Detection of dried egg residues on a stainless steel surface using ELISA and sensitive ATP assays

F. Al-Taher[1], L.S. Jackson[2], Robert S. Salter[3]

[1]Illinois Institute of Technology, National Center for Food Safety & Technology (NCFST), 6502 S. Archer Rd., Summit-Argo, IL 60501; [2]Food and Drug Administration, NCFST, 6502 S. Archer Rd., Summit-Argo, IL 60501; [3]Charm Sciences Inc, Lawrence, MA

Abstract

Rapid methods are needed to verify that food soils are removed during equipment cleaning to prevent allergen cross-contact. Allergen-specific ELISA tests are used to detect the presence of allergens on swabbed equipment surfaces, but these tests require technical competence and 30 min or more to perform. Sensitive ATP tests are not allergen-specific but offer non-technical users a 30 sec evaluation of surface cleanliness. The objective of this work was to compare a sensitive ATP test to ELISA assays for detecting the presence of dried egg residues on a stainless steel surface. Spray-dried egg powder was diluted in ATP-free water to prepare solutions containing 0-1000 µg/mL (ppm) egg. The egg solutions were tested to determine the limit of detection using two different egg-specific ELISA kits and a sensitive ATP test. In addition, 1 mL of egg solutions was pipetted onto the surface of stainless steel plates. The plates were placed into an oven at 20-23ºC, 60ºC or 80ºC and heated until the egg solutions formed a dried residue. The residues were swabbed simultaneously for ELISA and ATP testing. The detection limit was defined as the level of egg which tested positive in all trials.

The detection limits for the sensitive ATP and ELISA tests using egg solutions were 25-50 ppm and 1-5 ppm egg, respectively. When egg solutions were dried onto the stainless steel surface, the detection limit was 250-500 µg egg for ATP and ELISA tests. The temperature at which egg solutions were dried onto the plates did not affect the detection limit for either assay. While ELISA assays were more sensitive than the ATP test for egg solutions, both assay methods had similar sensitivity to dried egg residues. Research is needed to evaluate the ability of ELISA and sensitive ATP tests to detect allergens on other food-contact surfaces.

Introduction

Since the early 1990s, the food industry has devoted considerable resources into developing allergen control plans with the goal of preventing unintended allergen contamination of food (Taylor and Hefle, 2005). Validation of the cleaning protocol is essential to an allergen control program. This is the process of assuring that a defined cleaning procedure is able to effectively remove the specific allergenic food from the specific food processing line or equipment (Holst, 2006). Validation/verification of cleanliness is often performed using analytical methods.

Immunoassays, such as ELISAs, have played a major role in validating allergen cleaning procedures. They are able to detect the presence of at least five different allergenic foods (egg, milk, peanut, soy, some tree nuts). Allergen-specific ELISA tests are used to detect the presence of allergens on swabbed equipment surfaces, but these tests are expensive, require technical competence and 30 min or more to perform.

ATP (adenosine triphosphate) tests are being used by the food industry along with ELISA-based (allergen-specific) tests to validate and verify cleaning program effectiveness. These tests, based on detection of ATP and total protein detection, provide a rapid detection and are inexpensive when compared to the ELISA tests. Sensitive ATP tests are not allergen-specific but offer non-technical users a 30 sec evaluation of surface cleanliness. The ATP tests detect microbial ATP as well as ATP associated with residual foods and therefore, only can be used to verify the effectiveness of wet cleaning procedures. Research is needed to compare immunochemical (allergen-specific) methods to non-specific methods (ATP and total protein) for determining cleaning efficiency.

Objective

To compare a sensitive ATP test to ELISA assays for detecting the presence of dried egg residues on a stainless steel surface.

Table 1. Detection of egg solids dried onto the surface of a stainless steel plate at different temperatures (20-23°C, 60°C and 80°C) using sensitive ATP and ELISA tests.

Amount of egg solids (µg)	Egg dried onto plate at 20-23ºC			Egg dried onto plate at 60ºC			Egg dried onto plate at 80ºC		
	Sensitive ATP¶	ELISA 1#	ELISA 2*	Sensitive ATP¶	ELISA 1#	ELISA 2*	Sensitive ATP¶	ELISA 1#	ELISA 2*
0 (Control)	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-
250	-	-	-	-	+	+	-	+	+
500	+	+	+	+	+	+	+	+	+

¶ Detection of egg residue with Charm Sciences AllerGiene® (sensitive ATP) test was considered positive when results were greater than the background level (>181,000 RLU) in all trials.
# Detection of egg residue with Neogen Alert for Egg test was considered positive when results (≥ 5 ppm egg) were reported in all trials.
*Detection of egg residue with R-Biopharm Ridascreen® Fast Egg Ei/Egg Protein Quantitative test was considered positive when results (≥ 5 ppm egg) were reported in all trials.

Materials and Methods

Spray-dried egg powder (Henningsen Foods, Omaha, NE) was diluted in ATP-free water to prepare solutions containing 0-1000 µg/mL (ppm) egg. The egg solutions were tested to determine the limit of detection using two different egg-specific ELISA kits (Ridascreen® Fast Ei/Egg Protein Quantitative test, R-Biopharm AG, Darmstadt, Germany; and Alert for Egg Allergen, Neogen Corp., Lansing, MI) and a sensitive ATP test. (AllerGiene ®, Charm Sciences, Lawrence, MA). In addition, 1 mL of the egg solutions (10, 50, 100, 250, and 500 µg/ml) was pipetted onto the surface of stainless steel plates. The plates were dried at 20-23ºC, 60ºC or 80ºC until the egg solutions formed a dried residue. The residues were swabbed simultaneously for the two ELISA tests and sensitive ATP testing. The detection limit was determined to be the level of egg which tested positive in all trials. All trials were done at least six times.

Results

- The detection limits for the sensitive ATP and ELISA tests using egg solutions were 25-50 ppm and 1-5 ppm egg, respectively (data not shown).
- When egg solutions were dried onto the stainless steel surface, the detection limit was 250-500 µg egg for ATP and ELISA tests (Table 1).
- In general, the temperature at which egg solutions were dried onto the plates did not affect the detection limit for either assay (Table 1).

Conclusions

- While ELISA assays were more sensitive than the ATP test for egg solutions, both assay methods had similar sensitivity to dried egg residues.
- Research is needed to evaluate the ability of ELISA and sensitive ATP tests to detect allergens on other food-contact surfaces.

References

Holst, B. 2006. Developing a cleaning process: Cleaning in development. In "Cleaning Validation Handbook," pp.4-22, Institute of Validation Technology, Duluth, MN.

Taylor, S.L. and Hefle, S.L. 2005. Allergen Control. *Food Technol.* 59(2): 40-43.