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Campden BRI
food and drink innovation

ALLERGEN TESTING AND CLEANING VERIFICATION STUDIES

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1. INTRODUCTION

Cleanliness within the fast-food ready-to-eat business' is a high priority for food companies and consumers alike. Having a quick and easy way of determining how clean a surface is would be beneficial in ensuring the safety of foodstuffs made to order. Hygiena International wishes to primarily market their ATP product in the ready-to-eat sector (RTE), in which the use of ATP is currently the default hygiene verification. In Hygiena International's direct experience with customers, this approach has delivered very good cleaning practices. Hygiena International would now like to investigate the use of a high sensitivity ATP test in combination with protein tests and specific allergen tests to measure the presence of food residues and allergens on product contact surfaces.

2. AIMS AND OBJECTIVES

2.1 Aims

The aim of this investigation was to produce a report assessing the effectiveness of different cleaning verification methods to determine the reduction of allergens and soil on stainless steel surfaces.

Campden BRI compared the effectiveness of ATP swabs – Ensure with Supersnap, high sensitivity Protein test = ALLERSnap (Hygiena) and four commercially available allergen lateral flow tests for use in food factories (2 x Neogen, 2 x R-Biopharm).

The intent of this ATP testing is not to replace specific allergen tests but rather to augment them and make cleaning verification more comprehensive, cost effective and faster.

2.2 Objectives

The objective was to take measurements of ATP, proteins and 4 allergens at appropriate stages during a simulated cleaning cycle. A foodstuff soil on a stainless steel surface was tested for 4 different allergens: gluten, casein, egg and peanut throughout the cycle. The proposed study was not intended to be an allergen cleaning validation exercise, but a simple simulated laboratory exercise to compare the results using the methods described above.

3. MATERIALS

3.1 Swabbing materials

3.1.1 Supersnap (Hygiena Cat. No. SUS3000) – 44 x ATP detecting swabs. The ATP swab readings were taken using an accompanying Hygiena Luminometer device known as EnSURE provided by Hygiena (serial number 021975). The device measures ATP concentration in relative light units (RLU).

3.1.2 ALLERSnap (Hygiena Cat. No. ALS-100) - 44 x Protein detecting swabs. These swabs are based on a reactant inside the swab tube changing colour based on the amount of protein residue detected. It is a semi-quantitive result based on colour.

3.1.3 Reveal 3-D Casein Test (Neogen Corporation Art. No. 902075M) – 44 x Horizontal lateral flow detection strips for casein detection.

3.1.4 RIDA® QUICK Gliadin RS Immune Chromatographic Test (R-Biopharm AG Art no. R7003) – 44 x Vertical dip test strips for gliadin (gluten) detection.

3.1.5 (R-Biopharm AG, Lateral Flow Egg Art. No. BL 608-25) - 44 x Vertical dip test strips for egg.

3.1.6 (R-Biopharm AG, Lateral Flow Peanut Art. No. BL 606-25) - 44 x Vertical dip test strips for peanut.

3.1.7 Enviromental/Surface Swabs (Imutest) – 10 x for gluten and peanut allergen determination.

3.1.8 RIDA SCREEN® Gliadin immunoassay (R-Biopharm AG Art no. R7001) – Plate ELISA for detection of gliadin**.**

3.1.9 RIDA SCREEN® FAST Peanut Immunoassay (R-Biopharm AG Art no. R6202) – Plate ELISA for detection of peanut.

3.2 Detergent (Somplex Fatsolve)

Somplex Fatsolve from Johnson Diversity is a pale yellow, alkaline foam detergent for use in the food, beverage and allied industries. For daily routine cleaning it is used at a 1-2% solution concentration and it has a contact time of 10 -15 minutes.

3.3 Disinfectant (Suma D-10 J-flex)

Suma D10 J-Flex from Johnson Diversity is a concentrated dark purple, QUAT based detergent disinfectant for cleaning and disinfection of all surfaces in food premises. It is used in a 1% solution concentration with a 30 seconds disinfection contact time.

3.4 Product slurry -

The food product used was a ready-to-eat beef with noodles meal (450g)

Ingredients: (high to low)

Egg Noodles (34%)
Yellow bean and chilli sauce
Marinated beef (16%)
Beansprouts
Red pepper
Spring onion

Allergens stated on the packaging are egg, wheat (gluten), soya and 'unsuitable for peanut allergy sufferers'. This food product was processed for approximately 2 minutes in a Moulinex Masterchef Delicio food processor until smooth then 0.3g of freeze dried peanut powder was added to provide detectable levels. The homogenised product was diluted down 1:1:1; food solids: semi skimmed milk (1.8% fat): tap water. The suspension was then pressed through a commercial sieve to remove any larger particles that may be present. The product slurry was made fresh on the morning of the trial.

3.5 Surfaces

The surfaces consisted of ten stainless steel (AISI 304 with a 2B finish) sheets individually measuring 50x50cm and were marked out using black permanent marker into 10x10cm squares creating a grid 5x5. The surfaces were thoroughly cleaned with detergent and rinsed before the trials took place.

3.6 High pressure washer

The high pressure washer used was a K.E.W model 1702K operating at a pressure of 25bar or 1PSI (Pounds per Square Inch). The water used was provided from the potable water mains.

3.7 Pre-trials

Pre-trials were carried out to determine a) the right distance and spray times to sufficiently cover and then rinse the stainless steel sheets, b) that a gradual reduction of food particles on the surface was achieved during the simulated cleaning cycle, by taking ATP swabs, c) that all allergens were detectable in the slurry using lateral flow allergen tests, and d) to determine timings for the trials and to plan a schedule.

4. METHOD

4.1 Slurry to surface

A 10g portion of slurry was poured on to each stainless steel sheet and spread out, as evenly as possible, using a small paint brush. After initial swabs were taken, the sheet was placed in an incubator set at 57°C for 10 minutes to dry.

4.2 Foaming of cleaning agents

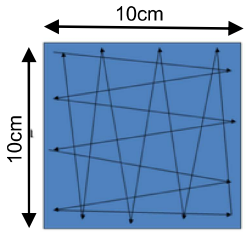
A stationary power hose was set up at 90cm away from the stainless steel sheets (see Appendix 1). The spray pressure of the water/detergent/disinfectant sprays was 25bar. A bucket of fresh water was present for purging the connecting hose after each chemical spray. This connecting hose used a venturi to draw up the concentrated chemical to then be combined with mains water to make the percentage concentration desired. This was adjusted using a dial on the front of the power hose.

D10 solution and Somplex Fatsolve solution were used at a concentration of 1%, which gave sufficient foaming against the stainless steel sheets at the distance used.

4.3 Method of swabbing

The swabs from Supersnap and ALLERsnap were supplied pre-moistened. The other swabs used were not pre-moistened due to the high level of surface water following cleaning. Each 10x10cm square was swabbed by rotating the swab in at least 2 different directions and covered at least 4 times (Figure 1)

Figure 1: Swabbing technique.



All swab types were placed in a randomised pattern generated in Minitab 16 Statistical software. This information was plotted into ten 5x5 grids on paper so it was obviously randomised on the day of the trial (see Appendix 2). The swab labels were colour coded for each cleaning time interval; swabbing consisted of;

- Before drying,
- After drying and pre-rinse,
- After washing with detergent and post rinse,
- After washing with disinfectant and post rinse.

For each detection method, 40 swabs were taken during the whole trial (10 swabs after each cleaning step).

Due to the cost, the number of swabs for allergen plate ELISA testing was kept to a minimum. Squares for allergen ELISA swabbing were randomised, as for all swab types, but a total of 10 swabs were collected;

- 1 swab before drying
- 3 swabs after pre-rinse of sheet after drying
- 3 swabs after washing with detergent and rinsing
- 3 swabs after washing with disinfectant and rinsing

Each swab was individually labelled corresponding to the time of swabbing (before, after etc) and with the sheet number and square number.

4.4 Washing method

The method used for the spraying of the sheets after drying included (in order);

- 2x sprays of water and a second set of swabs taken,
- 2x sprays of detergent (left for contact time of 10 mins) and hose purged,
- 2x sprays of water and a third set of swabs taken,
- 2x sprays of disinfectant (left for contact time of 30seconds) and hose purged,
- 2x sprays of water and a forth set of swabs taken.

Each spray was slowly drawn over the surface of the sheet to ensure complete coverage and contact. During the 10 minutes detergent contact time, the next sheet was covered with slurry and put in the incubator for drying.

4.5 Reading results

4.5.1 Supersnap –

The results were taken straight after swabbing, only snapping when the machine was ready to take a reading. The machine was stood upright on the counter during the reading and the first reading was always recorded.

4.5.2 ALLER-snap –

All of the protein swabs were snapped at the end of the trials and placed in a 37°C incubator for 30 minutes.

4.5.3 Allergen lateral flow tests -

These tests were carried out as the trial was carried out. A small incubator was used for the R-Biopharm egg and peanut dip tests (37°C), the incubator was provided by Hygiena (Mini incubator 10 slots serial number 3754). All the testing was done according to the manufacturers instructions.

4.5.4 Swabs for allergen plate ELISA testing

Carried out by the Biochemistry Section of Campden BRI:

Gluten: A total dilution of 1/75000 was conducted in order to determine the level of gluten in the ready-meal slurry. The lower limit of quantification (LLOQ) for swabs is 0.01mg gluten/L and the upper limit of quantification (ULOQ) is 0.16mg gluten/L. Testing for false negative results was conducted by analysis of a suspension of a

gluten positive sample extract with cleaning fluid at the working concentration-the LLOQ for sample extracts using 1/500 nominal dilution is 5mg gluten/kg, and the ULOQ is 80mg gluten/kg.

Peanut: A total dilution of 1/100 was conducted in order to determine the level of peanut in the ready-meal slurry. The swabs (regarded as an indicative of the presence/absence of peanut) have a LLOQ of 0.13mg peanut/L, and a ULOQ of 1mg peanut/L. Testing for false negative results due to cleaning fluid interference was conducted by analysis of a suspension of a peanut positive sample extract with cleaning fluid at the working concentration – LLOQ for sample extracts using 1/20 nominal dilution is 2.5mg peanut/kg, and the ULOQ is 20mg peanut/kg.

Testing of the false positives due to cleaning fluid interference was conducted by analysis of the cleaning fluid at the working concentration (1%).

All of the allergen plate ELISA kits and swabs were carried out according to the manufacturers' instruction leaflets provided with the kits.

5. CONTROLS

Controls were carried out to confirm that the tests detect the presence of allergens in the slurry. Controls to identify false positive and false negative results interference by cleaning fluids were carried out before the trial. Specifically, the controls carried out were:

5.1 Working concentration of slurry

The slurry made fresh on the day of the trial was tested with all the allergen lateral flow kits, the ALLER-snap protein swabs (snapped straight away and incubated in mini-incubator in lab) and the ATP Supersnap swabs (reading taken straight away).

5.2 False negative cleaning fluid interference testing

Disinfectant: This control was prepared by adding 5g ±0.7g of double working concentration disinfectant (2%) to double concentration of slurry (4.7g). Double concentration disinfectant was obtained by changing the dial on the high pressure washer to 2% instead of 1% and letting the disinfectant solution flow through the machine for a minute before taking a sample. These two concentrations were mixed together and an ATP reading taken straight away. All the allergen tests were also taken except peanut lateral flows as there were not enough peanut kits to fulfil the trials.

Detergent: This control was prepared by adding 5g ±0.7g of double working conc. detergent to 5.7g of double concentrated slurry. Double concentration of detergent was obtained by changing the dial on the high pressure washer to 2% instead of 1% and letting the detergent solution flow through the machine for a minute before taking a sample. These two concentrations were mixed together and an ATP reading taken straight away. All the allergen tests were also taken except peanut lateral flows as there were not enough peanut kits to fulfil all the trials.

5.3 Cleaned stainless steel sheet

The stainless steel sheets were cleaned before the trails using detergent and rinsed thoroughly ensuring no residue was left on the surface, and then dried using paper towels. An ATP swab was taken at the centre of the sheet and a reading taken straight away. Gluten and protein tests were also carried out on the sheet to make sure the surface was clean of food residues.

5.4 False positive cleaning fluid interference testing

This control consisted of taking samples of 1% disinfectant and 1% detergent (working concentration) and testing with all of the tests except peanut lateral flow.

5.5 False positive water testing

Mains water: water was purged through the high pressure washer and a sample taken. This sample was then tested with all the tests used in the trial (except plate ELISAs) for any false positives.

Filtered water: was tested using the lateral flow kit for egg after all the other controls due to the spurious results (Section 6, Table 3). (Produced at Campden BRI using a PURELAB Prima 15 machine which is demineralised and passed through a reverse osmosis membrane).

Sterile distilled water: was tested using the lateral flow kit for egg after all the other controls due to the results (Section 6, Table 6). It was a matter of taking a sample of sterile water produced at Campden BRI and following the steps accordingly in the lateral flow kit. An ATP test was also conducted.

5.10 ATP blanks

Five ATP Supersnap swabs were broken and read immediately without exposing the swab matrix. These were averaged as the ATP blank.

6. RESULTS

Table 1: The results from the control swabs taken before starting the experiment; ATP, protein and lateral flow tests (excluding plate ELISA results).

Control Results						
Substance	Results					
Working conc. slurry and disinfectant	**Gluten**	**Casein**	**Egg**	**Protein**	**ATP (average of 3 readings)**	
	+	+	+	+	0 (day 1) 7446 (day 2)	
Working conc. slurry and detergent	**Gluten**		**Protein**		**ATP**	
	+		+		7664	
Cleaned stainless steel sheet	**Gluten**		**Protein**		**ATP**	
	-		-		4	
Working conc. slurry	**Gluten**	**Casein**	**Peanut**	**Egg**	**Protein**	**ATP**
	+	+ *	+*	+	+	8543
Working conc. disinfectant	**Gluten**	**Casein**	**Peanut**	**Egg**	**Protein**	**ATP**
	-	-	-	+°	+×	11
Working conc. detergent	**Gluten**	**Casein**	**Peanut**	**Egg**	**Protein**	**ATP**
	-	-	-	+	-	0
Mains water	**Gluten**	**Casein**	**Peanut**	**Egg**	**Protein**	**ATP**
	-	-	-	+°	-	11
Filtered Water	**Egg**					
	+					
Sterile distilled water	**Egg**			**ATP**		
	-			0		
ATP swab blank				0		
*= Faint Line, °= Test repeated twice, × = Turned Grey (Faintly positive)						

The control results in Table 1 show that the disinfectant had an effect on the operation of the ATP swabs, showing false negative, at one out of two days when the controls were measured, for the working concentration of the disinfectant with slurry. The same situation occurred with the plate ELISA method (see Tables 8b and 9b) where the false negative results were also recorded. The protein and allergen tests did not show false negatives when testing disinfectant with slurry. When testing the disinfectant at its working concentration, the egg allergen kit and protein kit showed false positives. The detergent mixed with slurry gave acceptable results with all tests, though the detergent alone gave false positive results for the egg kit. Table 1 also shows that the stainless steel sheets were clean before starting the trial with a very low ATP level. Water was negative for all except egg (that is positive for all tests).

Additional tests were performed for the egg lateral flow allergen kit. Filtered water was tested and also showed positive and only sterile distilled water showed a negative result for egg allergen.

Table 2: Results from the Supersnap ATP swabs for all 10 stainless steel sheets at 4 different stages in the cleaning cycle.

ATP **Supersnap** swabs results (rlu–relative light units) at intervals of cleaning cycle				
Grid no / time interval	Before Drying	Pre-rinse after drying	After washing with detergent and post rinse	After washing with disinfectant and post rinse
1	8150	6791	10	43
2	7407	7442	28	5
3	7112	8233	623	22
4	8755	8255	1198	24
5	8373	8118	126	9
6	7838	8446	46	15
7	8477	8007	38	11
8	7284	8549	143	12
9	8897	7603	87	11
10	8777	8148	72	137

The results displayed in table 2 show that before drying, and pre-rinse after drying, have no real change in the amount of ATP present. After washing with detergent and post rinse shows a substantial drop in the amount of ATP on the surface and an even further reduction after washing with disinfectant and post rinse. Sheets 3 and 4 have high counts of ATP after detergent and rinsing; however these are reduced to lower levels after disinfection and rinsing. The levels of ATP after the final sanitation step were low, however ATP was still present on the surfaces at values higher than the swab blank.

Table 3: Results for the ALLER-snap protein swab on all 10 stainless steel sheets at 4 different stages in the cleaning cycle. Results displayed the same as the colour code on the ALLER-snap swab.

Protein **ALLER-snap** swabs at intervals of cleaning cycle				
Grid no. / time interval	Before Drying	Pre-rinse after drying	After washing with detergent and post rinse	After washing with disinfectant and post rinse
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Purple = definite positive reading, Grey = Faintly positive, Green = Negative				

The colour coded results above in table 3 show that protein was definitely present before washing with detergent and rinsing. After washing its presence declined, although some surfaces recorded faintly positive or 1 positive result, which follows the trend seen in Table 2 of a higher amount of ATP being present on sheets 3 and 4. There were no positive results after washing with disinfectant and final rinse.

Table 4: The results from R-Biopharm gliadin (gluten) lateral flow swabs for all 10 stainless steel sheets at 4 different stages in the cleaning cycle.

Gluten (gliadin) **R-Biopharm** lateral flow tests at intervals of cleaning cycle				
Grid no. / time interval	Before Drying	Pre-rinse after drying	After washing with detergent and post rinse	After washing with disinfectant and post rinse
1	-	+	+	-
2	+	+	+	-
3	+	+	+	-
4	+	+	+	-
5	+	+	+	-
6	+	+	+	+/-*
7	+	+	+	+/-*
8	+	+	+	-
9	+	+	+	-
10	+	+	+	-
* = Half red line appeared, invalid result.				

Results presented in Table 4 show that gluten was present on most of the surfaces until washing with detergent and rinsing. Sheet 1 before drying shows an anomaly as compared with all the other sheets and as the pre-rinse after drying shows a positive result. Most of the results after washing with disinfectant and rinsing were below detectable levels; there were 2 tests which showed only a half red line which could not be classified as a negative or positive result, therefore these tests are classified as invalid.

Table 5: The results from R-Biopharm peanut lateral flow swabs for all 10 stainless steel sheets at 4 different stages of the cleaning cycle.

Peanut **R-Biopharm** lateral flow tests at intervals of cleaning cycle				
Grid no. / time interval	Before drying	Pre-rinse after drying	After washing with detergent and post rinse	After washing with disinfectant and post rinse
1	+*	-	-	-
2	+	+*	-	-
3	+*	+*	-	-
4	+	-	-	-
5	-	+*	-	-
6	+	+*	-	-
7	+	+	-	-
8	+	+*	-	-
9	+	+	-	-
10	+	-	-	-
* = Faint line appeared				

The test results for peanut lateral flow tests displayed in Table 5 show that after washing with detergent and post rinse all peanut was removed from the surface of the sheets below the detectable level of the kit (typically 10 mg/L). The same results were observed for the final disinfectant step.

Table 6: The results from R-Biopharm egg lateral flow swabs for all 10 stainless steel sheets at 4 different stages of the cleaning cycle.

Egg **R-Biopharm** lateral flow tests at intervals of cleaning cycle				
Grid no. / time interval	Before drying	Pre-rinse after drying	After washing with detergent and post rinse	After washing with disinfectant and post rinse
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+

The test results for egg in Table 6 show all positive results for all stages of the cleaning cycles.

Table 7: The results from Neogen casein lateral flow swabs for all 10 stainless steel sheets at 4 different stages of the cleaning cycle.

Casein **Neogen** lateral flow tests at intervals of cleaning cycle				
Grid no. / time interval	Before drying	Pre-rinse after drying	After washing with detergent and post rinse	After washing with disinfectant and post rinse
1	+	+	-	-
2	+	+	-	-
3	+	+	-	-
4	+*	+	-	-
5	+*	+	-	-
6	+	+	-	-
7	+	+	-	+
8	+	+	+	+
9	+	+	+	+
10	+*	+*	+*	+
* = Faint line appeared				

The casein results displayed in Table 7 show a full removal of the casein after washing with detergent and post rinse for sheets 1- 6. Sheets 8-10 show recurring positives for all the stages and sheet 7 shows up positive after disinfection after a negative after washing with detergent.

Table 8 (a): Results from the plate ELISA swabs, gluten detection for sample slurry and swabs taken during the trial

Grid number	Sample description	Result
-	Slurry applied to surface	7200 mg/kg
4	**Swab before drying**	**>0.16 mg/L**
10	Swab after Rinsing	>0.16 mg/L
8	Swab after Rinsing	>0.16 mg/L
6	Swab after Rinsing	>0.16 mg/L
5	Swab after washing with detergent and rinse	>0.16 mg/L
9	Swab after washing with detergent and rinse	0.03 mg/L
6	Swab after washing with detergent and rinse	>0.16 mg/L
6	Swab after washing with disinfectant and rinse	0.02 mg/L
5	Swab after washing with disinfectant and rinse	0.04 mg/L
7	Swab after washing with disinfectant and rinse	0.03 mg/L

Table 8 (b): Results from the plate ELISA swabs, gluten detection of cleaning fluid interference testing

	Sample description	Result
-	Working conc. disinfectant for false positive	<5mg/kg
-	Working conc. detergent for false positive	<5mg/kg
-	Working conc. disinfectant + gluten extract for false negative (expected amount 15mg gluten/kg)	<5mg/kg
-	Working conc. detergent + gluten extract for false negative (expected amount 15mg gluten/kg)	<5mg/kg

The results in Table 8 (a) and (b), for gluten detection by the plate ELISA method show a positive result for the slurry and no undesirable false positive results for the working concentrations of detergent and disinfectant. Testing of the working concentrations of the disinfectant and detergent with gluten showed false negative results (Table 8 (b)). From the results (Table 8 (a)) during the trial a gradual reduction in the amount of allergen detected was seen as the cleaning cycle progressed, but residues were still detected after disinfection and rinsing.

Table 9 (a): Results from the plate ELISA swabs, peanut detection for slurry and swabs taken during the trial

Grid number	Sample description	Result
-	Slurry applied to surface	800 mg/kg
4	**Swab before drying**	**>1mg/L**
10	Swab after Rinsing	>1 mg/L
8	Swab after Rinsing	>1 mg/L
6	Swab after Rinsing	>1 mg/L
5	Swab after washing with detergent and rinse	<0.13 mg/L
9	Swab after washing with detergent and rinse	<0.13 mg/L
6	Swab after washing with detergent and rinse	0.13-1 mg/L*
6	Swab after washing with disinfectant and rinse	<0.13mg/L
5	Swab after washing with disinfectant and rinse	NT
7	Swab after washing with disinfectant and rinse	NT
*Semi quantitative result for this sample only NT = not tested		

Table 9 (b): Results from the plate ELISA swabs, peanut detection for cleaning fluid interference testing

	Sample description	Result
-	Working conc. disinfectant for false positive	<2.5mg/kg
-	Working conc. detergent for false positive	<2.5mg/kg
-	Working conc. disinfectant + peanut extract for false negative (expected amount of 4mg peanut/kg)	<2.5mg/kg
-	Working conc. detergent + peanut extract for false negative (expected amount of 4mg peanut/kg)	<2.5mg/kg

The results in Tables 9 (a) and (b) for peanut detection by the plate ELISA method show a positive result for the working concentration of slurry and no undesirable false positive results for the working concentrations of detergent and disinfectant. Testing of the working concentrations of disinfectant and detergent with peanut showed false negative results (Table 9 (b). From the results during the trial a gradual reduction in the amount of allergen detected as the cleaning cycle progressed was seen, with 2 of the 3 swabs after washing with detergent and rinsing showing results below the lower limit of detection.

7. DISCUSSION

Table 10: Summary results for all detection techniques tested

Simulated cleaning process	**High Sensitivity ATP**		**High Sensitivity Protein**	**Plate ELISA Allergen tests**			**Lateral Flow Allergen tests (#<LOD-passes/ Positive-Fails)**			
	EnSURE SuperSnap		AllerSnap 37°C, 30min	R-Biopharm			R-Biopharm	R-Biopharm	R-Biopharm	Neogen
	Average RLU	# Passes/ Fails	# Passes / Fails	mg gluten/L	mg peanut/L		Gluten	Egg	Peanut	Casein (milk)
Before Drying	8107	0 / 10	0 / 10	>0.16	>1.0		1 / 9	0 / 10	1 / 9**	0 / 10****
Pre-Rinse	7959	0 / 10	0 / 10	>0.16	>1.0		0 / 10	0 / 10	3 / 7***	0 / 10 *****
Detergent and rinse	237	1 / 9	5 / 5	0.03 (x1) >0.16 (x2)	<0.13 (x2) 0.13 - 1.0 (x1)		0 / 10	0 / 10	10 / 0	7 / 3 *****
Disinfectant and rinse	29	2 / 8	10 / 0	0.03 (average of 3)	<0.13 (x1) Not tested(x2)		8 / 0*	0 / 10	10 / 0	6 / 4
	ATP Fails = samples >10 RLU (according to the ATP kit manufacturer recommendation)						*2 invalid	Negative controls all positive, results invalid	**2 faint ***5 faint	**** 3 faint ***** 1 faint

Table 10 summarises all results achieved.

7.1 Controls

The solution of slurry and disinfectant showed false negatives for the plate ELISA method and ATP tests (one out of two occasions), however in the trials all of the sheets showed a reading for ATP after disinfection (Table 2). This may be due to the disinfectant having been efficiently rinsed off and the actual ATP/soil left on the surface was picked up by the ATP Supersnap swabs. From this it can be taken that when using disinfectant in the sanitising cycle to thoroughly rinse as a false negative may be given when using some testing kits. The protein and allergen lateral flow tests did not show false negatives when testing with the disinfectant with slurry.

For the purposes of this work, any ATP readings greater than the swab blank of 0 (zero) are taken as positive indications of the presence of ATP, however, following the manufacturer's instructions, in these trials, only results greater than 10 RLU are considered as a fail result.

The detergent had no or little effect on the ATP and the other test's results when mixed with slurry. The false negative testing of the detergent showed a positive result for ATP testing which was to be expected, showing the detergent had no/little effect on the Supersnap swab (the result was not as high as the pure slurry but it was close) and can be more readily trusted. Also the average ATP result for the slurry was 8107 RLU with a standard deviation of 8.2% (Table 2, column 1). The RLU results in the presence of detergent or disinfectant were 7664 RLU and 7446 RLU respectively, which is a difference of 5.5 % and 8.2% of the average respectively and within standard variation. Similar results were observed for the protein and gluten lateral flow tests. Detergent alone gave false positive results for the egg kit only. Additional tests were performed for the egg allergen kit to determine whether this was a rouge result. Filtered water was tested which also showed positive, and only sterile distilled water showed a negative result for the egg allergen. Table 1 also shows that the stainless steel sheets were clean before starting the trial with very low ATP levels. Water was negative for all tests except the egg lateral flow (which was positive for all tests). After cleaning and drying of the stainless steel sheets the ATP swabs showed a minimal reading of only 4 stating that it is a clean surface to begin the trials with. There was also no protein and gluten detected by lateral flow.

The working concentration of the slurry showed positive for all the tests which is highly desirable, however the casein and peanut lateral flow tests showed only a faint line either representing a low amount of the allergen or an overloading of the allergen test. Therefore for casein, a 100x dilution was tested, which showed negative, and 100% semi-skimmed milk was tested showing up with a faint line as before (therefore the test could be overloaded but still showing a positive result).

The working concentration of the disinfectant showed negative results for casein, peanut and gluten. A small ATP reading of 11rlu was detected which was the same as the suggested water reading, that disinfectant alone did not cause false positive ATP readings. However the tests for protein and egg showed false positive readings. The protein ALLERsnap test showed up green/grey which is on its lowest end of the

detection scale and could reflect upon its detection capabilities due to a possible compound in the disinfectant. The egg test was carried out twice to ensure no contamination and carried out as aseptically as possible; they both showed up positive.

The water tested showed negative for all tests except ATP which is a very small amount (11rlu) and again R-Biopharm egg. Again this test was carried out as aseptically as possible using the water from the mains (used in trial) and repeated twice more with filtered water; these tests also showed up positive. It was not possible to clean the water for the experiment any further because high volumes were needed to clean the stainless still sheets. The only negative results for egg were achieved when testing sterile distilled water.

7.2 Cleaning stages results

The results from the egg allergen lateral flow kit were positive throughout all tests and controls, even for the water from the mains. A problem with the test kit is possible, which may not be reflective of the kits abilities. For the purpose of this work therefore, the egg allergen results are not further discussed.

7.2.1 Fresh slurry applied

The Supersnap swabs for ATP detection picked up high levels of ATP on the surfaces were the slurry was freshly applied; the average ATP reading on all surfaces was 8107rlu. The protein test and all allergen lateral flow tests also showed positives on most of the surfaces. This was also confirmed by the plate ELISA testing where peanut and gluten were detected in swabs of surfaces with freshly applied slurry. There was a high amount of visible slurry on the surfaces prior to cleaning; therefore these results are as expected, without any deviations.

7.2.2 Pre –rinse after drying

This stage did not remove a significant amount of slurry from the surface because most of the food product was dried and strongly attached to the stainless steel surfaces. This step, therefore, served to moisten the surface and make further cleaning easier.

The presence of visible slurry was also reflected in the tests results. There were still high ATP levels on all surfaces (average 7959 rlu), also the protein, casein and gluten lateral flow tests were all positive. The peanut lateral flow test showed mostly positives however there were 30% negatives, which indicates that there may have been some reduction of allergen already tested. The plate ELISA testing showed results at detectable levels for both allergens. All of the methods at this stage showed comparable results.

7.2.3 Wash with detergent and rinse

Following the 'detergent and rinse' stage, there was no visible slurry remaining on the test surfaces. The levels of ATP dropped dramatically (ranging from 10 to 1198 rlu, average 237 rlu). Sheets 3 and 4 had the highest levels of ATP and this also reflects in the ALLERsnap protein readings with sheets 3 and 4 showing positive results. The overall protein readings showed 50% positive and 50% negative, which again correlates with the ATP results. The gluten lateral flow test, however, still showed all positive results which were also observed in the plate ELISA gluten test, where one result showed a reduction in allergen level but it was still present on the surfaces. According to the results from the peanut lateral flow test, the allergen was not detected on the surface after this stage; however, the plate ELISA test for peanut still detected its presence in one grid. For the Neogen casein kit, the first 7 sheets after detergent and rinsing are all negative; whilst the last 3 sheets 7-10 are all positive. These results are not usual, because the positive results are not randomly distributed within the 10 stainless steel sheets, they all occurred for the last 3 samples. This may suggest that the samples were contaminated towards the end of the experiment and all showed positive results, though there was an individual kit used for each swab which should rule out this hypothesis.

Whilst cleaning was performed in an identical fashion for all sheets, the position of the swabbed square may have had an effect on the results, which could be a reason why there are some variations within ATP readings, protein readings and plate ELISA results. Although most of the methods show that a contaminant is still present, only peanut and casein lateral flow showed negative results following detergent cleaning and rinsing.

The detergent cleaning should have removed most of the food residue. The reason why there was still a detectable level of food debris/allergens may be because no mechanical scrubbing of the surface was performed. Mechanical cleaning was not undertaken as it is difficult to reproduce between sheets; cleaning with the pressure hose is a more reproducible method.

7.2.4 Wash with disinfectant and post rinse

Following the 'disinfectant and rinse' stage, the average level of ATP dropped to 28.9 rlu (ranging from 5 to 137 rlu). The ALLERsnap protein test did not detect any protein residues, which was the same for gluten and peanut residues, which were below the limit of detection using the R-Biopharm lateral flow tests. 40% of the Neogen casein lateral flows showed up positive, but again, the results look abnormal due to the distribution of the positive results (last 4 sheets). The plate ELISA test confirms a reduction in the level of gluten on the surfaces; however gluten is still detected at this stage. The peanut allergen was below the lower limit of detection for the 1 sample tested, which correlates with the commercial lateral flow results.

In the control results, protein was positive (grey) when tested with disinfectant (false positive). To record all negative results after the disinfectant and rinse stage implies that all residues from the disinfectant were rinsed away.

The ATP (one out of two occasions) and plate ELISA tests showed false negatives when the disinfectant was tested with the disinfectant and slurry/allergen mixture. However, following disinfectant application and rinsing, the results for ATP were greater then 0 and there were some detectable amounts of allergens picked up by ELISA testing, which also suggests that the disinfectant was rinsed away by the final rinse.

8. CONCLUSIONS

The rinsing, cleaning and disinfection stages were designed to produce a gradual reduction in food soil on the test surfaces. This was reflected in the results produced from the ATP swabs, protein (ALLERsnap) swabs, casein (NEOGEN) lateral flows, peanut, gluten (R-Biopharm) lateral flows and plate ELISA testing for gluten and peanut (R-Biopharm).

However, after the disinfection and final rinse step only the plate ELISA test picked up the presence of allergens (all gluten swabs and 1 peanut) and the ATP Supersnap swabs gave an indication of ATP still being present. These tests were thus the most sensitive in the detection of their specific parameters.

For the test soil and cleaning methods performed in this work, there is correlation between the detection of low levels of allergens by plate ELISA tests and low levels of ATP by the ATP Supersnap test kit. Such correlations may not occur for all food soils and cleaning and disinfection systems, however, and this would have to be validated for every food processing situation in which the tests were to be used.

There appears to be a risk of getting false negatives, when performing plate ELISA and ATP testing, by interference from disinfectant or, in case of plate ELISA, also detergent residues. These tests are more likely to be robust, therefore, when in practical applications, the use of a disinfectant is followed by a final rinse. In food factories were the disinfectant is not rinsed off, validation tests for the use of all residue detection kits should be undertaken.

The egg detection kit did not produce any reliable results within the trial and the results can be discarded. Similarly, there may have been issues related to the Neogen lateral flow kit for casein detection, which showed a clear trend between the first and last sheets sampled.

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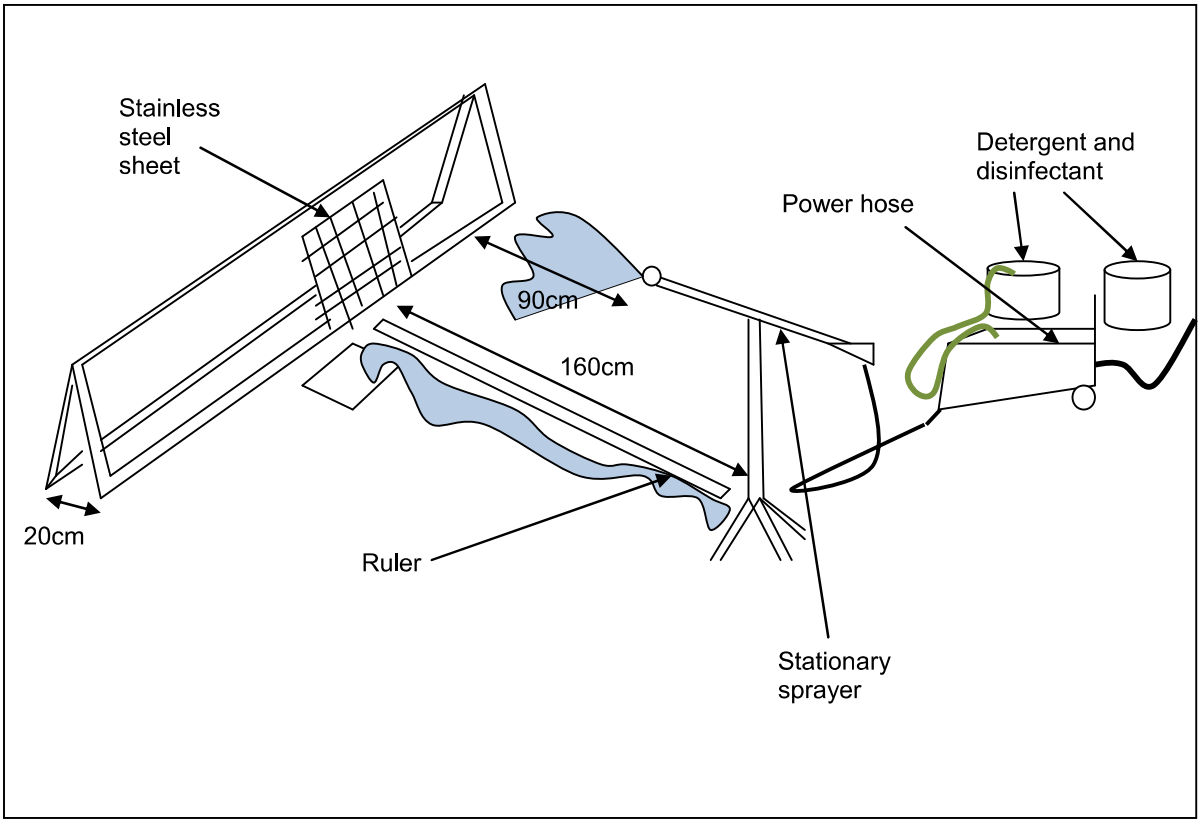
APPENDIX I

Spraying Layout

Spraying layout



APPENDIX II

Randomised swabbing method

Appendix 2 - Randomised swabbing method

--- Before drying
--- After pre-rinse
--- After detergent and rinse
--- After disinfectant and rinse
▉ Unused square

SS –ATP (Supersnap)
AS –Protein (ALLERSnap)
RG – Gluten (R-Biopharm)
NC – Casein (Neogen)
R – Egg and Peanut (R-Biopharm)
E – Plate ELISA testing

1

1	2	3	4	5	
NC	RG	AS	NC	NC	5
AS	AS	SS	RG	■	10
RG	NC	■	AS	R	15
RG	SS	R	■	■	20
SS	SS	R	R	■	25

2

1	2	3	4	5	
■	RG	R	R	■	5
■	NC	SS	R	RG	10
NC	SS	■	AS	R	15
AS	RG	AS	SS	SS	20
NC	RG	NC	■	AS	25

3

1	2	3	4	5	
■	AS	AS	■	R	5
SS	■	SS	■	AS	10
E	RG	NC	RG	SS	15
NC	R	R	NC	AS	20
SS	NC	RG	R	RG	25

4

1	2	3	4	5	
SS	R	RG	AS	AS	5
R	AS	NC	SS	RG	10
R	R	SS	E	RG	15
RG	■	■	■	NC	20
NC	SS	NC	■	AS	25

5

1	2	3	4	5	
■	NC	RG	AS	RG	5
R	E	SS	■	RG	10
SS	R	RG	R	R	15
■	SS	E	AS	AS	20
SS	AS	NC	NC	NC	25

6

1	2	3	4	5	
NC	RG	RG	AS	E	5
SS	■	■	SS	NC	10
R	AS	AS	RG	■	15
RG	R	R	SS	E	20
NC	AS	R	NC	SS	25

7

1	2	3	4	5	
NC	R	AS	■	RG	5
E	R	SS	NC	NC	10
AS	■	■	AS	SS	15
R	AS	RG	SS	RG	20
■	SS	RG	NC	R	25

8

1	2	3	4	5	
■	RG	RG	NC	AS	5
SS	RG	RG	SS	AS	10
NC	AS	■	AS	R	15
NC	■	E	SS	SS	20
R	R	■	R	NC	25

9

1	2	3	4	5	
E	■	■	AS	RG	5
R	SS	R	AS	NC	10
AS	RG	SS	■	R	15
RG	NC	E	NC	R	20
NC	RG	AS	SS	SS	25

10

1	2	3	4	5	
NC	R	■	NC	■	5
SS	AS	AS	SS	NC	10
R	SS	■	RG	AS	15
NC	R	AS	RG	RG	20
■	R	SS	RG	E	25