hygiena®

Cleaning and Sanitation

The Power of Quantification

Hazard Analysis Critical Control Points

Cleaning Verification & Validation

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.

Sanitation Standard Operating Procedures (SSOPs) detail the cleaning and sanitation steps, cleaning method validation and cleaning process verification necessary for a quality HACCP program.

Cleaning Method Validation

- Validates efficacy of cleaning verification method
- Conducted at SSOP adoption and at least annually thereafter
- Performed by reference laboratory (internal or third-party)
- Analyzed by high sensitivity, quantitative reference methods including:
  - Enzyme Linked ImmunoSorbent Assay (ELISA)
  - Real-Time Polymerase Chain Reaction (RT-PCR)

Cleaning Process Verification

- Verifies efficacy of cleaning procedures to remove microbes, protein and allergens
- Conducted at run or shift completion
- Performed on location by food sanitation staff
- Analyzed by high sensitivity, rapid test methods including:
  - Adenosine Trisphosphate (ATP) Testing
  - Protein Residue Testing
  - Lateral Flow (LF) Testing

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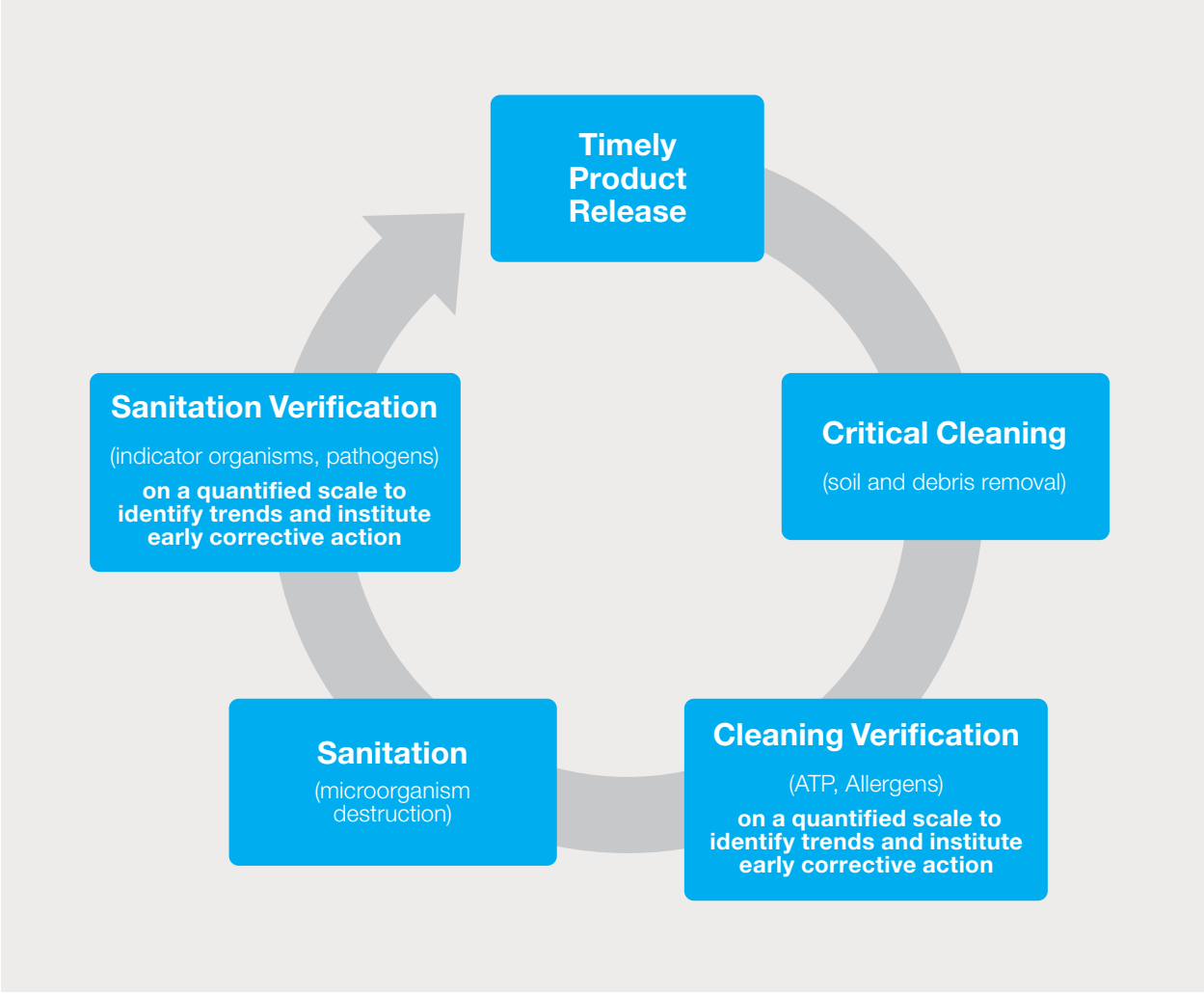
1 | The Role of Critical Cleaning in HACCP

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.

Effective HACCP is critical to expeditious release, maximized revenue and product recall avoidance.



Critical Cleaning describes 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 standard operating procedures vary by facility, internal processes, and product type, a quality critical cleaning process ensures adequate **T**emperature, **A**ction, **C**hemistry and **T**ime (TACT) to effectively remove debris and residual proteins.

TACT WINS

T **emperature:** Each cleaning solution has an optimal operating temperature for the best results. Monitoring and assuring the proper temperature range is essential.

A **ction:** Each cleaning procedure requires a specific amount of force to achieve the desired outcome. This action may be mechanical in nature, and/or include manual brushing, foaming and high-pressure washing.

C **hemistry|Concentration:** Each chemical cleaner should be used at the manufacturer's defined concentration. Achieving the proper solution strength may require dilution.

T **ime:** The amount of time a cleaning solution is in contact with the surface being cleaned is critical to the process. Too little time can result in inadequate cleaning. Too much time can result in reduced cleaning efficacy and/or undesirable residue build-up.

W **ater:** It is important to be aware of the ideal water type for cleaning purposes. For example, water hardness can negatively impact cleaning solution efficacy.

I **ndividual:** Adequate staff training for every individual is essential to ensure the facility has an optimal cleaning process in place.

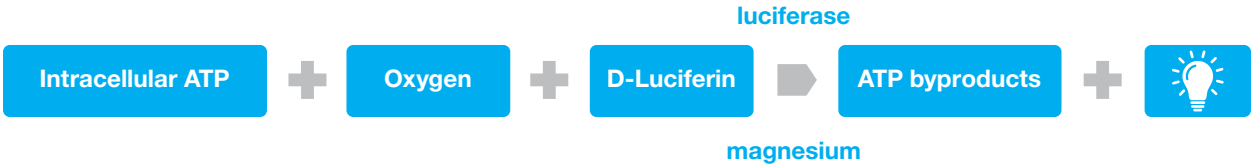
N **ature of Soil:** Each facility has its specific soil challenges (e.g., fats, proteins, minerals, sugars and complex carbohydrates). The nature of these soils will help determine the proper cleaning solutions.

S **urface:** The surface composition and critical control point locations will help determine the preferred cleaning solutions and testing tools.

2 | Cleaning Verification Methods

ATP Testing

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



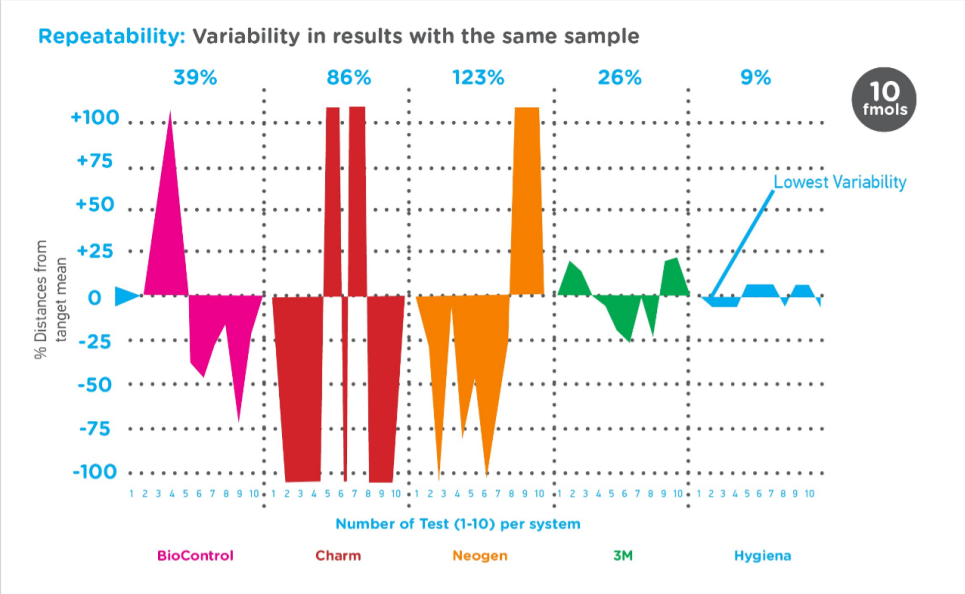
ATP testing provides quantitative insight into the effectiveness of cleaning procedures, the consistency of cleaning techniques and the relative amount of residual microbial debris remaining on the surface.

When considering an ATP method for cleaning verification, it is important to evaluate performance of the entire system including:

1. Precision (reproducibility/repeatability) of the luminometer
2. Sensitivity and linearity of the bioluminescence chemistry
3. The system's effective utility for its intended purpose

A quality test manufacturer partner will be enthusiastic about sharing these 3 critical performance data sets.

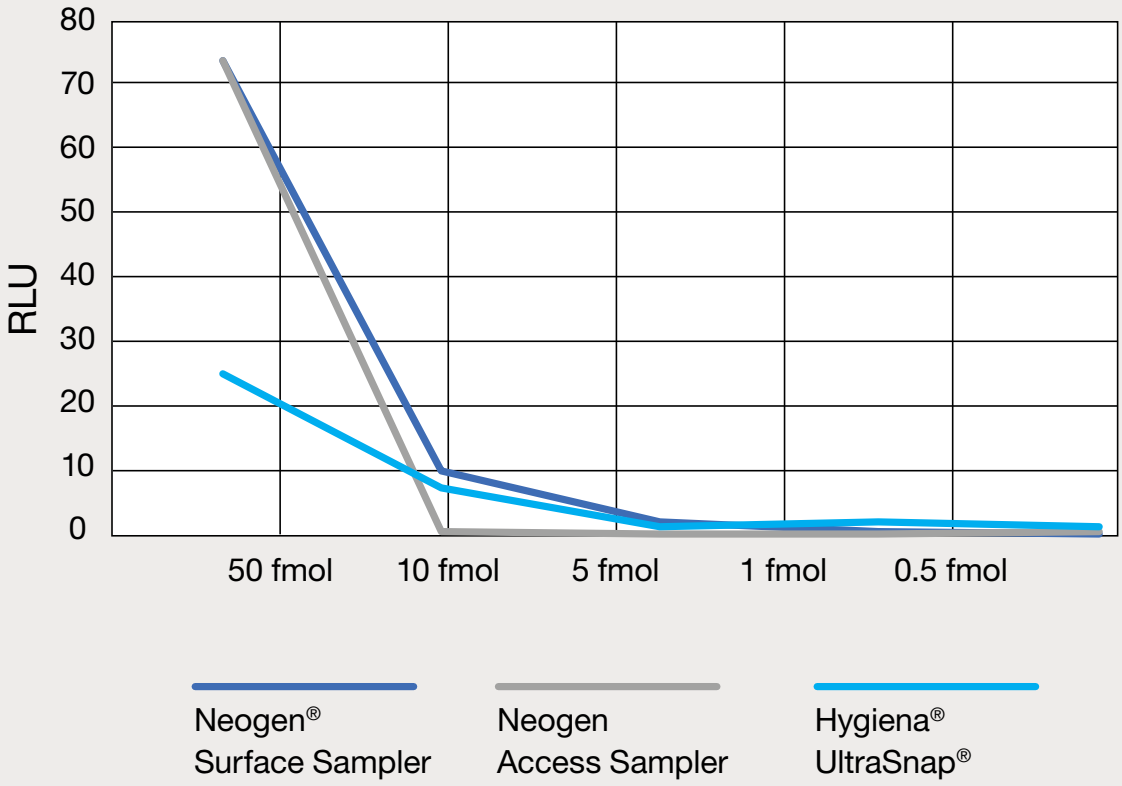
Comparative ATP System Performance[1]



[1] Food. Analysis & Control. Issue 3. 2009.

Bioluminescence Chemistry

Comparative Assay Linearity



ATP Test Device	5,000 fmol	500 fmol	50 fmol	10 fmol	5 fmol	1 fmol	0.05 fmol
Neogen's Surface Sampler	6,958.0	880.0	74.0	10.0	2.0	0.4	0.0
Neogen's Access Sampler	8,571.0	762.0	74.0	0.2	0.0	0.0	0.4
Hygiena's UltraSnap	3,170.0	259.0	25.0	7.1	1.2	2.0	1.0

Hygiena ATP Test Portfolio

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 101803

UltraSnap® Surface ATP Test
Designed as an all-purpose ATP detection device, UltraSnap is an operationally simple pen-sized device, ideal for virtually any food processing surface.

SuperSnap® High Sensitivity Surface ATP Test
Based on the foundational technology of UltraSnap, SuperSnap is 400% more sensitive, making it ideal for stringent SOP protocols.

AquaSnap® and AquaSnap Free
Designed for ATP detection in aqueous solution, AquaSnap detects total ATP (living cells, particulate matter, non-viable and non-microbial cells) while AquaSnap FREE isolates detection of non-microbial/non-viable cells (free ATP).

Superior Sample Collection

Patented Snap-Valve Design

Liquid-stable Chemistry

Easy as Swab, Snap, Squeeze

100% Recyclable

Protein Residue Testing

Protein is more difficult to remove from surfaces than glucose or other residues, so it is a good indicator of surface contamination. Coomassie Brilliant Blue dye is a useful and reliable reagent that does not require additional instruments to detect residual proteins. When bound to protein, the dye absorbs light at a new wavelength resulting in a characteristic, semi-quantitative color change.

When selecting a protein residue test, understanding the sensitivity of the assay is important. A low sensitivity can result in more false negatives, whereas a higher sensitivity is ideal for detecting low levels of protein. However, sensitivity depends on background noise, which PRO-Clean® and AllerSnap® tests minimize.

Notably, interference from cleaning solutions and sanitizers (e.g., quaternary ammonium compounds, hypochlorite) can result in false-positive results with some protein-residue testing methods. Although this is not a significant concern for PRO-Clean and AllerSnap tests, alternative technologies are commonly challenged by this limitation.

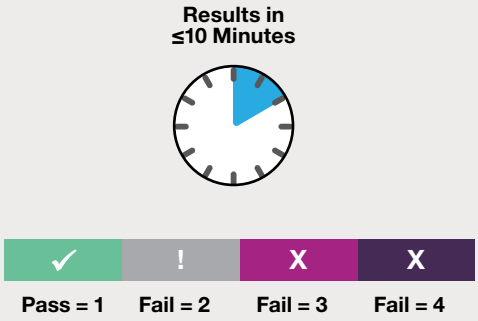
PRO-Clean Rapid Protein Residue Tests

PRO-Clean tests are the easiest and most accurate way to verify the cleanliness of food equipment surfaces. Cleaning verification is one of the most common applications of this test.

Sensitivity of PRO-Clean Tests

Time (minutes)	Limit of detection (µg of protein)
1	80
5	50
10*	20

* For PRO-Clean tests, instructions for use indicate a 10-minute, room temperature incubation. Additional data is presented for reference to demonstrate the LOD range at various times.



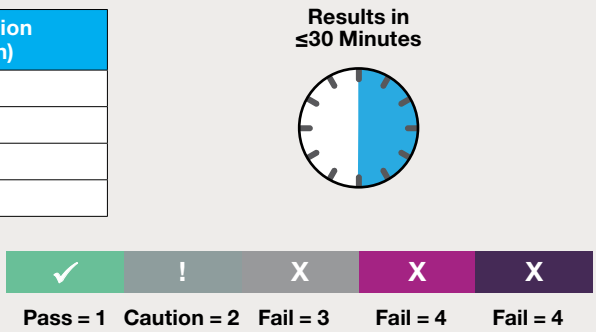
AllerSnap Rapid Protein Residue Tests

When needing higher sensitivity, AllerSnap is both cost-effective and time-saving: one swab test checks for all proteins, including allergenic residues, quickly confirming the hygiene of a surface and eliminating the need to run multiple, expensive allergen-specific tests. Protein can be detected in as little as 5 minutes, but incubations up to 30 minutes allow detection of as low as 3 µg of protein.

Sensitivity of AllerSnap Tests

Time (minutes)	Limit of detection (µg of protein)
5	10
10	5
15	5
30*	3

* For AllerSnap tests, color changes can be read as soon as they appear, but results should be read in 30 minutes or less.



Allergen Testing

Food allergy is no small problem. It affects approximately 2.5% of the general population worldwide, with reported prevalence rates ranging from 1% to 10%. As a result, the adoption of a robust, facility -and product-relevant allergen testing protocol plays an integral role in cleaning verification.

When selecting an allergen testing partner, it is important to consider the sensitivity (limits of detection) for each allergen protein. Because formal LOD guidelines do not exist outside of gluten, understanding LOD parameters specific to the selected assay will provide a clearer understanding of how and why the sensitivity thresholds were established.

In the case of Hygiena's line of allergen tests, LOD is established by using US FDA recommended Analytical, Safety and Risk Assessment methods.

1. Analytical Method: describes the sensitivity limits of the assay.
2. Safety Assessment Method: incorporates human challenge study data.
3. Risk Assessment Method: incorporates known or potential adverse events.

In support of this method, Hygiena refers to the scientific expert panel (VSEP) guidance behind Australia's Voluntary Incidental Trace Allergen Labeling (VITAL).[1] VITAL's 2019 allergen threshold guidance was developed from published and unpublished data of low-dose oral food challenges in the United States, Australia and the European Union.

This comprehensive approach defines the test sensitivity cut-off (limit of detection) to ensure the health and safety of ≥95% of the population.

AlerTox® and GlutenTox® Lateral Flow Tests

While AllerSnap tests provide general protein detection, sometimes there is a need for quick allergen-specific tests that have high sensitivity and no cross-reactivity, such as AlerTox and GlutenTox lateral flow tests. These tests are ideal for monitoring food, drinks and surfaces and can be a vital part of a comprehensive allergen prevention program.

[1] https://vital.allergenbureau.net/vital-science/

Hygiena Allergen Limits of Detection

Big 9 Allergens	AlerTox/GlutenTox Lateral Flow (ppm)	VITAL Reference Cumulative MED_{05} (mg/kg)
Gluten	≥1.0	20.0
Egg	1.25	2.4
Milk	2.5	3.1
Peanut	1.0	3.9
Tree Nut	from 2.3*	Varied*
Soy	10.0	10.0
Fin Fish	5.0	14.1
Shell Fish/Crustacean	10.0	429.0
Sesame	3.0	4.2

* LOD is variable dependent on nut type.

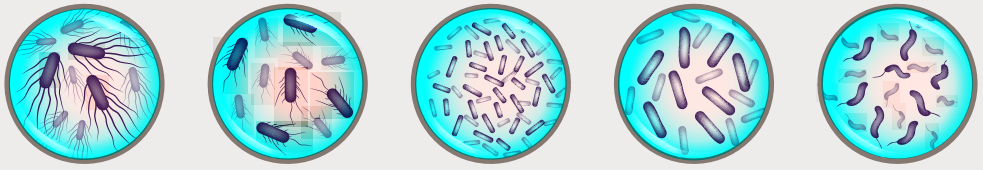
In each category of cleaning verification testing discussed (ATP, protein residue, allergen), quantification plays an important role; not only for establishing the proper limit of detection but to also provide a means to accurately identify and monitor trends over time. This latter aspect is discussed in more detail in Section 5. The Power of Quantification.

3 | The Role of Sanitation in HACCP

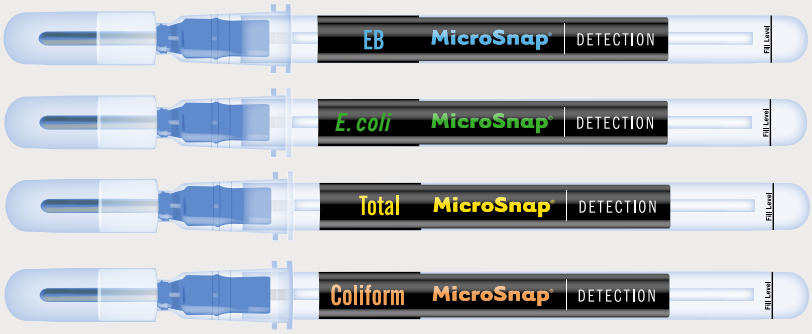
Sanitation follows the cleaning process. This step includes the procedures, practices and processes necessary to ensure sanitary conditions and thereby minimize or prevent hazards from environmental pathogens. As with Critical Cleaning, each facility must develop a product-relevant sanitation standard operating procedure (SSOP) to detail the appropriate steps. And in each case, testing methods to verify and ensure the efficacy of the SSOP are necessary.

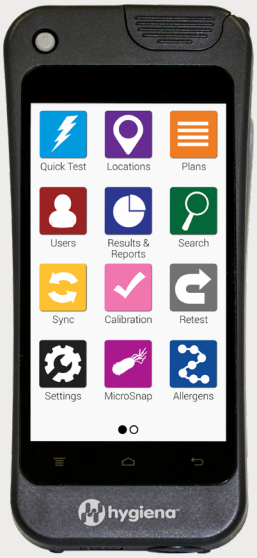
Indicator Organisms

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



Hygiena ATP Sanitation Verification Tests, Operations-Based Indicator Organisms





MicroSnap® Test	Detection Target/s	Application
MicroSnap *Enterobacteriaceae*	*Enterobacteriaceae* species	Food, Drink, Surface
MicroSnap *E. coli**	*Escherichia coli*	Food, Drink, Surface
MicroSnap Total*	Total viable count	Food, Drink, Surface
MicroSnap Coliform*	Gram negative non-spore forming rods	Food, Drink, Surface

* AOAC-RI *Performance Tested Methods*℠

Pathogens

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, including environmental monitoring through final product testing, to assure public safety.

Testing for indicator organisms and specific pathogens plays a critical role in the HACCP SSOP with testing protocols specifically designed to address the needs of the individual facility and product line.

4 | Sanitation Verification Methods

Although the International Standard ISO 18593, *Microbiology of the Food Chain-Horizontal Methods for Surface Sampling*, describes surface sample collection methods, it does not detail specific pathogen detection or testing frequency recommendations. Moreover, other than a zero-tolerance for *E. coli* in meat, existing global and regional regulations provide varying degrees of guidance.

This lack of definitive test frequency, test number and preferred method guidance thereby shifts responsibility to safety and laboratory management to devise a purpose-built plan optimized for the facility and product line.

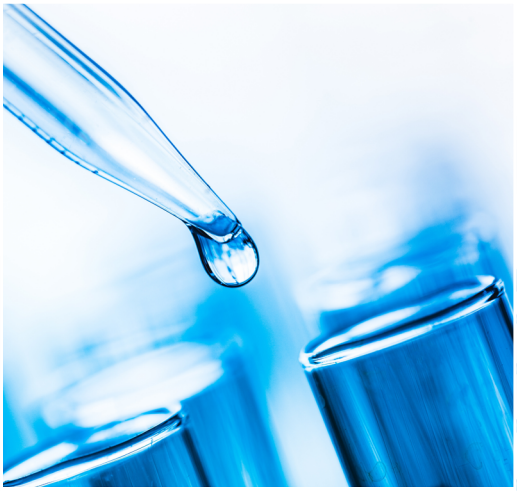
Although the traditional reference method for sanitation verification and validation involves agar plate microbiology and the Most Probable Number (MPN) method, this technique is highly subjective and labor- and time-intensive. Quantitative real-time polymerase chain reaction (RT PCR) has therefore become the preferred method at many facilities and reference and government laboratories including USDA's FSIS Field Service Laboratories; smaller facilities may rely on operations-based ATP for indicator organism and pathogen detection when properly validated by a reference laboratory method.

When evaluating a sanitation verification test partner, there are 3 primary factors to consider:

1. Relevant Quantitative Pathogen Menu
2. Multi-Matrix Validation
3. Industry Quality Certification

1. Relevant Quantitative Pathogen Menu

Ensure the test manufacturer has the appropriate test menu to fulfill the current SSOP requirements as well as any additional testing needs resulting from projected production or regulatory requirements.



Hygiena PCR Sanitation Verification and Validation

Powered by the modular BAX® System, Hygiena offers true quantification with shortened enrichment for more than a dozen pathogens; no additional reagents, components or training required.

BAX System Real-Time PCR Assays
Salmonella
Campylobacter
Listeria
L. monocytogenes
E. coli (O157:H7)
STEC
Shigella
Vibrio
S. aureus
Cronobacter
Yeast | Mold

Coming soon to the BAX System:
Aspergillus, Brucella, Yersinia enterocolitica, Streptococcus pneumoniae, Bacillus cereus

2. Multi-Matrix Validation

Food sample matrices present significant challenges to standard laboratory assays. Therefore, it is essential that test manufacturer partners have validated matrices for relevant products, both for sanitation verification as well as final product testing.

Food safety focused partners like Hygiena will also offer custom matrix validation programs to ensure reliable performance with the most unique matrices.

3. Industry Certification

Multiple quality programs provide independent third-party review and certification for proprietary test methods and validate test kit performance compared with the reference methods. Each of these validations (AOAC, AFNOR, etc.) ensures confidence in the quality and commitment of any test method by acting as a cleaning verification partner.

Representative Matrices Validated for Hygiena Products

AOAC: chicken, beef, turkey, spinach, lettuce, flour, frankfurters, smoked salmon, cheese, shrimp, yogurt, peas, strawberries, apple and orange juice, chocolate, peanut butter, pizza dough, dry pet food, environmental surfaces and more

NordVal: raw beef, raw pork, chicken and ovine meat, raw milk, fruits and vegetables, RTE, chicken, human and pet food, environmental surfaces

AFNOR: raw beef, raw pork, chicken and ovine meat, raw milk, fruits and vegetables, RTE, environmental surfaces

USDA FSIS: meats and carcasses, pasteurized liquids, frozen and dried egg products, fermented products, dried mixes, environmental surfaces

Health Canada: dry ingredients, soy, infant formula, dairy, seafood, bakery products, chocolate, fruits and vegetables, raw poultry, environmental surfaces

5 | The Power of Quantification

The ability to quantify results plays not only a role in assessing the efficacy of cleaning protocols, it plays a critical role in an effective HACCP program overall.

- **Trend Identification Allows for Early Intervention**
  Monitoring quantitative cleaning verification test results over time allows for the early identification of potentially troublesome trends. Increases in average test results point to a cleaning protocol that is not completely in control. Trend analysis, therefore, provides for expeditious, preventative intervention and continued timely product release.
- **Quantitative Results Allow for Root Cause Analysis**
  The location and enumeration of test results provide useful indicators of cause, allowing for targeted, preventative action with minimal process impact.
- **Integrated, Quantified Test Results Support Robust Audit Preparation**
  Tests and systems that allow for consolidated and quantified results offer a clear advantage during an audit. Not only does it demonstrate a scrupulous commitment to HACCP protocols, but it also allows for quick and easy access to the numbers.

Hygiena SureTrend® Cloud for Integrative Cleaning Verification Data

SureTrend® Cloud integrates ATP and allergen testing from multiple locations through a cloud-based data management system ideal for holistic HACCP visibility and insightful trend analysis.

Conclusion

Selection of an optimal test manufacturer with deep industry expertise, a true partnership philosophy and the quantified methods to support HACCP program efficiency is a vital decision requiring careful consideration.

A few of the most important factors include:

- A comprehensive and innovative menu across test methods (ATP, Protein Detection, Lateral Flow, ELISA, PCR)
- Detailed test performance data to demonstrate accuracy, sensitivity and reliable quantification
- Third-party quality and test performance certifications
- Deep food industry knowledge and focus
- Multiple food matrix test validation and the ability to custom validate unique matrices
- Modular test automation options to meet current demand and expand with growth
- Integrated results reporting and trend analysis to support HACCP goals
- Knowledgeable and responsive technical support

As a global leader in one health, holistic food safety solutions, Hygiena is dedicated to providing the exceptional test performance and support required for HACCP excellence and public health protection. To learn more, visit: www.hygiena.com