Practical measurements for allergen control and cleanliness

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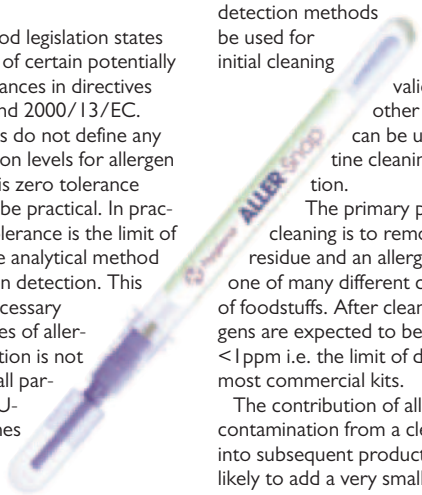
Whereas there is general concern and agreement for the control of allergens in foods to ensure food safety, there is also a lack of clarity leading to uncertainty and indecision.

Leading experts such as European Voluntary Incidental Trace Allergen Labelling (EU-VITAL) have summarised the situation for food content as follows:

"European food legislation states the declaration of certain potentially allergenic substances in directives 2007/68/EC and 2000/13/EC. These directives do not define any thresholds/action levels for allergen declaration. This zero tolerance appears not to be practical. In practice the zero tolerance is the limit of detection of the analytical method used for allergen detection. This has led to unnecessary labelling of traces of allergens. This situation is not acceptable for all parties involved. EU-VITAL establishes a standardised procedure for food producers in order to get a clear declaration for allergens. EU-VITAL defines action levels for labelling or not labelling. The proposed action levels are based on a recommendation of European and International scientific expert groups, who intensively examined this issue."



Declaration labelling of products is recommended when levels of allergens are generally >100ppm (mg allergenic substance per 1kg food) and a lower level of risk for 'may contain traces' is typically 10-200ppm.

A similar situation also exists for the post-cleaning verification processing equipment. There is a lack of clear guidance as to the methods and standards for allergen residues on product contact surfaces.

Technical expert groups and global standards organisation such as BRC recommend that specific allergen detection methods be used for initial cleaning validation but other methods can be used for routine cleaning verification.

The primary purpose of cleaning is to remove product residue and an allergen is just one of many different components of foodstuffs. After cleaning, allergens are expected to be present at <1ppm i.e. the limit of detection of most commercial kits.

The contribution of allergen cross contamination from a clean surface into subsequent product is therefore likely to add a very small non-detectable risk in the finished product.

Accordingly methods with the greatest sensitivity and broadest spectrum will give the greatest assurance of cleanliness and hence demonstrate a low cross contamination hazard and risk from allergens. As above, the concept of zero tolerance of allergen residues on product contact surfaces is neither reasonable nor practical.

Test methods

Specific detection methods alone give partial information about overall safety and risk, and should be used as a balanced analytical approach.

There are several methods for specific allergens of which immunological methods, for example quantitative plate ELISA tests and qualitative lateral flow devices (LTD) in dipstick formats are the most commonly used however the relatively high cost is often an impediment to their widespread adoption.

Plate ELISA test are more sensitive (typically <0.1ppm) and require a skilled analyst, whereas LTD are more convenient tests and have a limit of detection or 1-10ppm. Results from LTD can have variable performance and all methods for surface measurement are dependent on swabbing and sample recovery.

By contrast simple rapid hygiene test such as ATP bioluminescence and non-specific protein test are widely used by industry and are well established proven methods of cleaning validation and verification.

The benefit of ATP hygiene monitoring is also recognised in supplier specification, by BRC and regulatory agencies.

There have been many advances in ATP systems in recent years to make it simpler, more robust, more sensitive and more cost effective. The EnSURE instrument and SuperSnap reagent swab both provide higher sensitivity with low background noise and low variation for consistent high performance results.

This means that this system is x10 more sensitive than SystemSURE Plus with UltraSnap swabs and x100 more sensitive than other ATP systems (see Table 1).

SuperSnap also provides more robustness and tolerance to harsh materials at extremes of pH and in the presence of sanitiser, for example it is not affected by 1000ppm hypochlorite. Non-specific protein tests are also used to verify cleaning.

Most allergens are glycoproteins and a non-specific protein test can

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Table 1. Comparative sensitivity of new ATP systems.

Performance parameter	SystemSure UltraSnap	EnSure SuperSnap	Supplier A	Supplier B	Supplier C	Supplier D
Background noise (RLU)	0-1	0-1	2-11	100-570	0-511	0 - 48
Limit of detection (fmols)	1.0-1.4	0.1-0.2	1.3-2.7	1.1	10.0	10.0
%CV at 10 fmols ATP	6.2-10.4	6.9	17.1	52.6	213.8	114.4

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provide additional relevant information on residual contamination. High sensitivity protein tests such as AllerSnap can detect down to 1µg protein.

When using a combination of detection methods, case studies have shown that when high levels of cleaning are achieved and proven by both high sensitivity ATP and protein tests then specific allergens are not detected.

The combined methods approach provides a more rapid, comprehensive and cost effective approach with greater assurance of cleanliness.

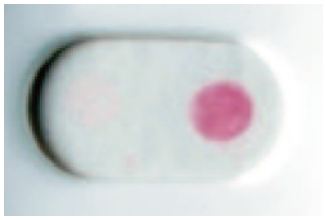
Allergen cleaning study

An extensive independent laboratory study was conducted in a pilot plant facility to simulate an industrial cleaning process. Several detection methods for the measurement of cleanliness and removal of food residue and allergens were compared.

A sieved slurry made from a commercial ready-to-eat noodle meal containing a variety of meat, vegetables, egg and other components was prepared as a model system. The allergen contents stated on the packaging are egg, gluten, soya and 'unsuitable for peanut allergy sufferers'. The slurry was supplemented with freeze dried peanut powder and semi skimmed milk to ensure that peanut and milk allergens were present at detectable levels.

A 10g portion of slurry was applied evenly to stainless steel sheets (50 x 50cm) and dried at 57°C for 10 minutes prior to implementing a cleaning procedure of pre-rinse, detergent treatment and rinse followed by a disinfectant treatment and rinse.

The cleaning procedure was administered via a static pressure hose running at 25 bar and at a distance of 90cm from the stainless steel sheets.



Test	Stages of cleaning			
	Before cleaning (wet residue)	After drying and pre-rinse	Detergent and rinse	Disinfectant and rinse
High sensitivity ATP EnSure SuperSnap	10/10 positive 8107 RLU	10/10 positive 7959 RLU	10/10 positive 237 RLU	10/10 positive 29 RLU
Plate ELISA Gluten	All positive >0.16ppm	All positive >0.16ppm	All positive 0.03 - 0.16ppm	All positive 0.03ppm
Plate ELISA Peanut	All positive >1.0ppm	All positive >1.0ppm	All positive 0.13 - 1.0ppm	All positive <0.13ppm
High sensitivity protein AllerSnap	10/10 positive >10µg	10/10 positive >5µg	5 positive >1µg 5 negative <1µg	10/10 negative negative <1µg
LTD allergen tests				
Gluten	9/10 positive 1/10 negative	10/10 positive	10/10 positive	8/8 negative 2/2 invalid
Peanut	7/10 positive 2/10 doubtful 1/10 negative	2/10 positive 5/10 doubtful 3/10 negative	10/10 negative	10/10 negative
Milk (casein)	7/10 positive 3/10 doubtful	9/10 positive 1/10 doubtful	2/10 positive 1/10 doubtful 7/10 negative	6/6 negative*

** 4/4 sample squares spoilt by cross contamination*

Table 2. Removal of residues and allergens during four stage cleaning procedure.

Each stainless steel sheet was marked into 25 squares each 10 x 10cm for testing purposes.

A randomised testing plan was devised such that each method had 10 replicate samples at randomised locations and at each stage of cleaning procedure.

The test methods included;

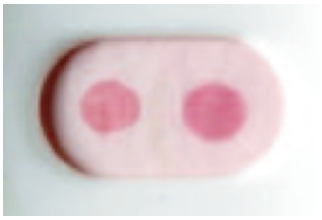
- High sensitivity ATP (EnSURE with SuperSnap swab).
- High sensitivity non-specific protein (AllerSnap).
- Plate ELISA allergen tests for gluten (Gliadin) and peanut (supplier 1).
- Lateral flow allergen dipstick test for gluten (Gliadin), peanut and egg (supplier 1) and milk (Casein, supplier 2).

Results

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 shown in Table 2 for the high sensitivity ATP test, protein (AllerSnap) test, lateral flow tests for casein peanut, gluten and plate ELISA testing for gluten and peanut. The egg detection kit did not produce any reliable results within the trial.

The FlowThrough system.



The ATP and plate ELISA methods were the most sensitive tests and were able to measure food residues after the final disinfection step. Only the plate ELISA tests for gluten and peanut detected the presence of allergens at all stages of cleaning and after the disinfection step.

The average ATP result after the disinfectant step was 29 RLU and any result above 2 RLU (0.1 fmols ATP) could be considered a positive. Accordingly this ATP test has extremely high sensitivity to detect the very small amounts of product residue. This would need to be verified for other foodstuffs and processing conditions.

After the disinfection step, none of the LTD allergen tests detected residues.

After the detergent cleaning step, only the gluten LTD detected residues in all 10 replicates, at which stage only two out of 10 replicates gave a positive detection with the milk LTD. The peanut LTD detected two clear positives samples out of 10 samples after the pre-rinse stage although five other samples were weakly positive. No peanut residues were detected by the LTD after the detergent or disinfectant stages but were detected by the ELISA peanut test.

Alternative, more robust, semi-quantitative versions of the LTD chemistry are being devised such as the FlowThrough system. These are more user friendly, cost effective and suffer less from interference at heavy sample loadings.

The non-specific protein tests AllerSnap detected residues at all stages of cleaning except the disinfectant stage and gave results equivalent or better than the LTD tested.

Accordingly, using a combination of high sensitivity ATP and non-specific protein tests verified the effectiveness of cleaning procedures to a high standard such that;

- ATP levels were <1.0 fmols.
- Protein residues were <1µg protein.
- Plate ELISA allergen test for gluten and peanut were < 0.03 and 0.13ppm respectively.
- Specific allergen for gluten, peanut and milk were not detected (<1-10ppm).

Benefits of rapid screening

The ATP test gives results in 15 seconds so that immediate corrective action such as re-cleaning can be implemented prior to conducting any additional, more expensive specific allergens tests, thereby saving time, cost and minimising down time.

When the results of high sensitivity ATP and protein tests show that there is very little residual contamination then there is a corresponding low level of specific allergen remaining that are below the limit of detection of LTD.

Using a combination of detection technologies gives a comprehensive assurance and verification of cleaning procedures to minimise the hazards and risks from residual allergen contamination. ■

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