



Validation Report

REVEAL 3-D Mustard Test

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1.0 INTRODUCTION

The REVEAL 3-D Mustard Test is uniquely designed with 3 lines of detection and can be used virtually anywhere to screen environmental swabs and CIP rinses for the presence of allergen contamination. The test employs the principles of lateral flow chromatography EIA and is a highly sensitive and specific test designed to screen for very low PPM (parts per million) levels of mustard.

The 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.

2.0 SUMMARY

The REVEAL 3-D Mustard utilises highly specific antibodies to detect mustard protein.

The REVEAL 3-D test is designed to detect low parts per million (ppm) levels of mustard content in CIP rinses.

Swabbing recovery experiments found that the Reveal 3-D Mustard test could detect as low as $10\mu\text{g}/100\text{cm}^2$ from Teflon, plastic and stainless steel surfaces.

The limit of detection for the REVEAL 3-D Mustard in CIP rinses was 5ppm. Please note the presence of chemicals or sanitisers in CIP rinses may affect the LOD.

The limit of detection for the test was established as 0.5ppm mustard in buffer.

When 3 operators tested 3 batches of devices over 2 days, specificity was found to be 97.2% while sensitivity was 100%.

The REVEAL 3-D Mustard Test was able to detect deheated mustard at levels comparable with the mustard internal-reference flour. The mustard internal-reference flour was prepared using a mixture of yellow, black and brown mustard seeds.

The REVEAL 3-D Mustard Test cross-reacts with canola and rapeseed due to their similarity in structure to mustard protein.

Mustard standard solutions containing 0ppm, 5ppm, 100ppm, 500ppm and 1000ppm were prepared from the mustard internal-reference flour. Standard solutions were verified by running alongside the Veratox mustard ELISA assay, product code 8400.

3.0 SWABBING RECOVERY

A range of surfaces, commonly found in industry, were artificially contaminated with known levels of mustard. The surfaces tested were Stainless Steel, Teflon and Plastic. Mustard solutions equivalent to 0, 10, 20, 50, 100 and 500 $\mu\text{g}/\text{cm}^2$ 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.

Scoring of the lines

Throughout the data presented in this report, line intensity of the control, test and overload line is scored by comparing the device to a reference card.

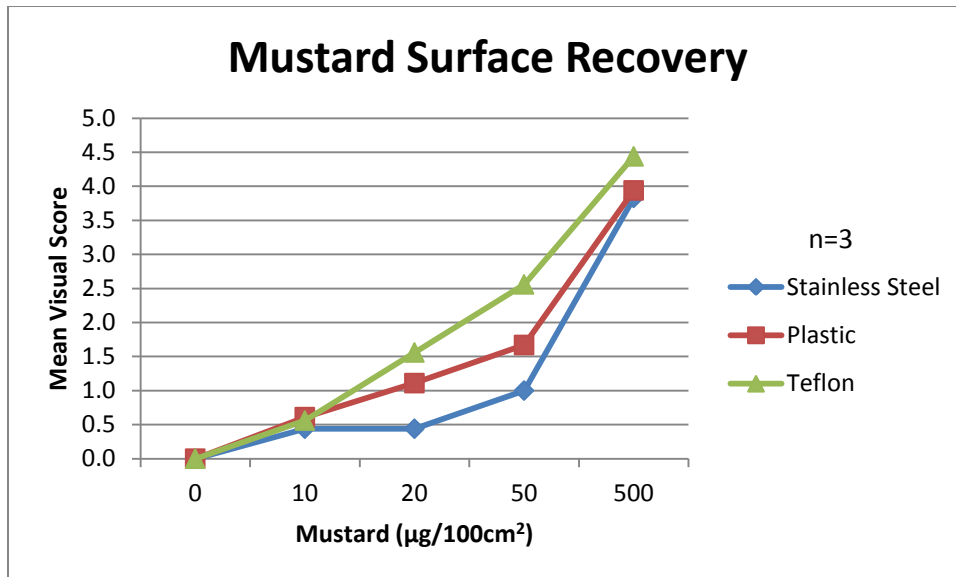
The scale is measured between 0 (no line intensity) – 5 (highest line intensity).

3.1 Results Summary

Swabbing recovery experiments found that Teflon surfaces gave the greatest recovery followed by plastic and stainless steel.

- % Positive

	0 $\mu\text{g}/100\text{cm}^2$	10 $\mu\text{g}/100\text{cm}^2$	20 $\mu\text{g}/100\text{cm}^2$	50 $\mu\text{g}/100\text{cm}^2$	500 $\mu\text{g}/100\text{cm}^2$
Stainless Steel	0	77.8	91.7	100	100
Plastic	0	100	100	100	100
Teflon	0	100	100	100	100



4.0 ROBUSTNESS - INTER & INTRA ASSAY VARIABILITY

Inter-assay and intra-assay variability was evaluated by testing standards at 0, 5, 100 and 1000ppm, with 3 different operators over 2 days using 3 different batches of devices. All standards were presented to each operator randomised and blind.

4.1 Results

- % Positive

Operator	0ppm		5ppm		100ppm		1000ppm	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	0	0	100	100	100	100	100	100
2	16.7	0	100	100	100	100	100	100
3	0	0	100	100	100	100	100	100

- Statistical analysis revealed specificity to be 97.2% while sensitivity was 100%.

4.2 Summary

Operator 2 scored two of the 0ppm standards at 0.5 on Day 1, however on Day 2 operator 2 scored all 0ppm negative. All remaining operators scored negative for 0ppm on both days.

All operators scored 5, 100 and 1000ppm positive on both days.

5.0 ROBUSTNESS - PARAMETER VARIABILITY

To determine the level of robustness of the REVEAL 3-D Mustard Test, various parameters of the test were identified and varied. These were performed alongside the normal running conditions and tested at various ppm levels (0, 5 and 1000ppm) 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 analysed effectively.

5.1

Testing Variations

	Variations	1*	2	3	4	5
Sample volume	±10% (0.1ml)	1	0.9	1.1	1	1
Buffer volume	±5% (200µl)	4	4	4	3.8	4.2
Buffer temp	2-8°C .v. ambient	ambient	ambient	ambient	ambient	ambient
Extraction time	±50% (±30 sec)	60	60	60	60	60
Extraction motion	Hand mix/No mix	hand	hand	hand	hand	hand
Running time	±20% (±1 min)	5	5	5	5	5
CIP	CIP A/CIP B/CIP C	CIP A	CIP A	CIP A	CIP A	CIP A

	Variations	6	7 *	8	9	10
Sample volume	±10% (0.1ml)	1	1	1	1	1
Buffer volume	±5% (200µl)	4	4	4	4	4
Buffer temp	2-8°C .v. ambient	2-8°C	ambient	ambient	ambient	ambient
Extraction time	±50% (±30 sec)	60	60	30	90	60
Extraction motion	Hand mix/No mix	hand	hand	hand	hand	no mix
Running time	±20% (±1 min)	5	5	5	5	5
CIP	CIP A/CIP B/CIP C	CIP A	CIP A	CIP A	CIP A	CIP A

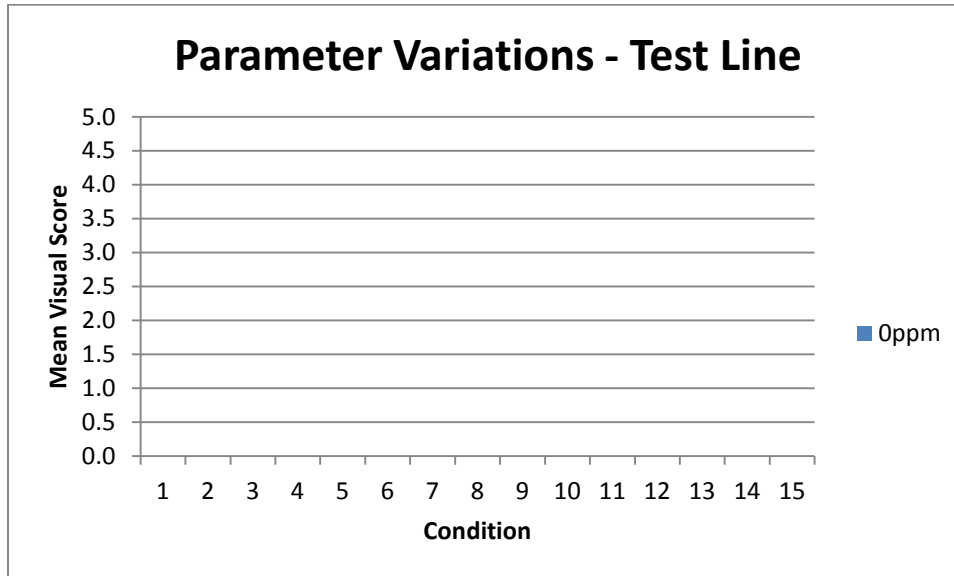
	Variations	11	12	13	14	15
Sample volume	±10% (0.1ml)	1	1	1	1	1
Buffer volume	±5% (200µl)	4	4	4	4	4
Buffer temp	2-8°C .v. ambient	ambient	ambient	ambient	ambient	ambient
Extraction time	±50% (±30 sec)	60	60	60	60	60
Extraction motion	Hand mix/Vortex	vortex	hand	hand	hand	hand
Running time	±20% (±1 min)	5	4	6	5	5
CIP	CIP A/CIP B/CIP C	CIP A	CIP A	CIP A	CIP B	CIP C

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

5.2 Results

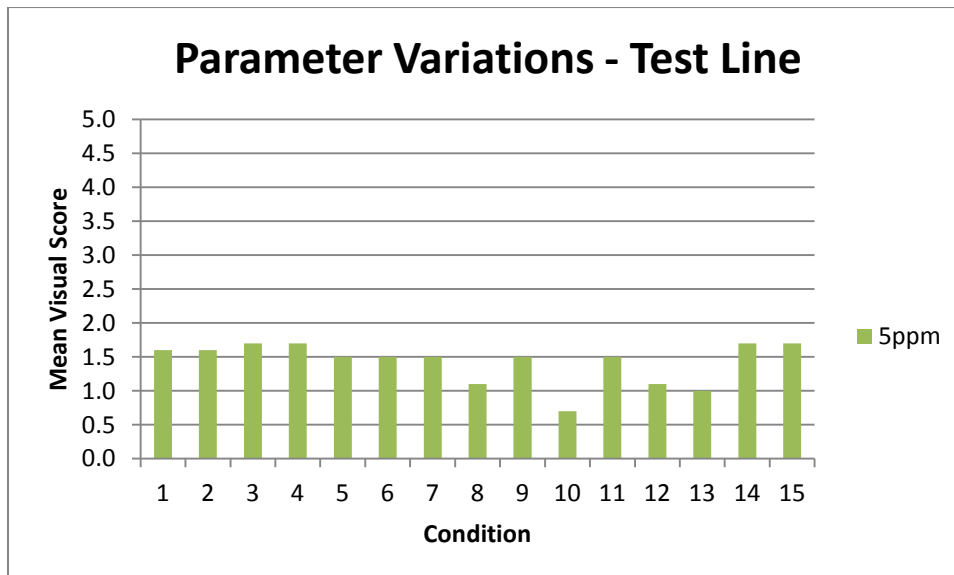
The mean of 5 replicates were calculated for the Test, Overload and Control lines. As an internal check the means of conditions 1 & 7, which were the same, were taken and used to compare any variations >0.5 (the smallest increment).

Test Line Data



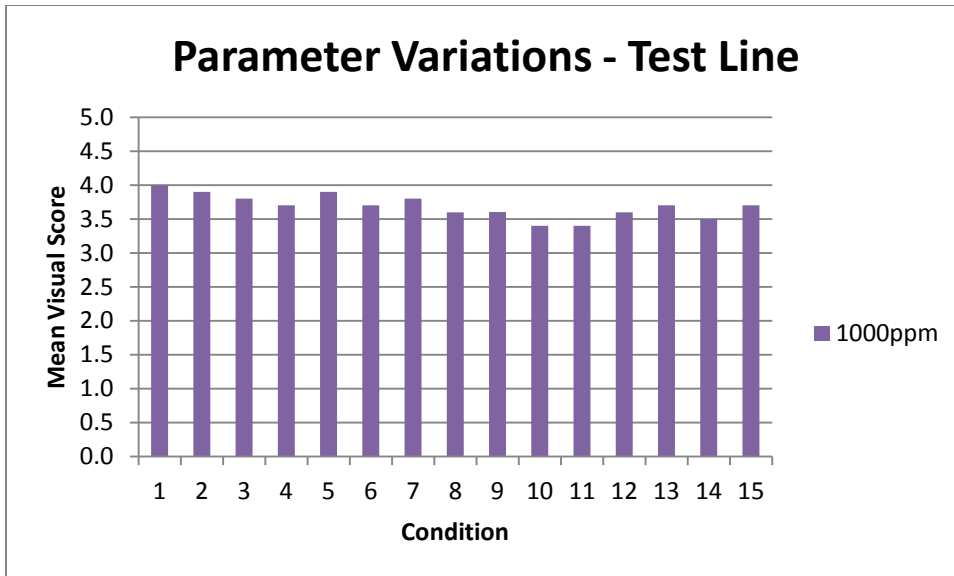
All conditions produced negative results at 0ppm.

No conditions varied >0.5 when compared to standard condition



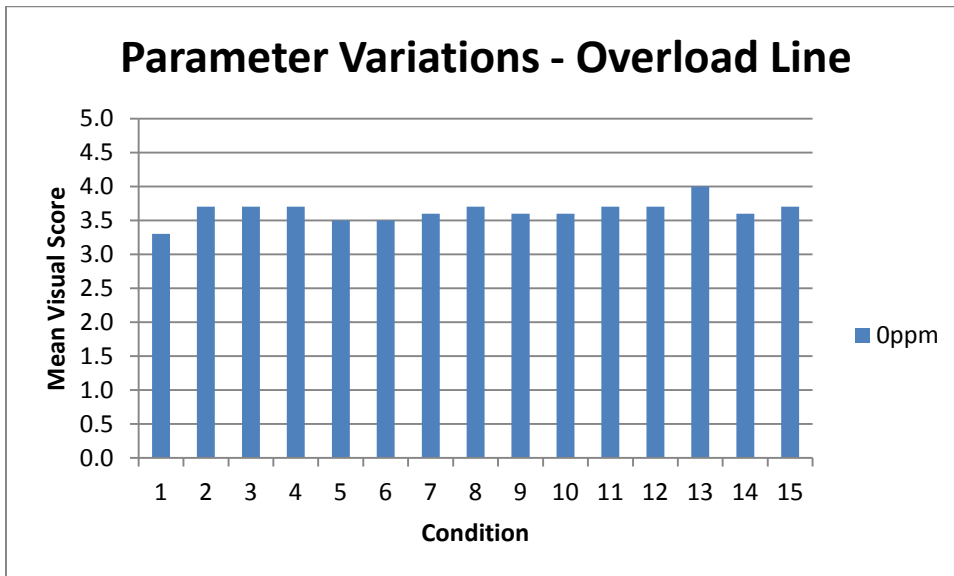
All conditions produced positive results at 5ppm.

Condition 10 (no mixing during extraction) produced the largest variation, followed by condition 13 (6 minute run time)

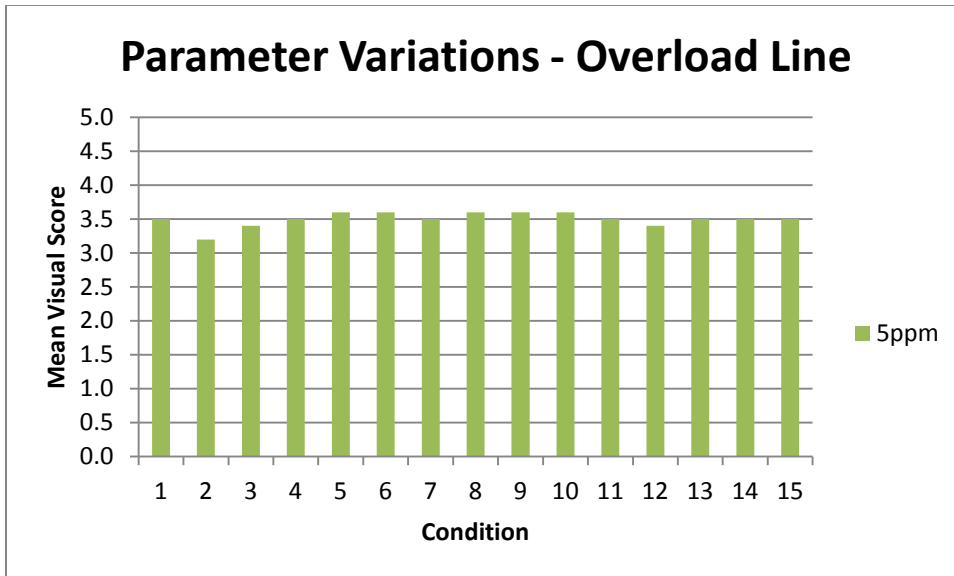


All conditions produced positive results at 1000ppm.
 No conditions varied >0.5 when compared to standard condition

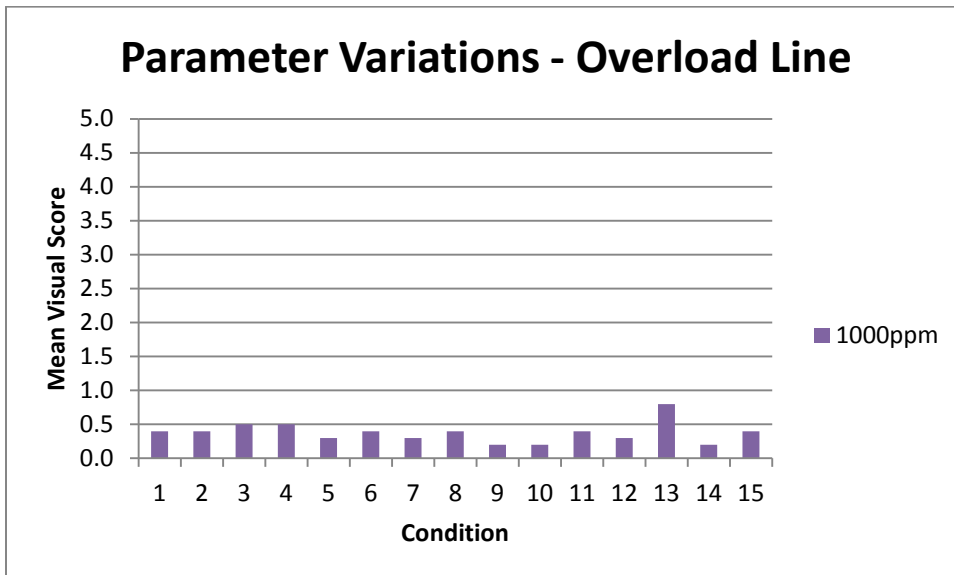
Overload Line Data



No conditions varied >0.5 when compared to standard condition

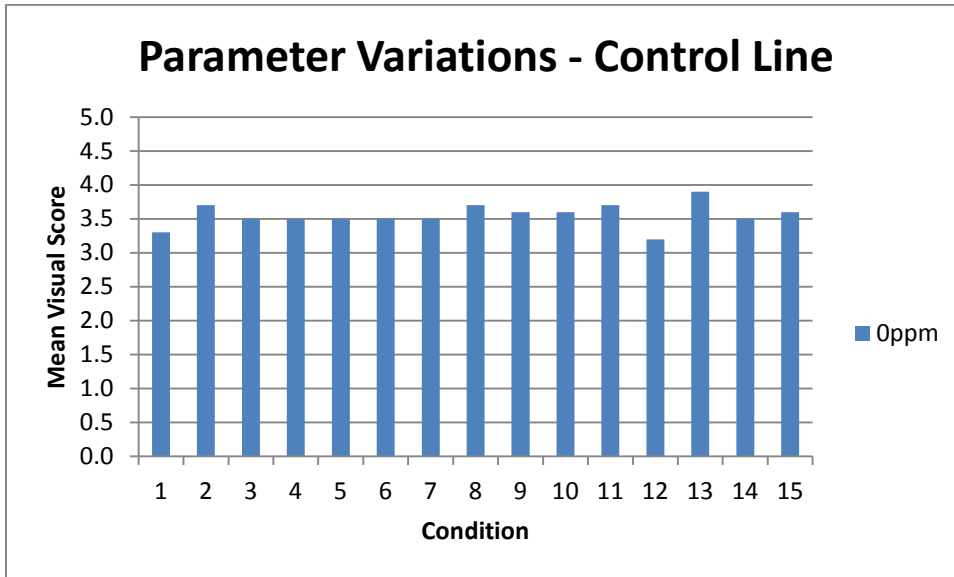


No conditions varied >0.5 when compared to standard condition

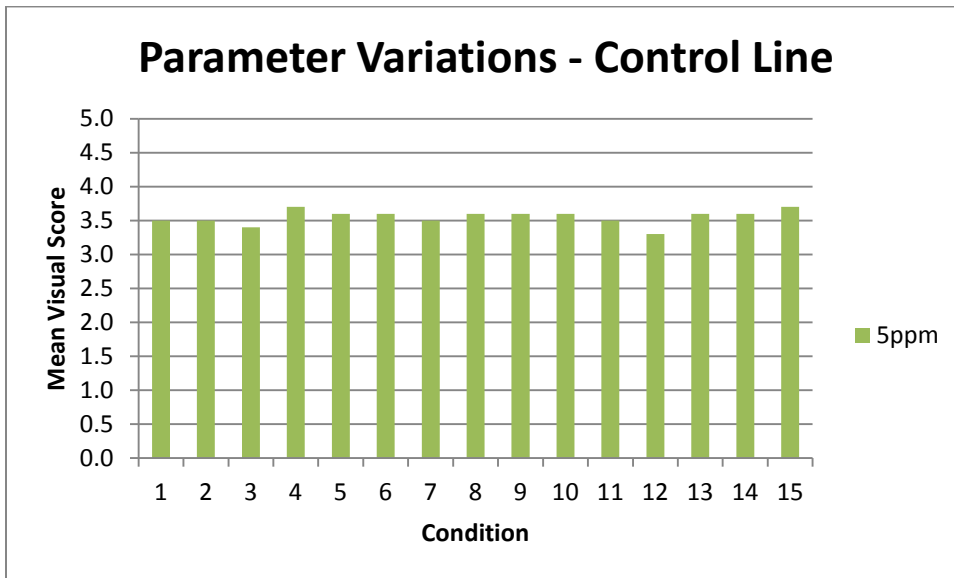


No conditions varied >0.5 when compared to standard condition

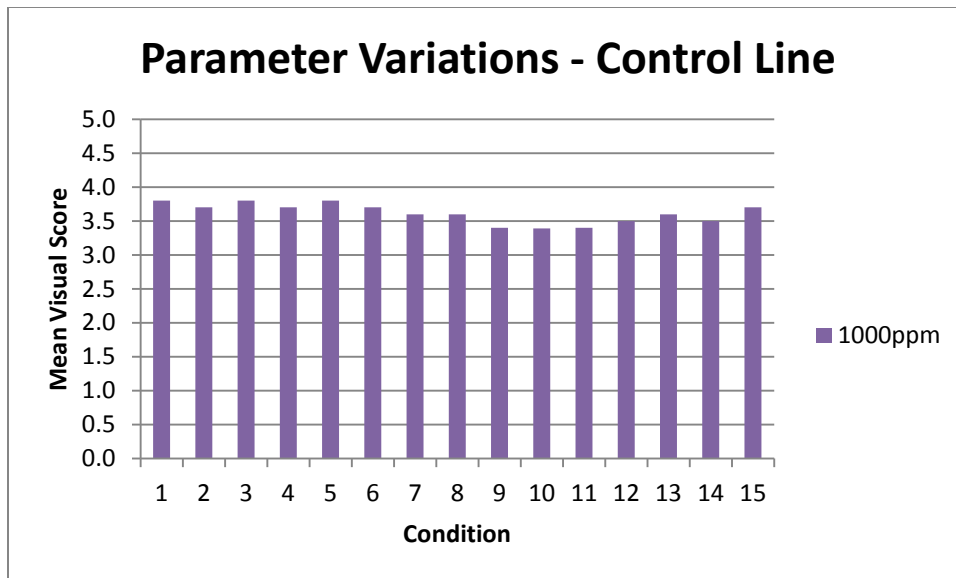
Control Line Data



No conditions varied >0.5 when compared to standard condition



No conditions varied >0.5 when compared to standard condition



No conditions varied >0.5 when compared to standard condition

5.3 Conclusions

It is due to the consistency and reproducibility of the test that the variation being compared is so small, 0.5, the smallest increment in the visual scoring chart. The results demonstrate a very high level of robustness for the REVEAL 3-D Mustard Test.

Extraction motion (10) produced the largest variation when there was no mixing during the extraction procedure as opposed to the recommended shaking by hand for 1 minute.

Running time (13) produced the second largest variation when the devices ran for 6 minutes, as opposed to the recommended 5 minute running time.

For conditions 1 and 7 where devices were run under identical conditions following the kit protocol only between 0 and 0.3 variation was seen across the three standards 0ppm, 5ppm & 1000ppm.

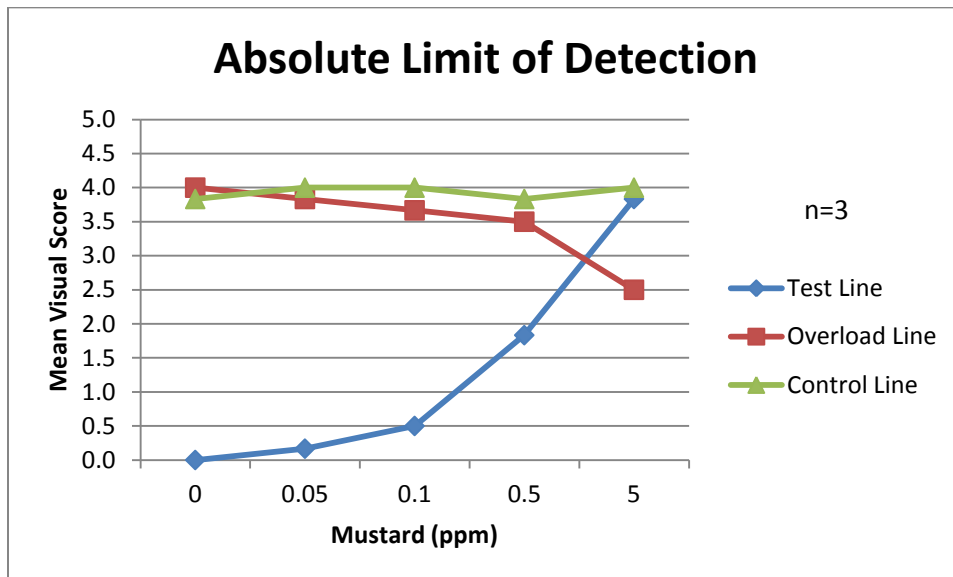
6.0 SENSITIVITY - ABSOLUTE LIMIT OF DETECTION

To determine the absolute limit of detection, a stock standard of mustard was diluted to give the required ppm levels and extracted in extraction buffer (REB7) directly. Standards tested were 0, 0.05, 0.1, 0.5, 5, 100 & 1000ppm mustard.

6.1 Results

The means of 3 devices per concentration were calculated for each line.

- At 0ppm, all devices were negative.
- 1 in 3 devices produced a line at 0.05ppm.
- All 3 devices produced a test line at 0.1ppm and above.
- The overload and control lines performed as expected.



6.2 Conclusions

The absolute limit of detection is 0.1ppm. When taking into account the in-use dilutions as per the instructions, the absolute limit of detection equates to an in-use limit of detection of 0.5ppm.

7.0 DEHEATED MUSTARD

Deheated yellow mustard flour is widely used as a blender, thickener and flavour enhancer in a wide range of products from cheese to sausages. The yellow mustard flour is heat-treated to 110-180°C with an enzyme to deactivate the chemical causing the hot sensation.

A sample of ground deheated mustard produced from #1 grade Canadian Yellow Mustard Seed (*Brassica Hirta*), in which the myrosinase enzyme had been inactivated by timed temperature treatment, was obtained for testing to determine if the Reveal 3-D mustard device could detect deheated yellow mustard flour.

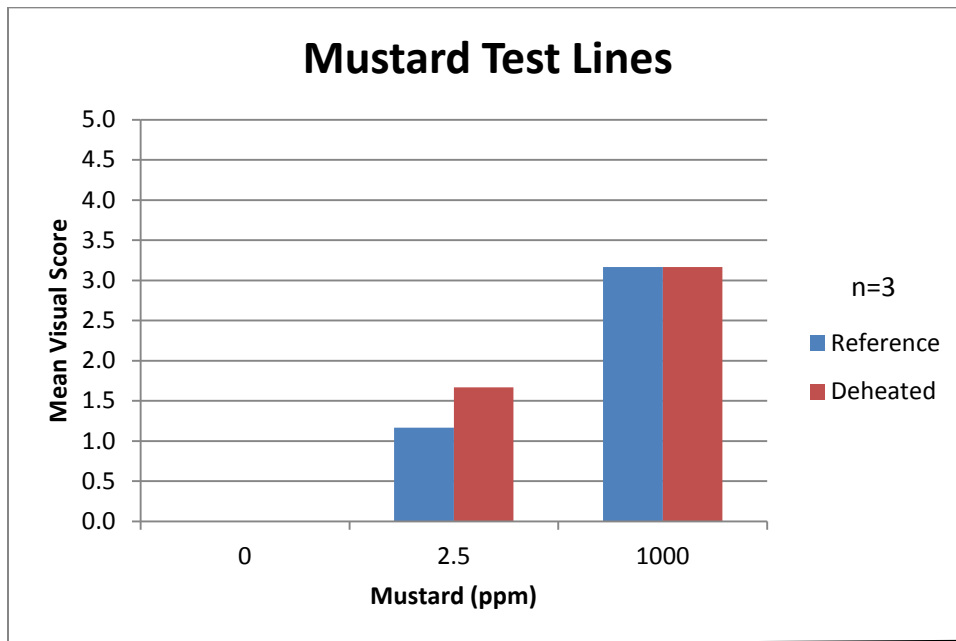
7.1 Methods

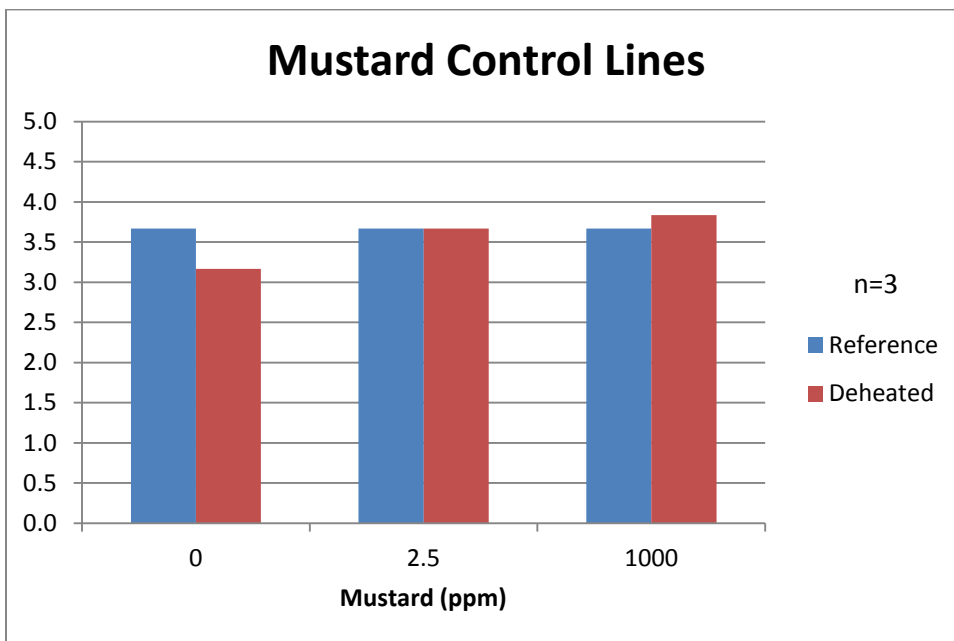
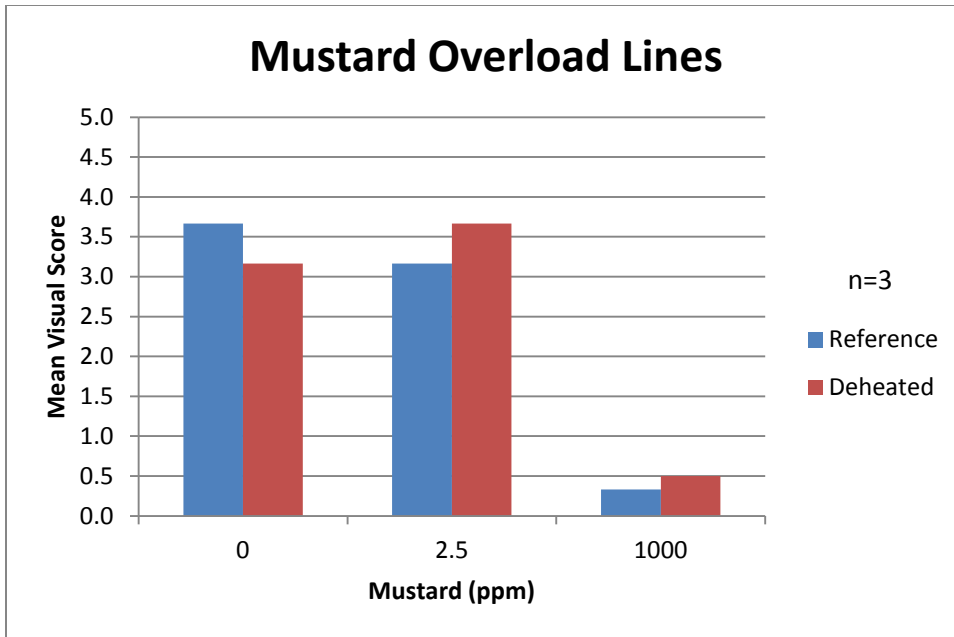
Mustard standards

Both deheated and mustard reference flour were prepared in water to 0, 2.5, 100 and 1000ppm and then extracted as per kit instructions.

7.2 Results

At 0ppm, all devices were negative. All 3 devices on both mustard types produced a test line at 2.5ppm and above. The overload and control lines performed as expected.





7.3 Conclusions

The REVEAL 3-D Mustard Test was able to detect deheated mustard. The reference and deheated mustard samples gave comparable results and did not differ by more than 0.5 (the smallest scoring increment) from each other.

10.0 CROSS-REACTIVITY

The REVEAL 3-D Mustard Test cross-reacts with canola and rapeseed due to their similarity in structure to mustard protein.

11.0 CONCLUSION

The REVEAL 3-D Mustard test is a sensitive & robust lateral flow device suitable for screening both CIP rinses and environmental samples to detect low levels of mustard.