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Introduction:

A sensitive ATP (Adenosine Triphosphate) test has been shown to detect food soils from milk, eggs, and peanuts at ppm (part per million) levels similar to ELISA for allergenic proteins (1,2,3). ATP is present in most foods and is not a specific indicator for allergens but testing for ATP at the sensitive 0.02 femto mole level predicts whether dried allergenic food ingredients would be detected at similar $\mu\text{g}/100\text{cm}^2$ levels as ELISA (4). Since sensitive ATP tests take only 30 seconds, it is a fast and useful indicator of cleaning effectiveness to reduce cross-contamination in allergen avoidance programs (5).

Purpose:

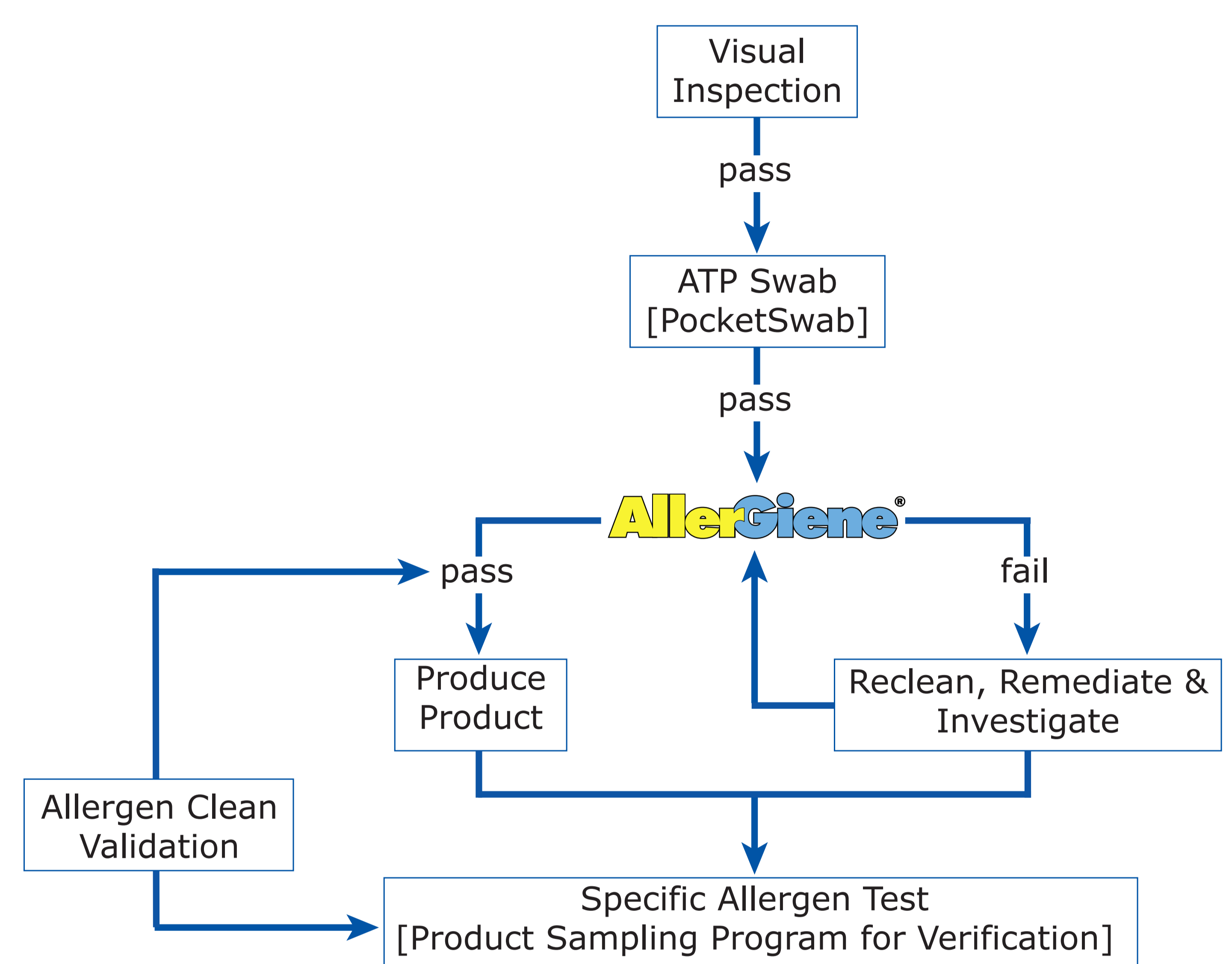
This work demonstrates application of ATP tests in allergen-cross-contamination avoidance following the integrated hygiene protocol shown in Figure 1.

Method:

Sensitive ATP, AllerGiene[®] (Charm Sciences Inc., Lawrence, MA, USA) is performed after cleaning verification by conventional ATP, PocketSwab[®]. The method uses a luminometer channel for sensitive ATP. At 20 seconds, the relative light units, RLU, are compared to a limit and if exceeded surfaces are re-cleaned. Surfaces that pass are verified clean using protein specific ELISA. The limit is established based on food ATP levels in proportion to allergenic ingredients.

Figure 1.

Integrated Hygiene Monitoring for Allergen Control



Results:

Filling equipment, used for milk and juice, was tested using ATP and a milk-protein ELISA. Testing was before and after implementation of a mechanical cleaning SSOP (Standard Sanitation Operating Practice) clean in place (CIP) operation. Surface swabs with ATP levels less than 70000 RLU compared with ELISA surface swabs testing $<5\mu\text{g}/100\text{cm}^2$, see **Table 1**. 70000 RLU limit successfully predicted negative results by ELISA surface swabs and improved the cleaning process. An eggnog producer detected egg-yolk solutions at 100 ppm with ATP, and eggnog solutions at 20 ppm. A limit of 30,000 was able to predict egg nog ELISA negative results $<5\text{ ppm}$, see **Table 2**. Similarly a processor using soy flour solutions found neither ATP (500 ppm) nor ELISA ($>1000\text{ppm}$) reached target detection at 20 ppm. However 100 ppm of food dried on a surface containing 6% soy-ingredient was detected by ATP. A limit of 0 RLU verified cleaning to $\sim 6\text{ ug}/100\text{ cm}^2$ dried-soy-ingredient using the ATP in the other food ingredients to amplify the risk of soy presence.

Table 1: Cleaning SSOP Validation for Bottle Filler During a Milk to Juice Transfer

Sample	AllerGiene*	ELISA (total Milk Protein)	Comments
Filler Valve 1 (older SSOP)	445219 (positive)	$> 5\mu\text{g}/100\text{cm}^2$ Positive	Surface swabs of 2 of the 4 filler heads had ELISA detectable residual milk protein
Filler Valve 4 (older SSOP)	1321653 (positive)	$> 5\mu\text{g}/100\text{cm}^2$ Positive	
Filler Elbow (older SSOP)	9650307 (positive)	$< 5\mu\text{g}/100\text{cm}^2$ Not Found	High ATP on elbow indicates increased risk of food residue. Milk protein was not detected.
Juice (older SSOP)	AllerGiene Not Applicable to finished product	$<5\text{ ppm}$ (not found)	Normal Cleaning did not yield detectable allergen in final product, but protein could still be detected on the surfaces of the equipment after cleaning.
Valve 1 (new SSOP)	0 (pass)	$< 5\mu\text{g}/100\text{cm}^2$ Not Found	*Mechanical cleaning step added into SSOP reduced ATP levels. A 70000 limit was established based on average of 6 cleaned sites + 2SD. Extra cleaned surfaces yielded ATP pass ($<\text{limit}$) and no detected milk protein by ELISA.
Valve 4 (new SSOP)	47688 (pass)	$< 5\mu\text{g}/100\text{cm}^2$ Not Found	
Filler Elbow (new SSOP)	0 (pass)	$< 5\mu\text{g}/100\text{cm}^2$ Not Found	

Table 2: Egg Products ATP and ELISA Detection Levels

Food Product /Ingredient (ppm)	AllerGiene ATP RLU (Average N=10)	% positive AllerGiene (N=10)	ELISA% Positive ($>10\text{ppm}$)(N=3)
50 ppm Egg Yolk	20515	60	100
100 ppm Egg Yolk	62218	100	100
10 ppm Egg Nog	18244	30	0
30 ppm Egg Nog	44463	90	0
100 ppm Egg Nog	78694*	100	Detectable but $<5\text{ppm}$

*The SD at this concentration was 23734. A 30000 limit $\sim 2\text{SD}$ less than the average can be established to predict cleaned surfaces below 5 ppm by ELISA

Significance:

Sensitive ATP, AllerGiene, is a rapid cleaning verification tool that is useful in avoiding cross-contaminating equipment with potentially allergenic ingredients during food processing changes. The low level ATP in foods can be used to effectively develop allergen cross-contact prevention strategies that are inexpensive, easy and predictive of allergen specific tests.

References:

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