# Detection of dried peanut and milk residues from stainless steel coupons using a sensitive ATP test, protein ELISA, and protein lateral flow tests.

Ken Jones[1], Chris Griffith[1], Janet Lewis[1] and Robert Salter[2]
[1]School of Applied Sciences, University of Wales Institute, Cardiff, Western Avenue, Cardiff CF5 2YB, email: kpjones@uwic.ac.uk
[2]Charm Sciences Inc, 659 Andover St, Lawrence MA, USA 01843

## Background

An estimated 1-2% of adults and 8% of children in the UK have a food allergy or intolerance. Food businesses are increasingly concerned about the risk to consumers stemming from accidental contamination of food. There has been a move away from end product testing (defect detection) to defect prevention including the assessment of cleaning efficacy to ensure the removal of allergens from contaminated surfaces. ELISA detection methods can be expensive, require specialist equipment and training and take time, ideally, a more rapid, equally sensitive surface test, would be beneficial.

The aim was to evaluate a highly sensitive ATP method, capable of detecting very low levels of food debris that might contain allergens within seconds, in comparison with more traditional methods that take longer.

## Methods

10 x 10cm stainless steel coupons, cleaned using a rigorous, previously validated, cleaning protocol, were coated with 100µl milk or peanut solutions and air dried. Solutions of EU standard peanut standard and NIST peanut butter standard, at 10, 7.5, 5, 4, 2 and 1ppm were tested. Skim milk powder and semi-skimmed milk at 10, 5, and 2.5 ppm were evaluated.

The peanut treated coupons were swabbed using a standard protocol and tested using the AllerGiene ATP system (Charm Sciences, Inc.), and some commercially available conarachin *ara h* specific ELISA kits (X and Y) as well as a rapid peanut lateral flow strip. For milk the AllerGiene ATP, β-lactoglobulin ELISA (kit M) and whey ELISA (kit N) were used. All tests were performed in quintuplicate and a positive interpretation required 5/5 detection at each dilution.

## Results and Discussion

Table 1 shows the limit of reproducible detection for the sensitive ATP system and ELISA Y were comparable for both the EU Peanut Standard and the USDA peanut butter (5ppm and 1ppm respectively). ELISA X did not give 5/5 detection at any concentration with either standard. The lateral flow strip did not detect any peanut antigen at any concentration.

**Table 1:** Dried peanut solution detection from stainless steel surface swabs using AllerGiene ATP and peanut *ara-h* protein specific tests

| | #Positive/#Tested | | | |
|---|---|---|---|---|
| Sample | **AllerGiene** ATP | **ELISA X** *ara-h* | **ELISA Y** *ara-h* | **Lateral Flow Strip** *ara-h* |
| EU Peanut Std | | | | |
| **10 ppm** | 5/5 | 4/5 | 5/5 | 0/5 |
| **7.5 ppm** | 5/5 | 0/5 | 5/5 | 0/5 |
| **5 ppm** | 5/5 | 2/5 | 5/5 | 0/5 |
| **4 ppm** | 0/5 | 0/5 | 0/5 | 0/5 |
| **2 ppm** | 0/5 | 0/5 | 0/5 | 0/5 |
| **1 ppm** | 0/5 | 0/5 | 0/5 | 0/5 |
| NIST Peanut Std | | | | |
| **10 ppm** | 5/5 | 4/5 | 5/5 | 0/5 |
| **7.5 ppm** | 5/5 | 4/5 | 5/5 | 0/5 |
| **5 ppm** | 5/5 | 1/5 | 5/5 | 0/5 |
| **4 ppm** | 5/5 | 0/5 | 5/5 | 0/5 |
| **2 ppm** | 5/5 | 0/5 | 5/5 | 0/5 |
| **1 ppm** | 5/5 | 0/5 | 5/5 | 0/5 |

Table 2 shows the limit of reproducible detection for the AllerGiene ATP system and the ELISA tests were broadly similar for both skim powder and semi-skimmed milk. The ELISA M detected 2.5 ppm pasteurized skim milk and the ATP and ELISA N detected 5 ppm. ELISA M detected all replicates at 10ppm and ELISA N was positive for 4 out of 5 with powdered skim milk. The sensitive ATP test detected 5 ppm.

**Table 2:** Dried milk detection from stainless steel surface swabs using AllerGiene ATP and milk protein specific ELISA tests

| | #Positive/#Tested | | |
|---|---|---|---|
| Sample | **AllerGiene** ATP | **ELISA M** B-lactoglobulin | **ELISA N** whey |
| Semi skim Milk | | | |
| **10 ppm** | 5/5 | 5/5 | 2/5 |
| **5 ppm** | 5/5 | 5/5 | 5/5 |
| **2.5 ppm** | 0/5 | 5/5 | 1/5 |
| Skim Milk Powder | | | |
| **10 ppm** | 5/5 | 5/5 | 4/5 |
| **5 ppm** | 5/5 | 2/5 | 1/5 |
| **2.5 ppm** | 0/5 | 0/5 | 2/5 |

## Conclusion

Management of food allergens and the prevention of cross contamination are of increasing concern and subject to new EU and US legislation. There is a need for improved controls and greater confidence in cleaning effectiveness when cross contact with allergens is a possibility. ATP is a general biologic indicator of organic debris not specific to allergens, but these results indicate that the AllerGiene sensitive ATP test, could be useful as an initial rapid indictor tool for assessing cleaning efficacy in removing food debris to the level of detection of protein specific allergen tests. Detected ATP could trigger immediate re-cleaning to lower levels before application of allergen specific tests. increasing concern and subject to new EU and US legislation. There is a need for improved controls and greater confidence in cleaning effectiveness when cross contact with allergens is a possibility. ATP is a general biologic indicator of organic debris not specific to allergens, but these results indicate that the AllerGiene sensitive ATP test, could be useful as an initial rapid indictor tool for assessing cleaning efficacy in removing food debris to the level of detection of protein specific allergen tests. Detected ATP could trigger immediate re-cleaning to lower levels before application of allergen specific tests. Further, specific allergen tests could be used to confirm that any high ATP readings were due to residual food debris containing allergens. This integrated approach to cross contact cleaning verification could speed cleaning effectiveness and validation testing at lower costs.

Further ATP evaluation work is needed in food production plants with a wider range of food items, including other nuts and composite processed products containing nut and dairy ingredients.