sensitive and robust lateral flow device suitable for screening both environmental samples and foods to detect low levels of gluten.

