

# Reveal® 3-D for Total Milk Allergen

## Validation Report for Reveal 3-D for Total Milk Allergen (Neogen item 8479)

Revision 1, June 2012

### Table of contents

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Summary .....	2
Materials and methods.....	2
Swabbing recovery.....	2
Clean-in-place (CIP) rinse and commodity testing.....	3
Beta site results .....	3
Sensitivity .....	4
Conclusion.....	4

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## SUMMARY

Reveal 3-D for Total Milk Allergen (Neogen item 8479) is intended for the simple screening of milk protein (casein and whey) residues in food products such as nondairy milk, juices and sorbets, clean-in-place (CIP) rinses and environmental swabs. Reveal 3-D employs the principles of lateral flow chromatography (enzyme immunoassay) and is a highly sensitive and specific test designed to screen for very low parts per million (ppm) levels of milk (casein and whey). This validation report details the findings of the experimental evaluation to establish product claims for the Reveal 3-D for Total Milk Allergen test.

**Swabbing recovery:** Experiments found the device can detect milk residue on stainless steel at the desired limit of detection, 20 µg/100 cm<sup>2</sup>.

**Commodity testing:** Commodity testing showed recovery and detection of milk in soy milk, orange juice, CIP rinses and sorbet samples.

**Beta site results:** Nine independent testing locations evaluated the method and performance of the Reveal 3-D for Total Milk Allergen kit on six samples. Each user interpreted 100% of the sample results accurately.

**Sensitivity:** The limit of detection of the Reveal 3-D for Total Milk Allergen test in buffer was found to be 5 ppm total milk.

## MATERIALS AND METHODS

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

### Scoring of the lines

Throughout this report, 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).

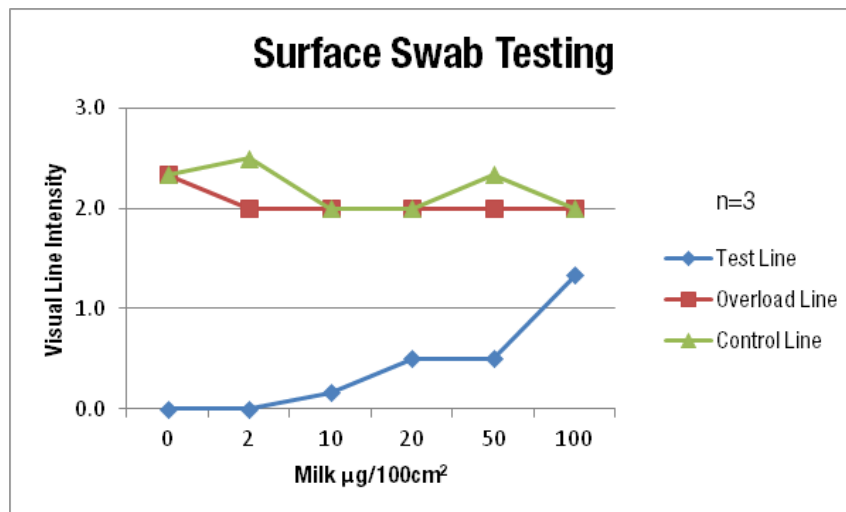
## SWABBING RECOVERY

Stainless steel was artificially contaminated with known levels of milk. Milk solutions equivalent to 0, 2, 10, 20, 50 and 100 µg/100 cm<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 samples were run on the device (n=3) and the line intensity of the overload, test and control lines were recorded.

### Results

Swabbing recovery on stainless steel is 20 µg/100 cm<sup>2</sup>.

	0 µg/100 cm <sup>2</sup>	2 µg/100 cm <sup>2</sup>	10 µg/100 cm <sup>2</sup>	20 µg/100 cm <sup>2</sup>	50 µg/100 cm <sup>2</sup>	100 µg/100 cm <sup>2</sup>
	% positive results					
Stainless steel	0%	0%	33%	100%	100%	100%



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

CIP rinses, orange juice, soy milk and sorbet samples were tested to determine recovery of total milk. The extraction volume for sorbet samples must be altered to reduce matrix effects.

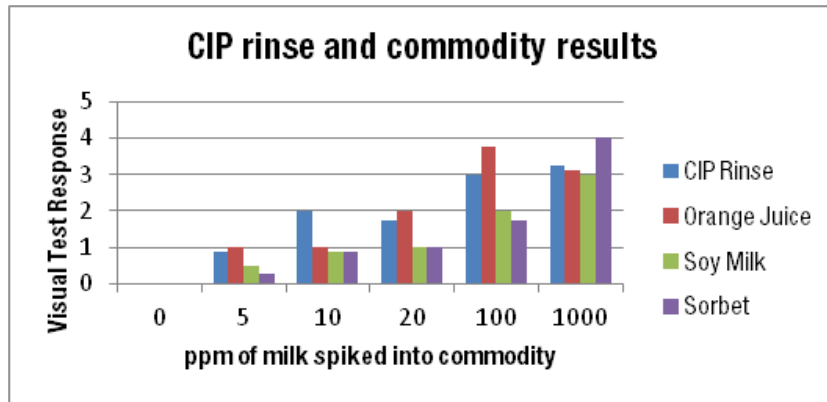
**Methods**

Nonfat dried milk was prepared in sample matrix to 0, 5, 10, 20, 50, 100 and 1000 ppm and extracted following test kit insert instructions.

**Results**

At 0 ppm, all devices were negative. CIP rinses, orange juice and soy milk samples all were visually positive at 5 ppm. Sorbet samples were positive at 10 ppm. Test line visual intensities continued to darken as the spike level increased. The overload and control lines performed as expected.

The graph shows the relationship of the test line response to the recovery of milk residue in the various commodities test. **NOTE:** Sorbet at 5 ppm was detected in 2 out of 4 replicates.



**BETA SITE RESULTS**

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

Samples were extracted and tested in triplicate using a new Reveal 3-D for Total Milk Allergen device. Each sample set included 27 devices, which were visually interpreted correctly.

**Beta site test results**

Sample	Total milk level	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
		<b>% correct</b>								
CIP	No detectable amount	100%	100%	100%	100%	100%	100%	100%	100%	100%
CIP	20 ppm	100%	100%	100%	100%	100%*	100%	100%	100%	100%
CIP	1000 ppm	100%	100%	100%	100%	100%	100%	100%	100%	100%
Juice	No detectable amount	100%	100%	100%	100%	100%	100%	100%	100%	100%
Juice	20 ppm	100%	100%	100%	100%	100%	100%	100%	100%	100%
Juice	1000 ppm	100%	100%	100%	100%	100%	100%	100%	100%	100%

\*Recorded negative results but commented the lines were present but light. However, any intensity of a line in the test zone is a positive result.

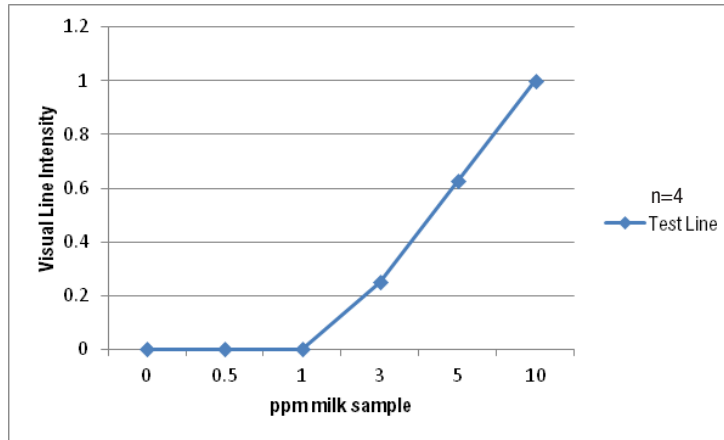
## SENSITIVITY

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To determine the absolute limit of detection, a stock solution was diluted to the required ppm level and extracted with type 8 extraction buffer.

### Summary

The limit of detection is 5 ppm. Of the samples spiked at 5 ppm, 100% were visually positive. At 3 ppm, 50% of samples were visually positive. The overload and control lines were present and responded as expected.



## CONCLUSION

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The Reveal 3-D for Total Milk Allergen test is a sensitive and robust lateral flow device suitable for screening CIP rinses, liquid foods and environmental samples to detect low levels of milk residue.