

# The Development and Validation of Reveal<sup>®</sup> 3-D for Sesame, a Lateral Flow Device for the Rapid Detection of Sesame in Clean-In-Place (CIP) Rinses and Environmental Samples

Greig Christie, Pauline Titchener, Karrie Melville and Jennifer Rice



## ABSTRACT

Neogen has developed a rapid lateral flow device for detection of sesame. This test is a sandwich-based assay utilizing one antibody for capture and a second antibody linked to a colored particle for detection. An extraction process has been developed to allow for detection of sesame in various sample types including clean-in-place rinses and environmental samples. Reveal 3-D for Sesame is an accurate, rapid and simple assay that requires no special tools or extensive training and yields results in less than 10 minutes from sample collection. The lower limit of detection is 5 ppm sesame in CIP rinse waters and 5 µg/100 cm<sup>2</sup> for sesame proteins on stainless steel, plastic and Teflon surfaces. The Reveal 3-D for Sesame lateral flow device features a unique overload line, which is included to signal the presence of a grossly contaminated solution or surface and eliminate the potential for false negative results in these samples.

**Purpose:** The purpose of this study was to develop and validate Reveal 3-D for Sesame for detection of sesame in CIP rinse waters and environmental samples and to determine the limit of detection of this assay.

## INTRODUCTION

Sesame is a highly allergenic food and the European Union and Canada have added it to their priority allergen lists, which include peanuts, tree nuts, milk, cereals, eggs, fish, crustacean, mollusks, soy and sulfites. Dishes containing sesame include tahini, gomashio, hummus and halva. Other sesame-containing foods include some noodles, dips, soups, sausages, samosas, processed meats, chutneys, salad dressings, mixed spices, spreads and confectionery bars.

Because of the widespread use of sesame in food production, there is a need to screen for the presence of sesame in food handling areas and equipment. Reveal 3-D for Sesame is a lateral flow device that provides rapid and accurate information regarding presence of sesame.

The assay takes 6 minutes which includes a 1 minute extraction and 5 minute device run time.

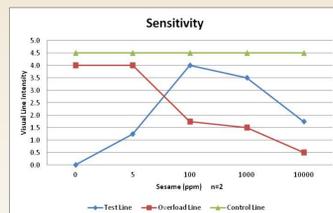
When analyzing rinses or liquid products, sesame residues are detectable at a level of 5–10 ppm. Levels of 5 µg/100 cm<sup>2</sup> of sesame protein on environmental surfaces can be detected.

## SCORING AND INTERPRETING LINE INTENSITIES

Throughout this testing, the line intensity of the control, test and overload line was scored by comparing the device to a reference card. The scale is measured between 0 (no line intensity) – 5 (the highest line intensity).

## SENSITIVITY

The limit of detection (LOD) was determined to be 5 ppm by evaluating levels of sesame from 0 ppm to 10,000 ppm in water. These samples were treated as rinse samples and follow the kit instructions for such a sample.



## SWABBING RECOVERY

Stainless steel, Teflon and plastic surfaces were contaminated with known levels of sesame protein to determine swab recovery. Sesame solutions equivalent to 0, 5 and 10 µg/100 cm<sup>2</sup> in buffer were deposited in triplicate in 100 µL amounts on a 10 cm x 10 cm area and left to dry for two hours. Surfaces were swabbed and extracted following the test kit insert instructions. The extracted samples were run in triplicate on the device and the line

## Reveal 3-D<sup>®</sup> for Sesame

### CIP RINSE SAMPLING

1. Open a Type 10 buffer sachet and add to a sample tube.
2. Add 1 mL of sample to the sample tube.
3. Secure cap and shake for 1 minute.

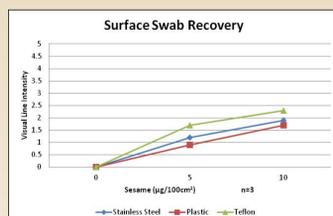
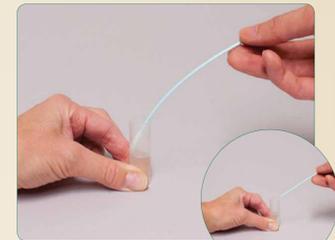
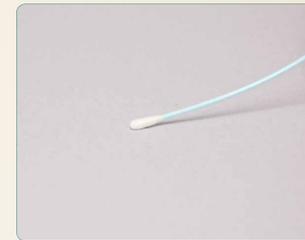
### SWAB SAMPLING

1. Open a Type 10 buffer sachet and add to a sample tube.
2. Gather the sample. For dry surfaces, moisten with extraction solution. Do not pre-moisten for wet surfaces.
3. Return the swab to the extraction buffer and break off into the tube.
4. Secure cap and shake for 1 minute.

### SAMPLE TESTING

1. Remove the lid and fill with liquid from the tube.
2. Dip the Reveal 3-D device into the liquid in the lid. Ensure that the cavity is saturated with the liquid.
3. Leave the cavity saturated until the liquid is observed into the test window.
4. Place the device on a flat surface and allow the test to develop for 5 minutes.
5. Interpret the results.

## Reveal 3-D for Sesame Procedure



intensity of the test, overload and control lines were recorded. Swabbing recovery of sesame protein was determined to be 5 µg/100 cm<sup>2</sup>.

	% Positive		
	0 µg/100 cm <sup>2</sup>	5 µg/100 cm <sup>2</sup>	10 µg/100 cm <sup>2</sup>
Stainless steel	0	100	100
Plastic	0	100	100
Teflon	0	100	100

## ROBUSTNESS – PARAMETER VARIABILITY

To determine the level of robustness of the Reveal 3-D for Sesame test, multiple parameters were varied. These were performed alongside the recommended running conditions and tested at 0, 5, 100 and 1,000 ppm sesame.

All variation results concurred with the recommended running conditions and mean visual line intensities differed by ≤0.5 score (the smallest measurable score). The results demonstrated a very high level of robustness for Reveal 3-D for Sesame.

Sample volume	Recommended	Variations
	1 mL	±10% (0.9 and 1.1 mL)
Buffer volume	4 mL	±5% (3.8 and 4.2 mL)
Buffer temperature	Ambient	2–8°C
Extraction time	60 seconds	±50% (30 and 90 seconds)
Extraction motion	Hand	No mix and vortex mix
Running time	5 minutes	±20% (4 and 6 minutes)

## ROBUSTNESS – INTER AND INTRA VARIABILITY

Inter-assay and intra-assay variability was evaluated by testing sesame at 0, 5, 100, and 1,000 ppm using three different operators on two days using three different batches of devices.

- Sensitivity and specificity was 100%. All operators scored the negative samples as negative and the 5, 100 and 1,000 ppm as positive over both days.
- Batch to batch variation was minimal. Within the individual operator groups, all mean visual intensities differed between batches by ≤0.5 score.
- Day to day variation was minimal. Within the individual operator groups, all mean visual line intensities differed between the days by ≤0.5 score.
- Operator to operator variation produced the most variability. Operator 3 consistently scored the lowest with averages being ≥0.5 score between operators in some instances. Operator 2 tended to score higher in most cases.

Regardless of variability, all negative samples were scored as negative and all positive samples were scored as positive.

## BETA SITE

Reveal 3-D for Sesame was tested by seven different industry professionals. Independent testing of four blind samples was performed in duplicate at each site. The samples included high positive, a moderate positive and two negative samples. The results returned were 100% accurate.

Site	% Positive			
	High Positive	Positive	Negative	Negative
Site 1	100	100	0	0
Site 2	100	100	0	0
Site 3	100	100	0	0
Site 4	100	100	0	0
Site 5	100	100	0	0
Site 6	100	100	0	0
Site 7	100	100	0	0

## CROSS-REACTIVITY

In total, 31 potential cross-reacting ingredients were tested on Reveal 3-D for Sesame at a concentration of 10,000 ppm. From the 31 tested, 26 ingredients produced negative results while 5 scored a very low positive result (estimated % cross-reactivity of 0.05%)\*.

### INGREDIENTS TESTED

**Nuts:** Macadamia, hazelnut, peanut, pecan, cashew, almond, pine, Brazil, pistachio, walnut\*

**Grain:** Rye, soya, soya protein mince, soya isolate, millet grain, wheat, oats, barley

**Pulses:** Green lentil, black eyed bean, chickpea, red lentil, green pea, red kidney bean\*, haricot bean\*

**Seeds:** Sunflower, pumpkin, cardamom, linseed\*, poppy\*

**Fruit:** Kiwi