



# Reveal 3-D for Almond Allergen

Technical Product Information

## Validation Report for Reveal 3-D for Almond Allergen (Neogen item 902086G)

Revision 2: December 2016

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TECHNICAL PRODUCT INFORMATION

# Reveal 3-D for Almond Allergen

## SUMMARY

The Reveal 3-D for Almond (Neogen item 902086G) is uniquely designed with three lines of detection and can be used virtually anywhere to screen environmental swabs, clean-in-place (CIP) rinses and liquid products for the presence of almond. This test employs the principles of lateral flow chromatographic immunoassay and is a highly sensitive and specific test designed to detect very low parts per million (ppm) level of almond. This lateral flow device contains an overload detection system designed to ensure highly positive samples are not reported as false negative.

This validation report details the findings of the experimental evaluation designed to validate the test parameters and establish the performance characteristics for the suitability of testing surfaces and CIP rinses in conjunction with Reveal 3-D Extraction Buffer Type 12.

**Limit of Detection:** The limit of detection in buffer was found to be 2.5 ppm total almond. Swab sensitivity was found to be 1.0 µg/100cm<sup>2</sup> almond protein on stainless steel, plastic and a non-stick surface with a rate of 100%. Partial recovery was observed at 0.5 µg/100cm<sup>2</sup>.

**Commodity testing:** Commodity testing showed recovery and detection of almond residue in spiked almond free commodities, CIP rinses and naturally incurred samples.

**Cross reactivity:** Cross reacts with apple seed an common *Prunus* genus foods (ie. apricot, cherries, nectarines, peaches and plums). Cross reactivity has been identified with the seed (stone); however the flesh (seedless fruit) does not cross react.

**Robustness:** To determine the robustness of the test method three operators tested three different lots of devices over two days. All samples on were reported correctly by each operator. Slight variations were seen across lots, days and operators but was attributed to interpretation in the intensity of the line scores as there was no trend identified across all variables. Overall the test is robust and reports accurately.

**Ruggedness:** Testing demonstrates that variation in sample interpretation within the device parameters for extraction time, run time, and presence of different CIP sanitizers does not greatly affect the detection behavior of the device. The variables that show a slight change from the standard parameters are sample and extraction buffer volume.

## MATERIALS AND METHODS

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

### Scoring of the line

Throughout this report, the relative line intensity of the test, overload and control line was scored by comparing the device to an internal reference card. The scale ranges from 0 (no line intensity) – 5 (the highest line intensity).

## LIMIT OF DETECTION

### Surface recovery

Surfaces commonly found in industry; stainless steel, plastic and non-stick (Teflon), were artificially contaminated with known levels of almond. Almond protein solutions equivalent to 0, 0.5, 1, 2, 4 and 8 µg/100cm<sup>2</sup> 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.

### Results

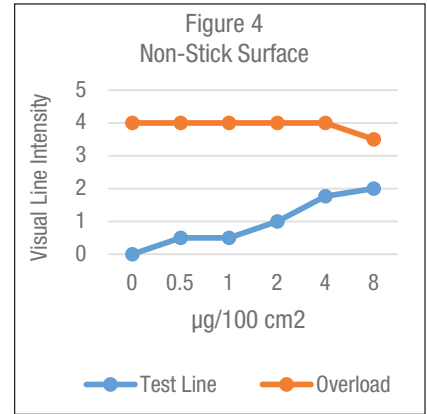
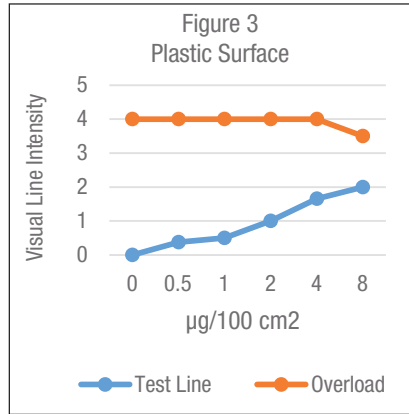
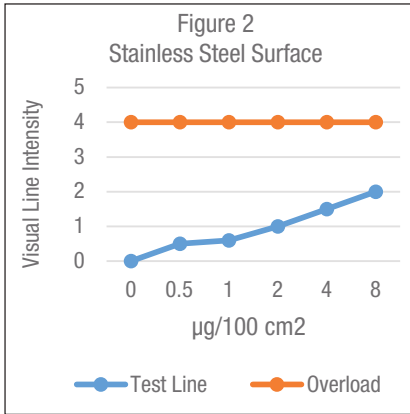
The mean of three squares was calculated for the test line, overload line and control line for each sample. On stainless steel and non-stick surface a mean visual score of 0.5 was seen at 0.5 µg/100cm<sup>2</sup>. On the plastic surface a mean visual score of 0.5 at 1 µg/100cm<sup>2</sup> with partial positives at 0.5 µg/100cm<sup>2</sup>. The overload and control lines performed as expected (not shown).

The level of detection on swabbed surfaces for Reveal 3-D for Almond is 1µg/100cm<sup>2</sup> with partial recovery at 0.5 µg/100cm<sup>2</sup>.

**Figure 1. Almond Surface Recovery - Limit of Detection**

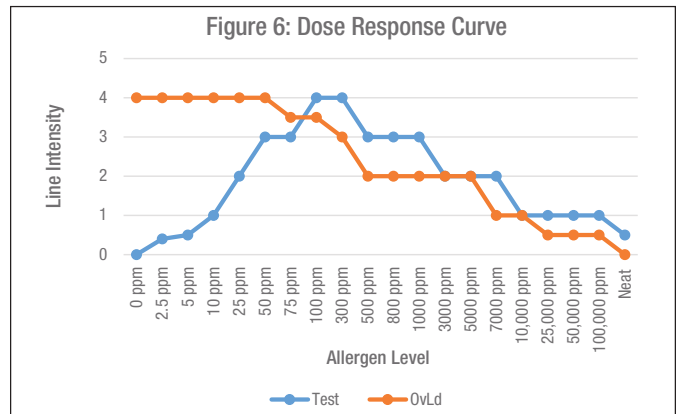
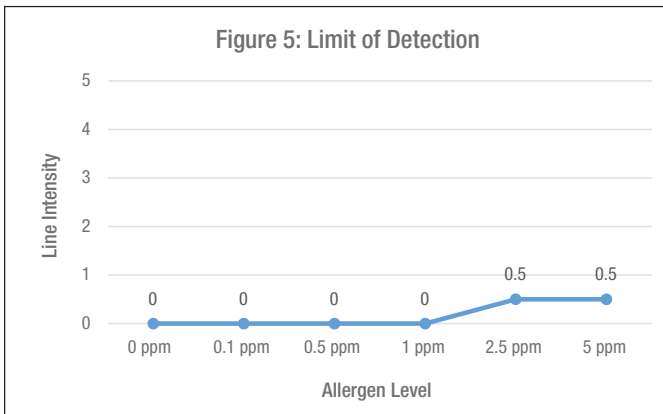
	0 µg/100 cm <sup>2</sup>	0.5 µg/100 cm <sup>2</sup>	1 µg/100 cm <sup>2</sup>	2 µg/100 cm <sup>2</sup>	4 µg/100 cm <sup>2</sup>	8 µg/100 cm <sup>2</sup>
	% Positive Result					
Stainless Steel	0%	100%	100%	100%	100%	100%
Plastic	0%	77%	100%	100%	100%	100%
Non-stick surface	0%	100%	100%	100%	100%	100%

## Surface Recovery - Almond Protein



## Limit of detection

To determine the absolute limit of detection and behavior of the device a qualified almond allergen solution was diluted in buffer to give a representative range of ppm levels and tested on the Reveal 3-D for Almond devices. Almond flour extract was utilized to display the overload behavior.



## Results

The data in Figure 5 and 6 demonstrate the relationship between the almond residue amounts (ppm) in a buffer spike and the visual device line intensities.

The absolute limit of detection is 2.5 ppm total almond in buffer (see Figure 5). The complete curve data shows that the test line peaks in line intensity between 100 and 300 ppm, with the overload line being lighter than the test line at a level near 25,000 ppm and completely disappearing with a 100% almond flour extract (see Figure 6). The control line is present for all test strips (not shown).

## COMMODITY AND CLEAN-IN-PLACE (CIP) RINSE TESTING

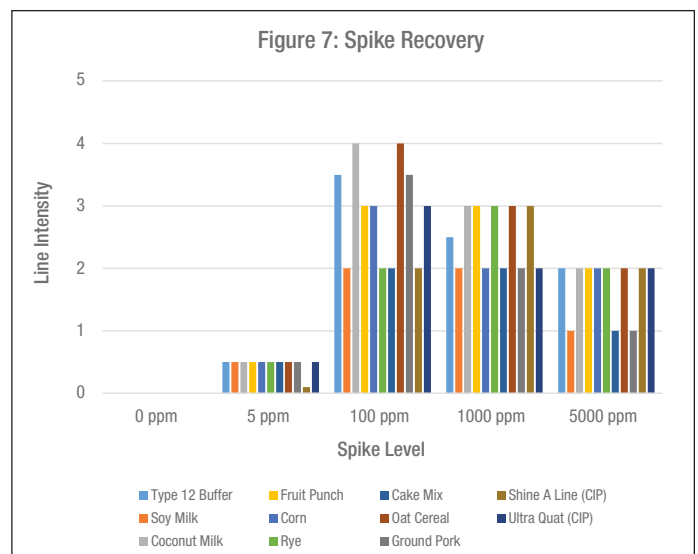
Various commodities and working strength sanitizers common to the food and beverage industry were tested to determine device response and recovery with the Reveal 3-D for Almond device. Known amounts of almond residue were spiked into dry foods, liquids, and sanitizers used as clean in place (CIP) rinses (Figure 7).

A screen of ultra-high temperature (UHT) almond beverages was also performed to confirm allergen detection. The dilution levels of each UHT sample was verified with by the Veratox for Almond test to confirm proper reporting correspondence between the Reveal 3-D and Veratox assays.

## Results

The Reveal 3-D for Almond devices were all negative at 0 ppm. Ten of the eleven samples were visually interpreted as positive at 5, 100, 1000 and 5000 ppm. The Shine-A-Line CIP matrix was only partially positive at 5 ppm and 100% visually positive at 100, 1000 and 5000 ppm indicating some reduction in sensitivity in Shine-A-Line matrix.

The graph shows the relationship of the test line response to the recovery of almond in various commodities and sanitizers.



## Reveal 3-D for Almond Allergen

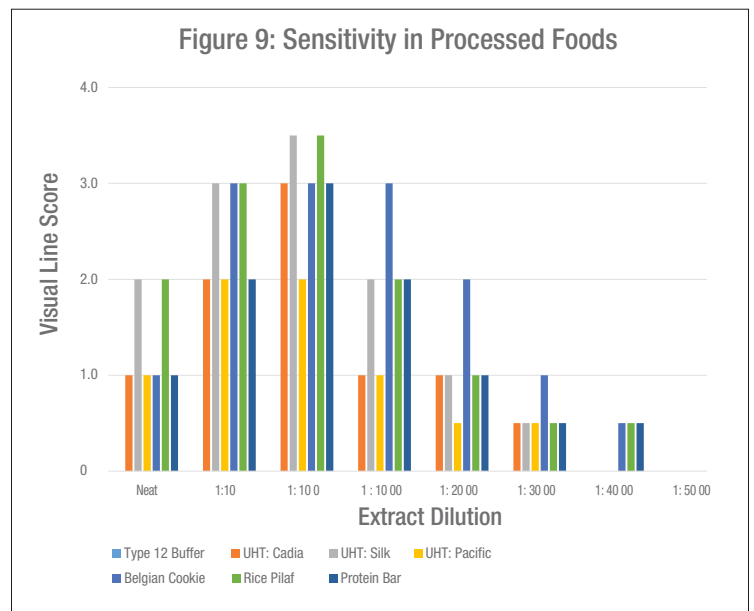
Additional testing was performed on three different working strength cleaners. One a quaternary ammonium base, a chlorine base and a phosphoric acid base. Almond material spiked into the quaternary ammonium and phosphoric acid base cleaner was detectable at 5 ppm and demonstrated an appropriate dose response. The chlorine base cleaner interferes with the assay and was not able to detect almond material in this study.

**Figure 8: Almond Detection in Sanitizer Solutions**

Clean in Place Rinses (at recommended working strength)									
Rinse (ppm spike)	Quaternary Ammonium Sanitizer / disinfectant (Ultra Quat)			Chlorine -based Cleaner (*Cirtec)			Phosphoric Acid Cleaner (Shine A Line)		
	Test	OvLd	Control	Test	OvLd	Control	Test	OvLd	Control
Type 12 Buffer	0	4	4	0	4	4	0	4	4
Cleaner Only	0	4	4	0	4	4	0	4	4
2.5 ppm	0.5	4	4	0	4	4	0	4	4
5 ppm	1	4	4	0	4	4	0.5	4	4
10 ppm	2	4	4	0	4	4	0.5	4	4
20 ppm	2	4	4	0	4	4	1	4	4
50 ppm	3	4	4	0	4	4	2	4	4
75 ppm	3	3	4	0	4	4	3	4	4
100 ppm	4	3	4	0	4	4	3	3.5	4

### Processed Food Detection

Samples including almond as an ingredient were evaluated neat and in a series of dilutions. The UHT beverages were detectable neat and up to a 1:3000 dilution. Belgian cookie, rice pilaf and the protein bar were detected neat and up to a 1:4000 dilution. All samples diluted 1:5000 were below the test limit of detection (Figure 9).



These samples were also evaluated on the Veratox for Almond Allergen test and found to contain 2-12 ppm levels of almond at the final dilution.

**Figure 10: ELISA Analysis of Almond Containing Foods**

Assay Reporting Summary			
Sample	Lowest Reporting Dilution on 3-D Device	3-D Device Test Line Score	Almond Veratox ppm
UHT: Cadia	1 : 3000	0.5	2.3
UHT: Silk	1 : 3000	0.5	2.0
UHT: Pacific	1 : 3000	0.5	2.0
Belgian Cookie	1 : 4000	0.5	5.7
Almond Rice Pilaf	1 : 4000	0.5	12.0
Protein Bar	1 : 4000	0.5	5.4

### CROSS REACTIVITY

Cross reacts with apple seed an common *Prunus* genus foods (ie. apricot, cherries, nectarines, peaches and plums). Cross reactivity has been identified with the seed (stone); however the flesh (seedless fruit) does not cross react. A panel of 36 additional samples including nuts, grain, proteins and seed were also tested and did not demonstrate any cross-reactivity with the Reveal 3-D for Almond allergen test.

**Figure 11: Cross-reactivity**

Sample	Result	Sample	Result	Sample	Result
Type 12 Buffer	Negative	Pistachio	Negative	Lentil	Negative
Chestnut	Negative	Poppy Seed	Negative	Great Northern Bean	Negative
Peanut	Negative	Brown Rice	Negative	Navy Bean	Negative
Macadamia	Negative	Sesame	Negative	Pinto Bean	Negative
Adzuki Bean	Negative	Split Pea	Negative	Rye	Negative
Anasazi Bean	Negative	Wheat Gluten	Negative	Kidney Bean	Negative
Black Eye Pea	Negative	Wheat Flour	Negative	Fresh Garlic	Negative
Walnut	Negative	Coconut Milk: Brand A	Negative	Pine Nuts	Negative
Cashew	Negative	Coconut Milk: Brand B	Negative	Black Turtle Bean	Negative
Chick Pea	Negative	Canned Coconut Milk	Negative	Ground Beef	Negative
Cocoa*	Negative	Pecan	Negative	Ground Pork	Negative
Lima Bean	Negative	Hazelnut	Negative	Ground Turkey	Negative

\* Commodity provided flow issues.

### BETA SITE RESULTS

Intensive validations were completed on multiple commodities, such as CIP rinses (e.g., working strength sanitation cleaners commonly used in food productions facilities) and environmental swabs. In each case, the recovery was excellent. The beta site evaluation included eight independent testing locations. Each site was asked to evaluate the method and performance of the test kit by evaluating blind samples provided by Neogen.

Eight samples were extracted and tested using a new Reveal 3-D for Almond device. Site 1 recorded an invalid result indicating the control line did not develop. All other sample results were interpreted correctly by all users.

**Figure 12: Beta Site Test Results**

Sample	Almond level	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	% accurate
A	0 ppm	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	100%
B	25 ppm almond	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%
C	100 ppm almond	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%
D	UHT Almond beverage diluted 1:50	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%
E	UHT Almond beverage diluted 1:100	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%
F	UHT Almond beverage diluted 1:50	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%
G	UHT Almond beverage diluted 1:100	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%
H	Almond Paste	Invalid	Pos	Pos	Pos	Pos	Pos	Pos	Pos	100%

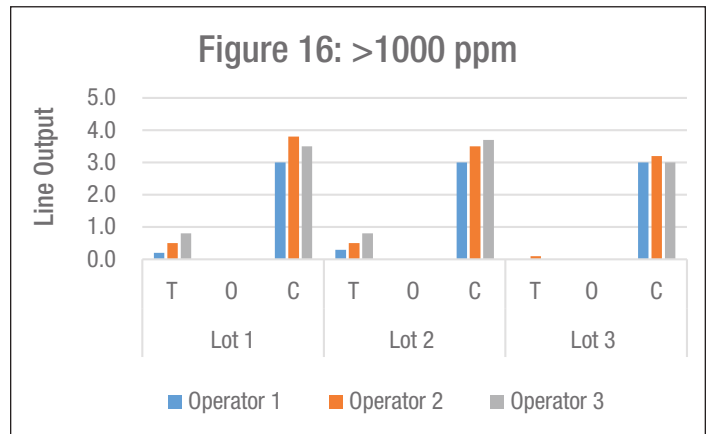
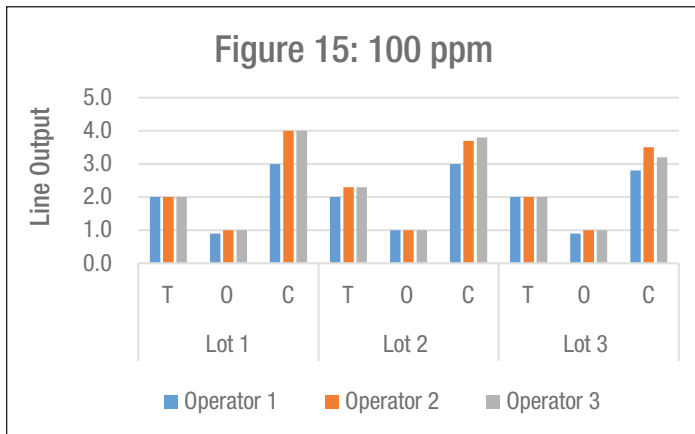
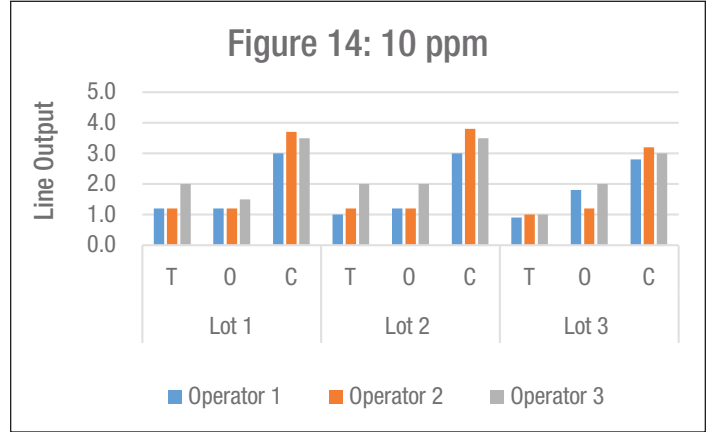
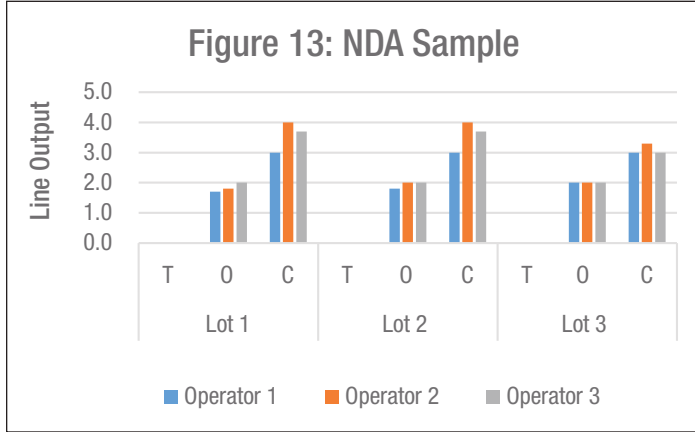
# Reveal 3-D for Almond Allergen

## ROBUSTNESS - INTER AND INTRA VARIABILITY

Inter-assay and intra-assay variability was evaluated by testing samples at 0, 10, 100 and 1000 ppm with three different operators on two days using three lots of Reveal 3-D for Almond devices. All samples were presented to each operator randomized and blind (see Figures 13 – 24).

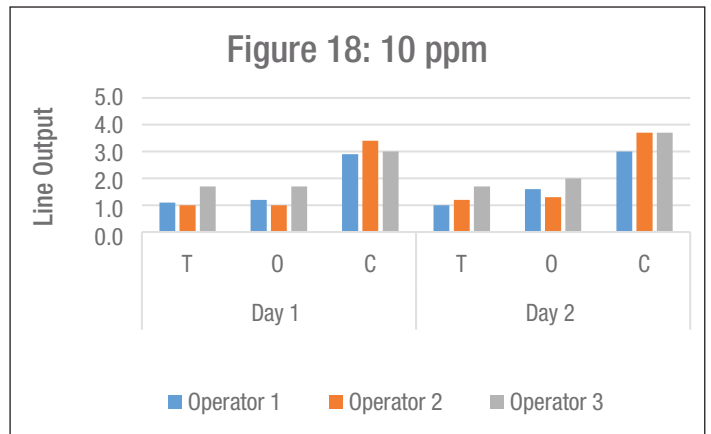
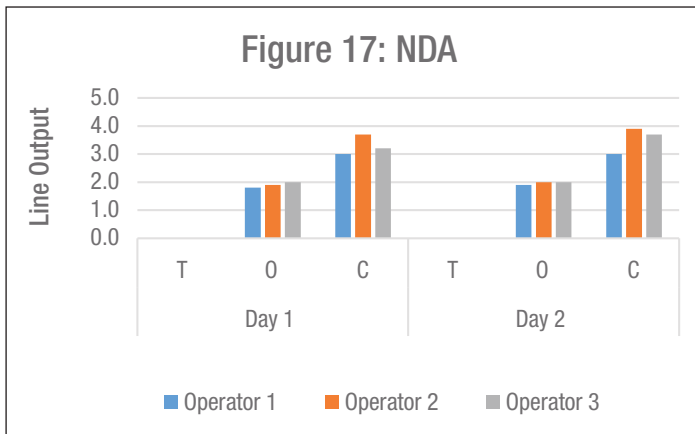
### Lot-to-lot variation

Note: Day one and two visual test line scored results averaged.

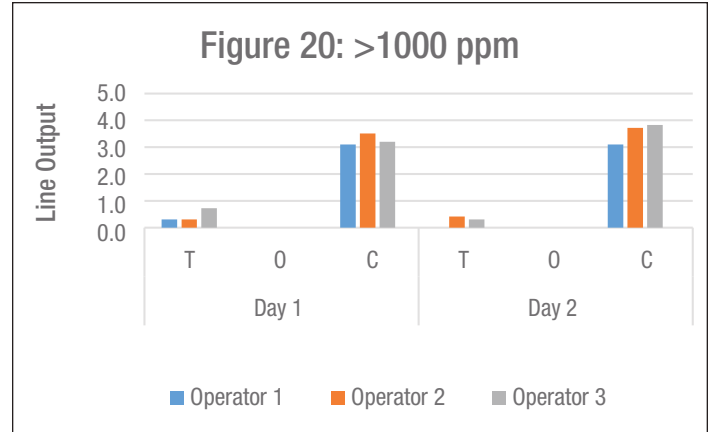
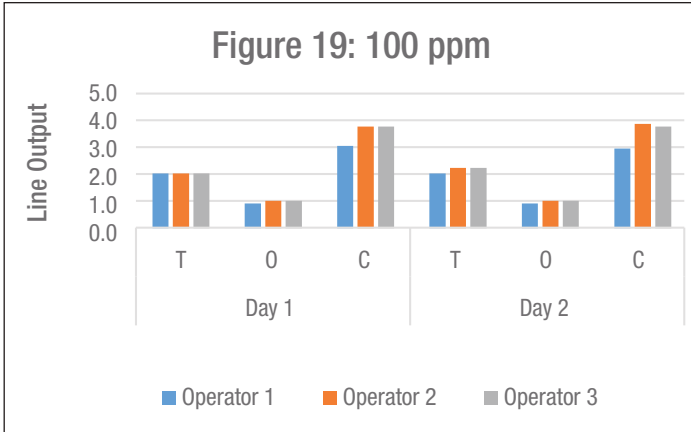


### Day-to-day variation

Note: Lots 1, 2 and 3 visual test line scored results averaged.

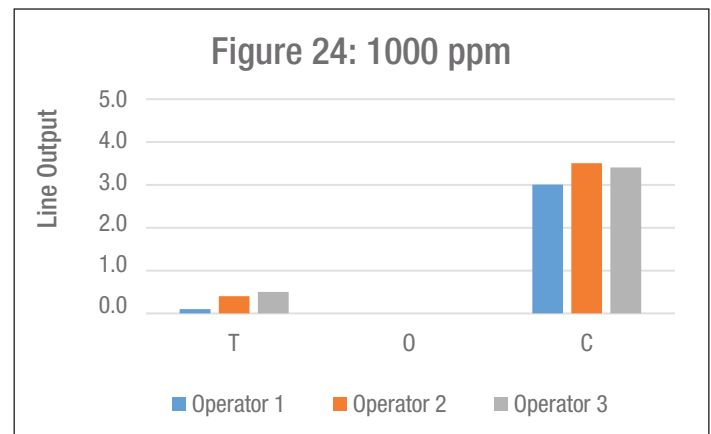
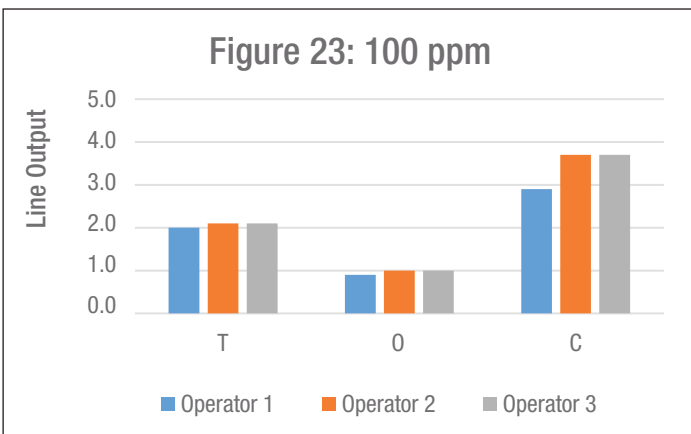
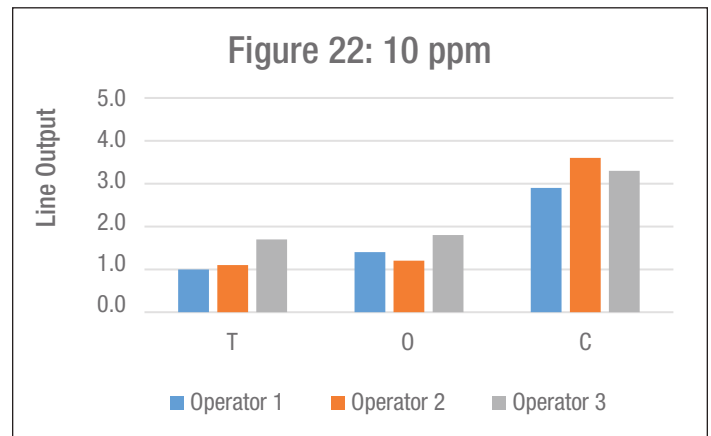
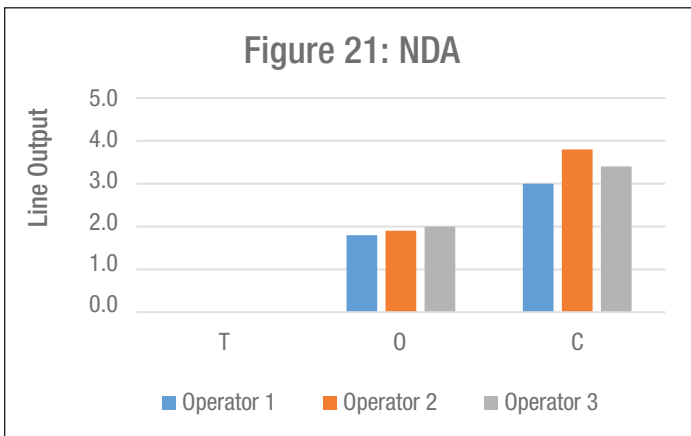


## Reveal 3-D for Almond Allergen



### Operator-to-operator variation

Note: Day 1 and 2 visual test line scored results averaged.



### Conclusion

The data in Figures 13 – 24 show all 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 and variations can be attributed to operator interpretation in the intensity of the line scores.

# Reveal 3-D for Almond Allergen

## RUGGEDNESS - DEVICE PARAMETER VARIATION

To determine the level of ruggedness of the Reveal 3-D for Almond Test, various parameters of the test were identified and varied. These were performed alongside the normal running conditions and tested at 0, 5, 100 and 1,000 ppm to determine any critical processes within the test.

**Figure 25: Ruggedness Testing**

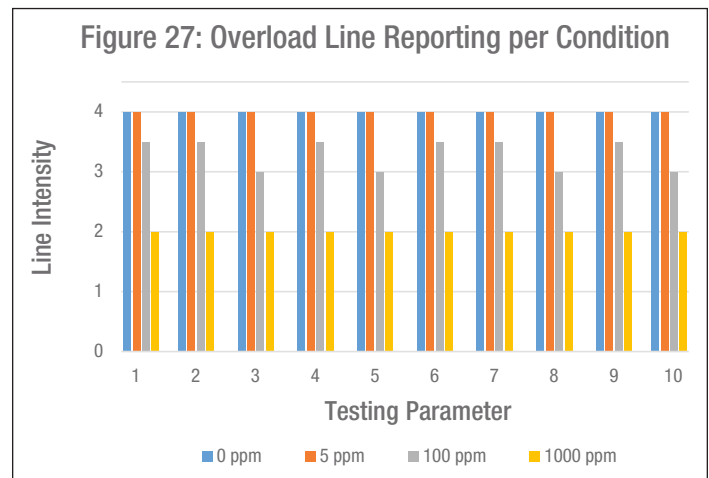
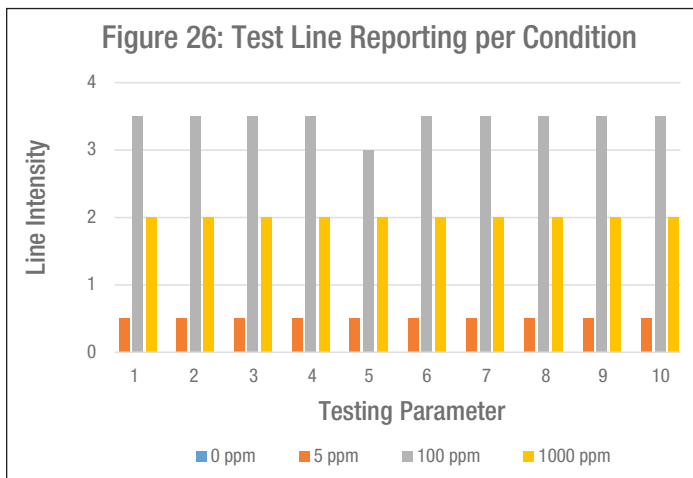
	Variations	1*	2	3	4	5	6	7	8	9	10
Sample Volume	± 40% (± 0.1mL)	0.25	0.15	0.35	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Type 12 Volume	± 12.5% (± 0.5mL)	4	4	4	3.5	4.5	4	4	4	4	4
Type 12 Temp	4°C vs 22°C	22°C	22°C	22°C	22°C	22°C	4°C	22°C	22°C	22°C	22°C
Extraction Time	± 50% (± 30 sec.)	60	60	60	60	60	60	30	90	60	60
Run Time	± 20% (± 1 min.)	5	5	5	5	5	5	5	5	4	6

\*Variations 1 is the standard device conditions

### Results

The mean of 3 replicates were calculated for the test, overload and control lines. As an internal check, the means of variation 1 was taken and used to compare any variations >0.5 (the smallest increment).

### Ruggedness Test Results



### Conclusion

For the 10 conditions tested, test line intensities demonstrated minimal or no variance. Overall, no significant deviations were observed and device results were accurate under all conditions. The results overall demonstrate a high level of ruggedness for Reveal 3-D for Almond Test.

### CONCLUSION

Reveal 3-D for Almond Allergen is a sensitive and robust lateral flow device suitable for screening CIP rinses, liquid products and environmental samples to detect low levels of almond residue.



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