TECHNICAL PRODUCT INFORMATION

Reveal® 3-D for Sesame

Validation Report for Reveal 3-D for Sesame (Neogen item 8535)

January 2014

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TECHNICAL PRODUCT INFORMATION

NEOGEN® CORPORATION

620 Lesher Place • Lansing, MI 48912
800/234-5333 (USA/Canada) • 517/372-9200
foodsafety@neogen.com • www.neogen.com

FD449-0114
NE1710

INTRODUCTION

Reveal 3-D for Sesame (Neogen item 8535) is uniquely designed with three lines of detection and can be used virtually anywhere to screen environmental swabs and clean-in-place (CIP) rinses for the presence of sesame allergen contamination. The test employs the principles of lateral flow chromatography enzyme immunoassay. It is a highly sensitive and specific test designed to screen for very low parts per million (ppm) levels of sesame.

This validation report details the findings of the experimental evaluation undertaken to determine the test parameters and establish the performance characteristics for the suitability for testing surfaces and CIP rinses in conjunction with type 10 extraction buffer.

SUMMARY

Reveal 3-D for Sesame utilizes highly specific antibodies to detect sesame protein. The test is designed to detect low ppm levels of sesame content in CIP rinses and from surfaces.

Limit of detection: Swabbing recovery experiments found Reveal 3-D for Sesame can detect as low as 5 µg/100 cm^2 from Teflon, plastic and stainless steel surfaces. The limit of detection (LOD) for Reveal 3-D for Sesame in CIP rinses was 5 ppm. The presence of chemicals or sanitizers in CIP rinses may affect the LOD. The LOD for the test was established as 0.5 ppm sesame in buffer.

Specificity/sensitivity: When three operators tested three batches of devices throughout two days, specificity and sensitivity were found to be 100%.

Internal reference: Reveal 3-D for Sesame was able to detect sesame at levels comparable with the sesame internal reference flour. The sesame internal reference flour was prepared using a mixture of white, brown and black sesame seeds. A portion of the sesame flour was chemically defatted as defatted sesame flour can be used as a food ingredient.

Cross-reactivity: A panel of 31 foodstuffs was tested for cross-reactivity with Reveal 3-D for Sesame. Cross-reactivity was found to be < 0.05%.

Dough study: Sesame dough was heat-treated at 100°C and 175°C. The Reveal 3-D for Sesame performed as expected, when taking the 1:3 dilution used to prepare the dough into account.

Toasted sesame study: Reveal 3-D for Sesame is not suitable for the detection of toasted sesame products or ingredients as toasted sesame is produced using a high temperature/time combination, which likely changes the protein structure. This makes detection by ELISA challenging.

Scoring of the lines: Throughout the data presented in this report, line intensity of the control, test and overload line was scored by comparing the device to a reference card. The scale was measured between 0 (no line intensity) – 5 (highest line intensity).

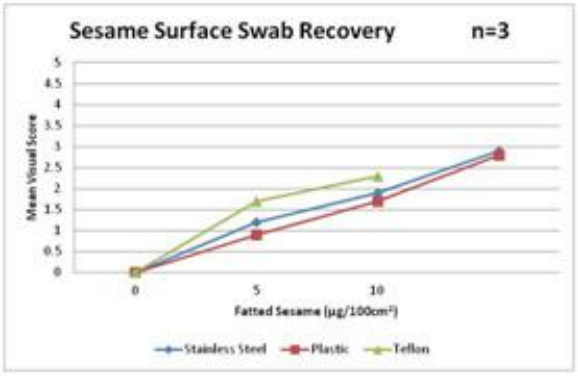
SWABBING RECOVERY

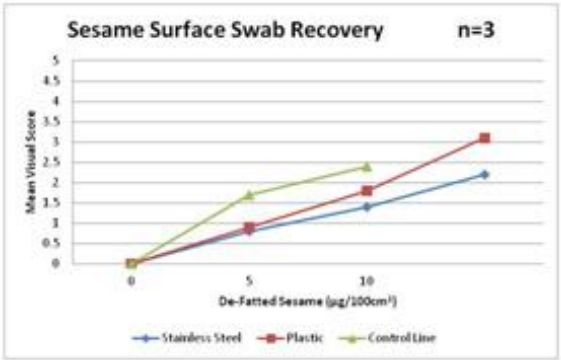
A range of surfaces, commonly found in industry, were artificially contaminated with known levels of sesame, prepared using fatted and defatted sesame internal reference flours. The surfaces tested were stainless steel, Teflon and plastic. Sesame solutions equivalent to 0, 5, 10, 20 µg/100 cm^2 were deposited onto each surface (n = 3) and left to dry. Each surface was swabbed and extracted following the kit insert instructions. Extracted samples were run on the device (n=3) and the line intensity of the overload, test and control line were recorded.

Results summary

Swabbing recovery experiments found that Teflon surfaces gave the greatest recovery followed by plastic and stainless steel, which had a similar recovery. All surfaces produced 100% negative results at 0 ppm and 100% positive results at 5 µg/100 cm^2 and higher.

	0 µg/100 cm^2		5 µg/100 cm^2		10 µg/100 cm^2		20 µg/100 cm^2	
	Fatted	Defatted	Fatted	Defatted	Fatted	Defatted	Fatted	Defatted
Stainless steel	0.0	0.0	1.2	0.8	1.9	1.4	2.9	2.2
Plastic	0.0	0.0	0.9	0.9	1.7	1.8	2.8	3.1
Teflon	0.0	0.0	1.7	1.7	2.3	2.4		





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INTER- AND INTRA-ASSAY VARIABILITY

Inter-assay and intra-assay variability were evaluated by testing standards at 0, 5, 100 and 1000 ppm with three different operators throughout two days using three different batches of devices. All standards were presented to each operator randomized and blind. Extracted samples were run on the devices and the line intensity of the overload, test and control lines were scored, averaged and recorded.

Results

Batch-to-batch variation

NOTE: Day one and two results averaged.

	0 ppm			5 ppm			100 ppm			1000 ppm		
Operator	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
1	0.0	0.0	0.0	0.6	0.7	0.7	3.4	3.1	3.6	3.4	3.6	3.3
2	0.0	0.0	0.0	1.2	1.4	1.3	3.5	3.2	3.5	3.1	3.1	3.5
3	0.0	0.0	0.0	0.8	0.5	0.6	2.9	2.7	2.8	3.0	3.1	2.9

Day-to-day variation

NOTE: Batches one, two and three averaged.

	0 ppm		5 ppm		100 ppm		1000 ppm	
Operator	**Day 1**	**Day 2**	**Day 1**	**Day 2**	**Day 1**	**Day 2**	**Day 1**	**Day 2**
1	0.0	0.0	0.7	0.7	3.2	3.4	3.3	3.5
2	0.0	0.0	1.0	1.5	3.4	3.4	3.2	3.3
3	0.0	0.0	0.5	0.7	2.6	2.9	2.9	3.1

Operator-to-operator variation

NOTE: Day one and two results averaged.

Operator	0 ppm	5 ppm	100 ppm	1000 ppm
1	0.0	0.7	3.3	3.4
2	0.0	1.3	3.4	3.2
3	0.0	0.6	2.8	3.0

Summary

Batch-to-batch variation appears to be minimal. Within the individual operator groups, all mean visual score averages differed between batches by <0.5. Day-to-day variation also appears to be minimal. Within the individual operator groups, all mean visual score averages differed between the days by ≤ 0.5. Operator-to-operator variation appears to give the most variation. Operator 3 consistently scored the lowest with averages being ≥ 0.5 between operators in some instances. Operator 2 tended to score higher in most cases.

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PARAMETER VARIABILITY

To determine the level of robustness of Reveal 3-D for Sesame, various parameters of the test were identified and varied. These were performed alongside normal running conditions and tested at various ppm levels (0, 5 and 1000 ppm) to determine any critical processes within the test.

The conditions varied are listed below. Each variable was tested independently of others to ensure the effect of one deviation from the protocol could be analyzed effectively.

Testing variations

	Variations	1 *	2	3	4	5
Sample volume	±10% (100 µL)	1.0	0.9	1.1	1.0	1.0
Buffer volume	±5% (200 µL)	4.0	4.0	4.0	3.8	4.2
Buffer temperature	2–8°C v. ambient	Ambient	Ambient	Ambient	Ambient	Ambient
Extraction time (sec.)	±50% (±30 sec.)	60	60	60	60	60
Extraction motion	No mix v. vortex	Hand	Hand	Hand	Hand	Hand
Running time (min.)	±20% (±1 min.)	5	5	5	5	5
CIP	CIP B v. CIP C	CIP A	CIP A	CIP A	CIP A	CIP A

	Variations	6	7 *	8	9	10
Sample volume	±10% (100 µL)	1.0	1.0	1.0	1.0	1.0
Buffer volume	±5% (200 µL)	4.0	4.0	4.0	4.0	4.0
Buffer temperature	2–8°C v. ambient	2–8°C	Ambient	Ambient	Ambient	Ambient
Extraction time (sec.)	±50% (±30 sec.)	60	60	30	90	60
Extraction motion	No mix v. vortex	Hand	Hand	Hand	Hand	No mix
Running time (min.)	±20% (±1 min.)	5	5	5	5	5
CIP	CIP B v. CIP C	CIP A	CIP A	CIP A	CIP A	CIP A

	Variations	11	12	13	14	15
Sample volume	±10% (100 µL)	1.0	1.0	1.0	1.0	1.0
Buffer volume	±5% (200 µL)	4.0	4.0	4.0	4.0	4.0
Buffer temperature	2–8°C v. ambient	Ambient	Ambient	Ambient	Ambient	Ambient
Extraction time (sec.)	±50% (±30 sec.)	60	60	60	60	60
Extraction motion	No mix v. vortex	Vortex	Hand	Hand	Hand	Hand
Running time (min.)	±20% (±1 min.)	5	4	6	5	5
CIP	CIP B v. CIP C	CIP A	CIP A	CIP A	CIP B	CIP C

**As an internal check, variation 7 was identical to variation 1.*

Results

The mean of three replicates were calculated for the test, overload and control lines. As an internal check, the means of variations one and seven, which were the same, were taken and used to compare any variations >0.5 (the smallest increment).

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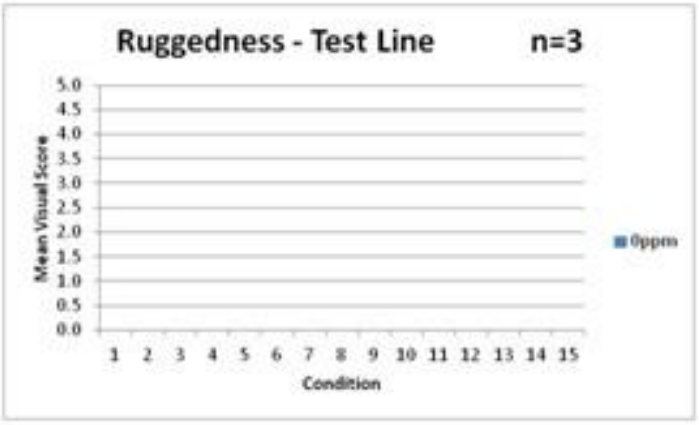
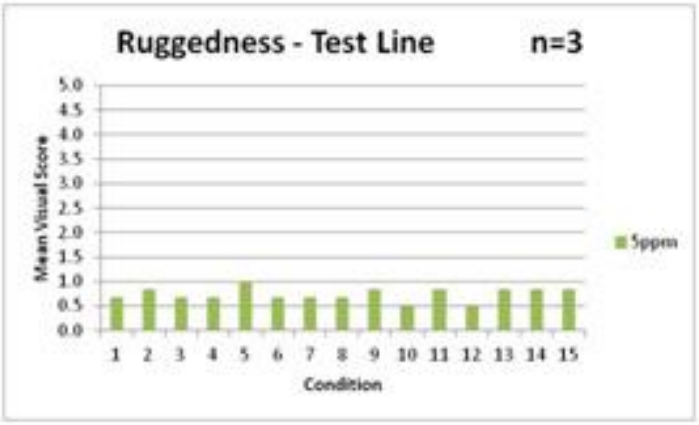
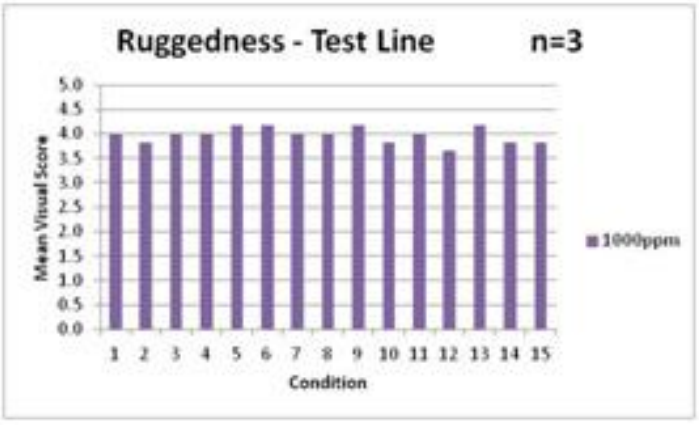
Test line data







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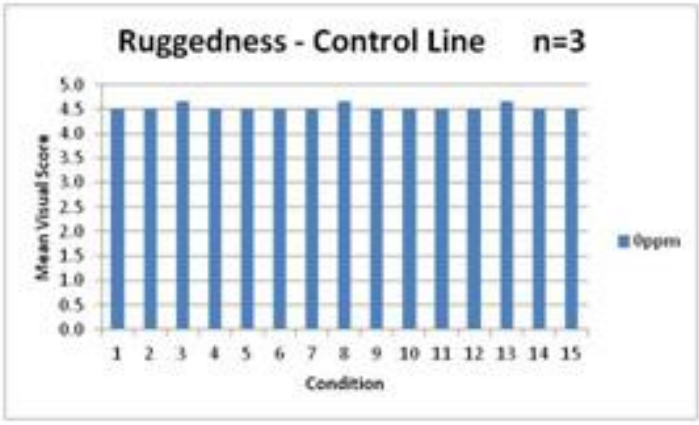

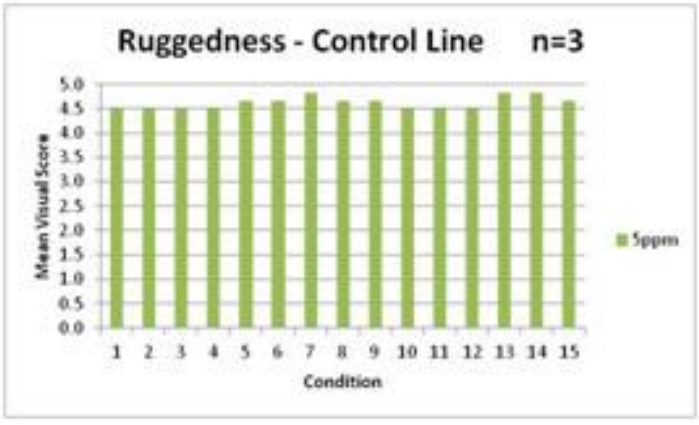

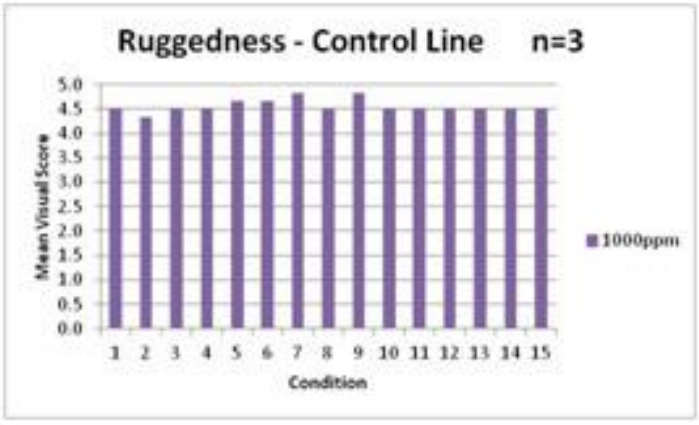

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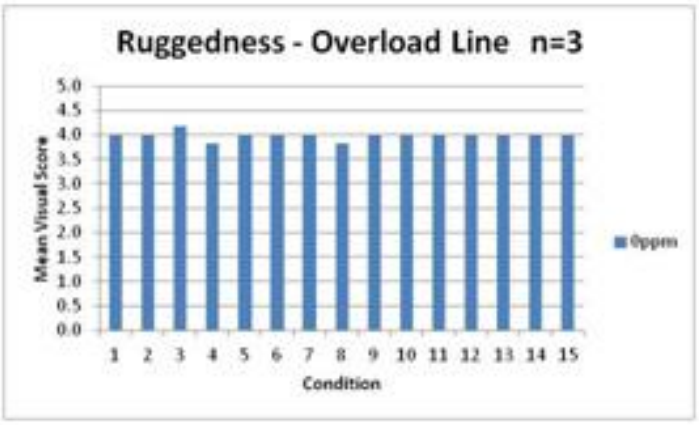
Overload line data



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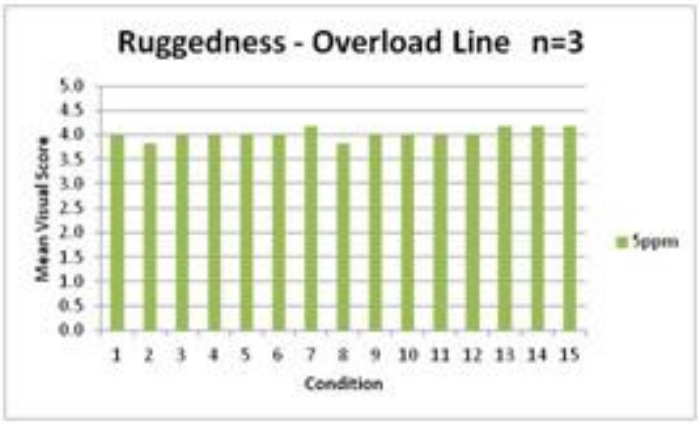
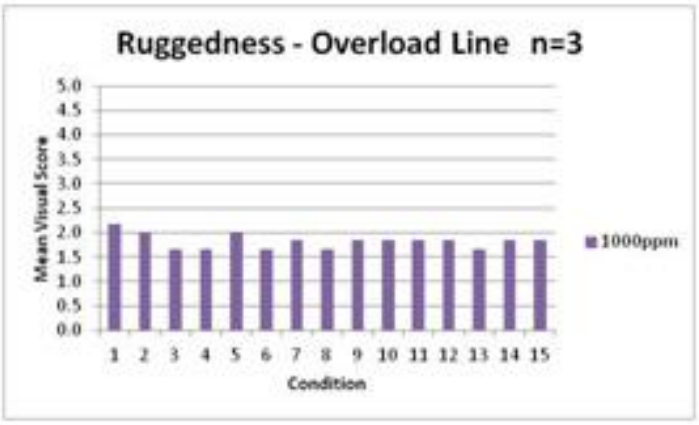




Conclusions

All conditions produced negative results at 0 ppm and positive results at 5 ppm and 1000 ppm, while no condition varied >0.5 from the mean of conditions one and seven. The results demonstrate a very high level or robustness for Reveal 3-D for Sesame.

SENSITIVITY

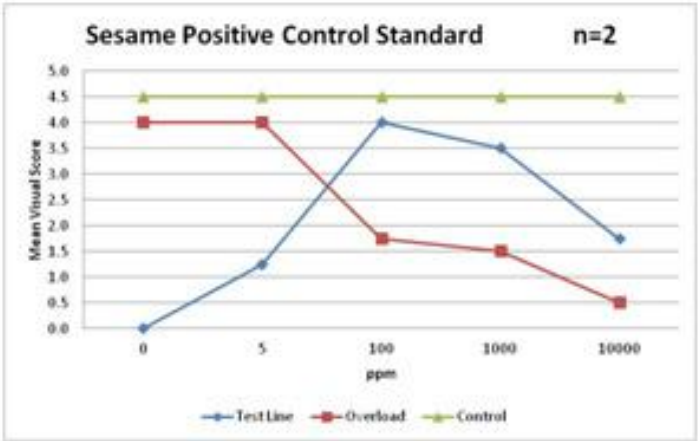
The sensitivity required for Reveal 3-D for Sesame is 5–10 ppm in CIP rinses. A sesame positive control standard was prepared to be representative of the various types and forms of sesame in use. A 1:1:1 mixture of sesame flour, natural sesame seeds and black natural sesame seeds was prepared and tested.

Results

The 0 ppm level was scored as negative. The 5 ppm level scored a clear positive, while the 100, 1000 and 10,000 ppm levels all scored positive results with overloading evident.

The decreasing overload line indicates a high level of sesame and, therefore, prevents false negative results (i.e., if the test line is zero and the overload line is zero, the device has overloaded because of high levels of sesame).

The test line increases as the allergen level increases; however, at high levels, the test line decreases. The overload line decreases as the level of allergen in the sample increases. When both the test and the overload are negative, overloading is in effect and indicates a very high level of sesame. Therefore, the overload line prevents a false negative if very high levels of sesame are present in the sample to be tested.



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BETA SITE STUDY

Beta site packs were prepared containing positive and negative samples. These were then distributed and performed by individuals outside of the scientific development team. The goal was to demonstrate the kit is usable in the real world and to evaluate beta site users' ability to interpret high and low positive and negative results.

Results

	Site 1		Site 2		Site 3		Site 4		Site 5	
Sesame level	Device 1	Device 2	Device 1	Device 2	Device 1	Device 2	Device 1	Device 2	Device 1	Device 2
	% correct									
Vial 1 (1000 ppm)	Positive	Positive	Positive	Positive	Positive (High)	Positive (High)	Positive (High)	Positive (High)	Positive (High)	Positive (High)
Vial 2 (100 ppm)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Vial 3 (0 ppm)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Vial 4 (0 ppm)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Conclusions

The results received from the five sites produced correct and expected results. This demonstrates the kit is fit for purpose.

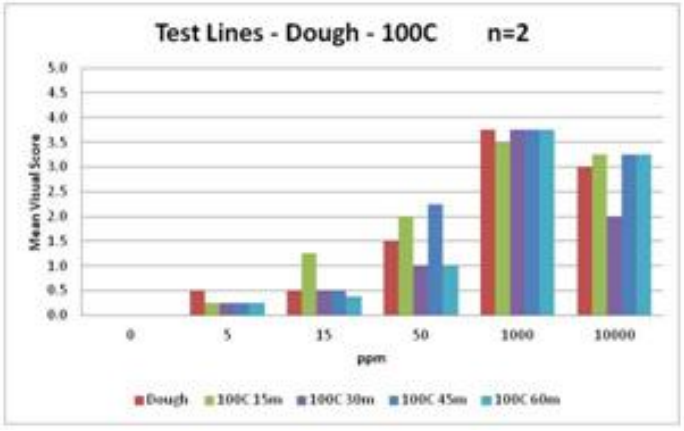
BAKING SESAME FLOUR AND DOUGH STUDY

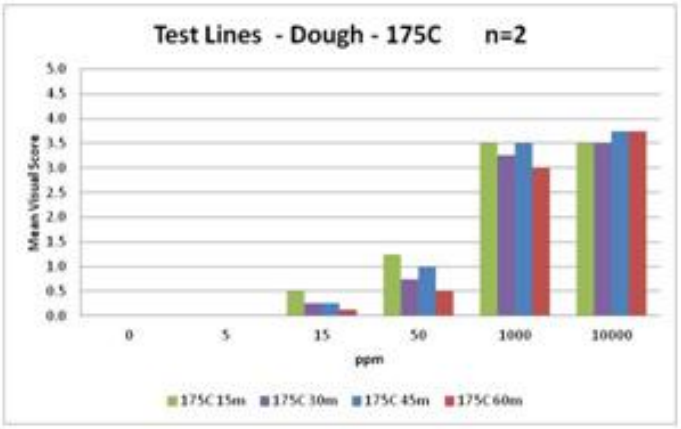
Sesame flour dough balls were baked in a conventional oven to mimic the effect of baking and determine the effect on sesame detection using Reveal 3-D for Sesame.

Sesame flour made with 200 mL of water and 100 g sesame flour was rolled into small balls approximately 3 cm in diameter. Experiments were designed to place dough balls in the oven at 100°C and 175°C temperatures for 0, 15, 30, 45 and 60 minutes. Each dough ball was crushed and thoroughly mixed. A representative sample from each dough ball was used to make a 10 mg/mL (10,000 ppm) solution prepared by adding 0.1 g to 10 mL water and vortex mixed for 2 minutes. The 10 mg/mL solution was then diluted to 1000, 50 and 5 ppm levels. Each level was tested using Reveal 3-D for Sesame devices. Water was used as a blank control.

Results

At 0 ppm, all devices were negative. All devices produced a test line at 5 ppm and above for dough treated at 100°C, while all devices produced a test line at 15 ppm for dough treated at 175°C. The overload and control lines performed as expected.





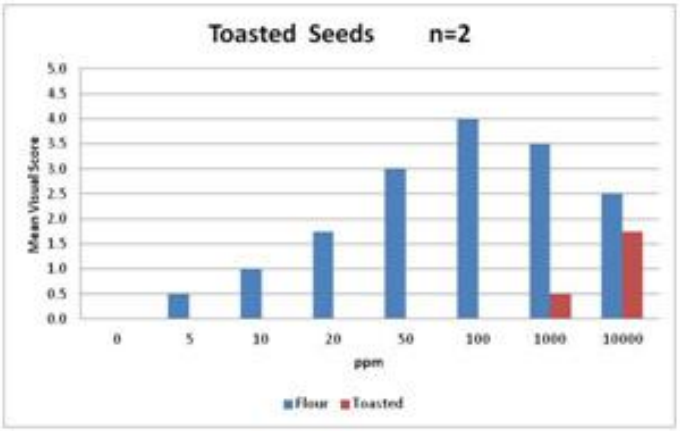
Conclusions

Dough heat-treated at 100°C was detected at 5 ppm at all time points. Dough heat-treated at 175°C was detected at 15 ppm at all time points. It is important to note that the production of the dough required a 1:3 dilution in water; therefore, the three-fold dilution must be taken into account.

TOASTED SESAME STUDY

Toasted sesame seeds were obtained and tested to determine the effect of toasting on sesame detection using Reveal 3-D for Sesame.

Toasted sesame seeds were crushed using a mortar and pestle, and a stock solution was prepared for serial dilutions. The diluted sesame stock solution was tested using Reveal 3-D for Sesame devices following the protocol outlined in the test kit insert.



Results

Toasted sesame was detected by the Reveal 3-D device at levels of 1000 ppm toasted sesame flour.

Conclusions

Toasting affects the detection within the assay. In this study, the high temperature involved in the toasting process reduced sensitivity to 1000 ppm.

CROSS-REACTIVITY

A total of 31 commodities were tested for cross-reactivity with the Reveal 3-D for Sesame test. Samples either demonstrated no cross-reactivity or negligible cross-reactivity.

No cross-reactivity: Kiwi fruit, macadamia nuts, hazelnuts, peanuts, pecans, cashews, almonds, pine nuts, green lentils, sunflower seeds, pumpkin seeds, Brazil nuts, black-eyed peas, chick peas, red split lentils, rye flour (organic), soya flour, millet grain, soya protein mince, soya isolate, wheat, oats, cardamom seeds, green split peas, pistachio nuts and pear barley.

Negligible cross-reactivity (0.3 test line): Poppy seeds, walnuts, golden linseed, red kidney beans and haricot beans.

Poppy seeds, walnuts, golden linseed, red kidney beans and haricot beans produced a very slight positive signal at 10,000 ppm. The high overload line confirms that it is a genuinely low result. All remaining foodstuffs were negative.

CONCLUSION

Reveal 3-D for Sesame is a sensitive and robust lateral flow device suitable for screening both CIP rinses and environmental samples to detect low levels of sesame.

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620 Lesher Place • Lansing, MI 48912 • **800/234-5333** (USA/Canada) • 517/372-9200
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