The Value of Quantification in HACCP Cleaning and Sanitation Verification

Introduction

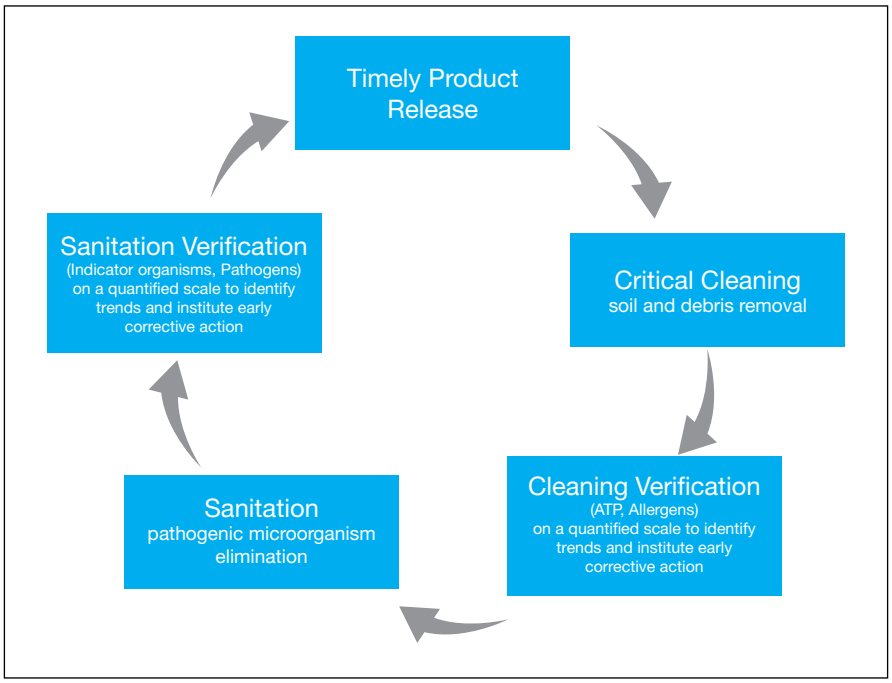
A Hazard Analysis and Critical Control Points (HACCP) plan is a quality management program designed to ensure food safety by detecting and analyzing biological and other hazards. In principle, HACCP is a systematic, purpose-driven approach to ensure the early identification, evaluation and control of food safety hazards to protect public health. In practice, HACCP allows food industry professionals to detect problems with cleaning and sanitation processes early enough to mitigate the development of more significant issues. Therefore, effective HACCP is critical to expeditious release, maximum revenues and product recall avoidance.

Selection of the proper tools for verification of HACCP cleaning and sanitation processes is an essential and necessary component of the program. Beyond test turn-around, accuracy, sensitivity, and the ability to quantify results play unique roles in program efficacy. As such, this white paper aims to elucidate the parameters and considerations around building an optimal verification plan and testing partnership.

Environmental Monitoring: What does clean mean?

In the food safety environment, cleaning and sanitation are two components of a single concept. While interconnected, each has a well-defined role. Without adequate cleaning, sanitation methods become ineffective. And without reliable methods to verify and monitor the efficacy of established hygiene standard operating procedures (SOPs), management insight is limited. Therefore, as vital as test accuracy is, without reliable test quantification, what may have been a simple corrective measure can become a large-scale disaster.

Figure 1. The Cleaning, Sanitation, and Verification Process



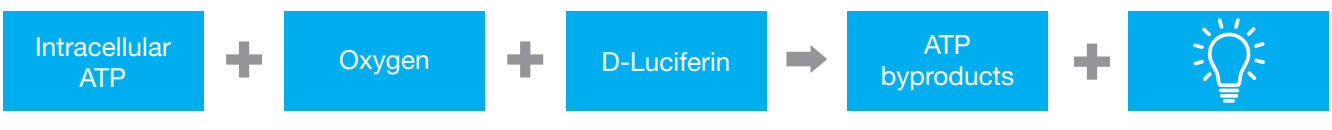
Critical Cleaning refers to the physical removal of soils by means of washing, rinsing and drying. In the food industry, critical cleaning is defined as a science-based protocol developed to reduce contamination risks to an acceptable level. Although SOPs necessarily vary by facility, an effective cleaning process considers Temperature, Action (the physical force required for soil removal), Chemistry, and Time (TACT). Once the optimal procedure has been developed, and technicians have been trained, a method of verification must also be incorporated to ensure the efficacy of the protocol and identify any concerning trends over time.

Cleaning Verification: The Total ATP Test Method

Accuracy, ease of use, time-to-results, and quantified, objective measures have made the adenosine triphosphate (ATP) bioluminescence method a widely adopted industry standard for cleaning verification.

Intracellular ATP represents the metabolic energy present within a biomass population. It can be identified and measured with great sensitivity and specificity utilizing the enzymatic luciferase reaction. A naturally occurring enzyme responsible for the firefly's glow, the coenzyme complex luciferase-luciferin converts ATP energy into photons of light. The detectable light energy is then measured with a luminometer and converted into relative light units (RLU) representative of the concentration of intracellular ATP in a sample.

Figure 2. ATP Technology



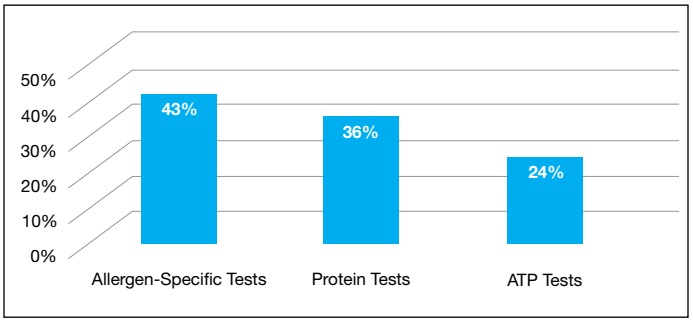
Although ATP testing is not intended to identify or quantify specific microbial species, it does provide quantitative insight into the effectiveness of cleaning procedures, the consistency of cleaning techniques, and the relative amount of residual microbial debris. Moreover, the speed at which results are available allows for immediate investigation and correction of failures. Another primary benefit of an ATP system is the potential for simple and integrated data management, analysis and reporting. Reliable results quantification over time allows for a clear understanding of environmental parameters and more control over production and hygiene processes. Where significant test result variance is noted, the cleaning process is no longer under control.

Allergen Detection

Another aspect of assessing cleaning protocol efficacy entails allergen protein testing. While an optimal solution for avoiding allergens can be found in dedicated, allergen-free facilities, this is not a feasible option for many operators. Therefore, environmental monitoring efforts should also include a robust allergen detection program.

It is estimated that approximately 80% of food processors incorporate allergen testing at some level.

Figure 3. Food Allergen Testing by Methods (US and Canada)*



***Data sourced from *Food Safety Magazine, Testing and Sanitation for Allergen Control* (Bob Ferguson, Feb 2018)**

A 2018 Food Safety Magazine survey of 275 U.S. and Canadian processors identified ingredient segregation and separation as the most effective component of their allergen program followed closely by validated cleaning processes, testing and monitoring.[1] Notably, one of the most problematic elements was cited as undeclared allergens, emphasizing the need for a consistent and reliable allergen testing program. Another challenging aspect of effective allergen testing lies in selecting the appropriate level of test sensitivity. Because there are very few established allergen threshold guidelines worldwide, it is important to ensure selected tests have undergone a rigorous analysis to determine an optimal assay cut-off. Mindful selection of the appropriate tests and a deeply knowledgeable testing partner like Hygiena™ can streamline the process. To learn more about allergen limits and test sensitivity values, review our white paper “Pushing the Limits of Food Allergen Detection: Allergen Threshold Guidance for Food Manufacturers and Processors.”

Sanitation follows the cleaning process and refers to the procedures, practices, and processes necessary to ensure sanitary conditions and thereby minimize or prevent hazards from environmental pathogens. Although sanitation standard operating procedures (SSOPs) are facility-specific, each will detail the chemicals, tools and processes required to achieve these goals. And as with cleaning procedures, methods for verifying the efficacy of the SSOP must be identified.

Sanitation Verification: Indicator Organism and Pathogen Testing

Indicator Organisms

The term "indicator organism" is used to describe representative non-pathogenic organisms that, if undetected in a sample, provide assurance that related pathogens are not present. A negative result is therefore considered representative of hygienic conditions and reflective of the microbiological quality of the product tested.

Quantitative analysis of indicator organism data over time can help identify trends that may allow facilities to take preventative action before a failure point is reached. It can foster a better understanding of seasonality effects and identify opportunities for operational and product improvements.

Pathogenic Organisms

US food safety experts, including the National Academy of Sciences and the National Advisory Committee on Microbiological Criteria for Foods, are in consensus with other regional authorities that meaningful pathogen reduction requires a holistic, farm-to-table approach.

The most common foodborne pathogens responsible for consumer illness include *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus, Salmonella enterica, Bacillus cereus, Vibrio spp., Campylobacter jejuni, Clostridium perfringens*, and Shiga toxin-producing *Escherichia coli* (STEC). These organisms may be present in a variety of products ranging from ready-to-eat foods to meats and seafood, thereby requiring a robust pathogen testing program to assure public safety.

Hygiena Cleaning Verification Solutions

"The more we used (the Hygiena EnSURE™ Touch ATP monitoring system) the more we came to depend on it - and now it's a very integral part of our program and cleaning in general. We've tested the ATP meter to the point where we're comfortable with it telling us what's clean and what's not clean."
—Quality Manager, Avery Brewing Company[2]

EnSURE Touch
User-friendly, handheld luminometer with integrated SureTrend™ Cloud data analysis and reporting

UltraSnap™ | AquaSnap™ | PRO-Clean™
Quantitative ATP and protein residue testing for cleaning verification

SuperSnap™ | GlutenTox® Pro ELISA
On-site and in-laboratory allergen detection and quantification for cleaning verification

Pathogen Testing Guidance

Regulatory guidance regarding sampling frequency and number of tested samples is nebulous in many regions with general testing recommendations described as risk-based and stage or contact zone-focused.

Figure 4. Indicator and Pathogen Organism Testing Frequency by Zone

Although the International Standard ISO 18593 does describe surface sampling methods, it does not detail specific pathogen detection or testing recommendations. Regional regulations also provide varying degrees of guidance. In many cases, the lack of definitive test frequency, test number and preferred method guidance necessarily puts the onus on safety and laboratory management to devise a purpose-built plan specific to each facility and product line.

As a basis for consideration, key pathogen testing concepts from representative regional regulatory bodies are in Table 1 on the next page.

Hygiena Sanitation Verification Solutions

"As we use (quantification) more and expand our companies' facilities, we are looking to continue to utilize the BAX (System) and its innovative methods to track organisms from the farm, or before, to the packaging point of our product."

—QA Laboratory Supervisor, Amick Farms

BAX® System
Validated PCR automation with modular capabilities for sample preparation through results reporting. BAX offers pathogen prevalence and quantification with no additional reagents or training.

Real-Time PCR Assays for Primary Pathogens
Salmonella, Campylobacter, Listeria and *L. monocytogenes, E coli (O157:H7), STEC, Shigella, Vibrio, S. aureus, Cronobacter, Yeast* and *Mold*

Table 1. Representative Regional Guidance

Region	Guidance	Source
Australia-New Zealand	Australia provides guidance regarding detection limits by food group for *Enterobacteriaceae, Campylobacter, Listeria, Salmonella, C. pefringens, S. aureus, V. parahaemolyticus,* and *E. coli.*	Australia-New Zealand Food Standards Code[3] Microbiological Quality Guide for RTE Foods[4] Dairy Pathogen Manual[5]
China	China provides guidance on sampling plans and detection limits for *Listeria, Salmonella, S. aureus, V. parahaemolyticus,* and *E. coli* in specific foods.	National Food Safety Standard for Pathogen Limits on Foods (Food Safety Law, China)[6]
European Union	EU provides guidance on sampling plans and detection limits for *Listeria, Salmonella, Cronobacter,* and *E. coli* in specific foods.	European Commission[7]
India	India specifies microbiological testing requirements and limits of detection for multiple pathogens including *Listeria, Salmonella, Shigella, V. cholerae, C. pefringens, S. aureus, V. parahaemolyticus, C. botulinum,* and *E. coli.*	Microbial Food Safety, Indian Regulations[8]
United Kingdom	UK guidelines describing the detection and enumeration of pathogens in ready-to-eat foods.	Health Protection Agency, U.K.[9]
United States	US guidelines indicate general pathogen testing practices for RTE food, meat, poultry and pasteurized eggs including: • Generic *E. coli* for carcasses • *Salmonella* for carcasses and raw ground meats • Generic *Listeria* for ready-to-eat products • *L. monocytogenes* and *Salmonella* for ready-to-eat and poultry products	U.S. Food and Drug Administration, Microbiological Testing by Industry for RTE Foods[10] Microbiological Testing Program for Meat and Poultry and Pasteurized Egg Products[11]

Sanitation Verification Quantified

Conventional pathogen detection approaches include traditional agar plate microbiology techniques and the Most Probable Number (MPN) method. An intense labor commitment and considerable wait time are attributes of both. Real-Time, or quantitative PCR testing, with the ability to assess and quantify prevalence, has become the preferred method for many facilities and a verified method at government laboratories, including USDA's FSIS Field Service Laboratories and Health Canada.

"Since it is not just the presence or absence of Salmonella, but the number of bacteria that can impact the likelihood of illness, FSIS will examine how quantification can be incorporated into this approach."[12]

Beyond establishing pathogen prevalence, quantification provides a more granular view of the degree of potential adulteration. Testing at each processing step allows for targeted interventions and informed process improvements. The combination of prevalence testing and quantification provides even deeper insights into causation and an informed pathway toward effective remediation.

For example, baseline quantification allows facilities to establish a data-driven detection limit to ensure consumer safety. When sanitation verification testing results in high prevalence rates with low pathogen levels, this indicates that the sanitation procedures may be inadequate, or process controls are failing. Continued quantification over time also provides an objective assessment of corrective action efficacy.

In Conclusion

Although the specifics of cleaning, sanitation and verification may vary from facility to facility and region to region, the value of test result quantification remains constant. Whether identifying the extent and source of a contamination event or monitoring the HACCP program's effectiveness over time, quantified data provides the focused insight required for appropriate and timely action. Moreover, selection of the right tools with a knowledgeable and responsive testing partner like Hygiena plays a vital role in keeping facilities running at peak performance: on target and on time.

With in-depth cleaning and sanitation verification expertise, Hygiena provides the tools and services to support food safety program excellence. Whether on the production floor or in the laboratory, Hygiena is your one-stop HACCP partner.

Cleaning Verification: Why Hygiena?

Hygiena's **One Health** Solution: Integrated and Scalable ATP and Allergen Testing

Figure 5. EnSURE Touch, SureTrend Cloud and EnSURE Touch Compatible Cleaning Verification Tests

EnSURE Touch is an advanced, hand-held luminometer-based monitoring system that collects, analyzes and consolidates data for a wide range of tests, across multiple locations, for rapid and reliable sanitation verification.

SureTrend™ Cloud is a value-added, secure cloud-based data analysis software program provided at no additional cost to EnSURE Touch users. It connects and consolidates ATP and allergen testing across locations to provide fully integrated cleaning verification data for timely intervention and full operational capacity.

EnSURE Touch compatible cleaning verification tests

Allergen Detection

As a vital component of cleaning verification, Hygiena offers a broad range of allergen detection and quantification assays.

Hygiena Operations-Based Allergen Tests:
Hygiena provides the tools and technology for quantitative on-site results in minutes. Fully compatible with the SureTrend Cloud data management system, consolidated cleaning verification reports and trend analysis are readily available from anywhere in the world.

Hygiena Laboratory-Based ELISA Allergen Tests:
Hygiena's enzyme-linked immunoassays for the detection and quantification of allergens in raw materials and final products provide laboratories with high sensitivity assays for 20 discrete allergenic proteins – all of which are fully compatible with standard ELISA automation.

In addition to the industry-leading GlutenTox ELISA Rapid G12, targeting the most toxic gluten fragment responsible for celiac disease sensitivity, Hygiena offers an expansive allergen product line addressing the Big 9 allergens and more.

Table 3. Hygiena Laboratory-Based Allergen Tests

Hygiena Allergen ELISA Tests:

- Almond
- BLG
- Casein
- Cashew
- Coconut
- Crustacean
- Egg
- Fish
- Gluten
- Hazelnut
- Lupine
- Lysozyme
- Macadamia
- Milk
- Mustard
- Ovalbumin
- Peanut
- Pistachio
- Sesame
- Soy
- Walnut

Coming Soon: Celery

Sanitation Verification: Why Hygiena?

Hygiena's **One Health** Solution: Integrated and Scalable Indicator Organism and Pathogen Testing

Hygiena Operations-Based Indicator Organism and Pathogen ATP Tests: Fully compatible with the EnSURE Touch and SureTrend Cloud data analysis software, Hygiena's MicroSnap™ line utilizes a novel bioluminogenic reaction for the detection and quantification of indicator organisms and *E. coli*.

Figure 6. EnSURE™ Touch Compatible Indicator Organism and Pathogen Tests

Table 4. EnSURE Touch-Compatible Indicator Organism and Pathogen Tests

MicroSnap Test	Detection Target(s)	Application
MicroSnap Total	Total Viable Count	Food, Drink, Surface
MicroSnap Coliform*	Gram negative non-spore forming rods	Food, Drink, Surface
MicroSnap *Enterobacteriaceae*	*Enterobacteriaceae* species	Food, Drink, Surface
MicroSnap *E.coli*	*Eshererichia coli*	Food, Drink, Surface

* AOAC-RI *Performance Tested Method*SM

Hygiena's Laboratory-Based PCR Tests: As a pioneer in reliable molecular pathogen detection for food safety laboratories worldwide, Hygiena's BAX System retains its leadership position with advanced, modular PCR automation from sample preparation to results reporting. With more than a dozen applications (e.g., SalQuant™, CampyQuant™), true quantification with shortened enrichment expands on the verification value of prevalence testing with no additional reagents, components or training required. Read more about the value of quantification in the case study, Amick Farms Tackles *Salmonella* and *Campylobacter* Challenges with the Hygiena® BAX System.

Figure 7. Hygiena BAX Press Express and BAX Q7 System

Powered by its line of AOAC performance-tested and NordVal-validated real-time polymerase chain reaction (RT-PCR) assays, Hygiena delivers reliable accuracy with even the most challenging matrices.

Table 5. BAX System-Compatible PCR Pathogen Tests

BAX System Real-Time PCR Assays	Coming Soon
Salmonella	*Aspergillus*
Campylobacter	*Brucella*
Listeria	*Yersinia enterocolitica*
L. monocytogenes	*Streptococcus pneumoniae*
E.coli (O157:H7)	*Bacillus cereus*
STEC	
Shigella	
Vibrio	
S. aureus	
Cronobacter	
Yeast / Mold	

At Hygiena, we are committed to delivering the right tools and tests to effectively support your HACCP cleaning and sanitation verification program regardless of size or complexity. With industry-leading applications expertise and live 24/7 support, we are dedicated to exceptional food safety partnership.

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[1] Source: https://www.food-safety.com/articles/5656-testing-and-sanitation-for-allergen-control

[2] Source: The Story of Avery Brewing's Quality Assurance ATP Testing Case Study

[3] https://www.foodstandards.gov.au/publications/Documents/Safe%20Food%20Australia/FSANZ%20Safe%20Food%20Australia_WEB.pdf

[4] https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/scienceandtechnical/microbiological_quality_guide_for_RTE_food.pdf

[5] https://www.dairysafe.vic.gov.au/publications-media/regulations-and-resources/guidelines/417-pathogen-manual/file

[6] https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=China%20Released%20the%20Food%20Safety%20Standard%20of%20Pathogen%20Limit%20for%20Foods_Beijing_China%20-%20Peoples%20Republic%20of_3-13-2018.pdf

[7] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02005R2073-20140601&from=DA

[8] http://www.ilsi-india.org/conference-in-microbiological-food-safety-management-details/Session-V/Mrs.%20Mabhullika%20Prakash%20&%20Mr.%20Chinmay%20Dwivedi.pdf

[9] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/363146/Guidelines_for_assessing_the_microbiological_safety_of_ready-to-eat_foods_on_the_market.pdf

[10] https://www.fsis.usda.gov/sites/default/files/media_file/2021-07/NACMCF_2018-2020_RTETesting.pdf

[11] https://www.fsis.usda.gov/science-data/data-sets-visualizations/microbiology/microbiological-testing-program-rte-meat-and-3

[12] Source: https://www.meatpoultry.com/articles/25667-usda-shares-plan-to-reduce-emsalmonella-em-illnesses