

Impact of ATP and microbiological indicator testing — in combination — on cleaning and sanitation effectiveness and food product quality

Results from a peer-reviewed study conducted by Cornell University and Neogen

Effective environmental monitoring programs (EMPs) rely on hygiene monitoring as an integral component. A robust hygiene monitoring program that utilizes both ATP testing and microbiological testing can help food manufacturers meet regulations, assess the effectiveness of their cleaning and sanitation operations, and support food safety and quality. To develop a framework in which both methods could be utilized by a variety of food manufacturing facilities, the Cornell University Department of Food Science and Neogen conducted a multi-phase study in a ready-to-eat (RTE) food manufacturing facility using the Neogen® Clean-Trace® Hygiene Monitoring and Management System and Neogen® Petrifilm® Plates. Results showed effective equipment surface cleaning and sanitation, and improvements in the facility's environmental hygiene and the microbiological quality of food produced. As the facility became cleaner, fewer failing test results occurred, enabling a decrease in the frequency of testing while keeping the cleaning and sanitation process under control. The Clean-Trace Hygiene Monitoring Software played a critical role by facilitating data collection and analysis as well as identifying and justifying opportunities for improvements in cleaning and sanitation. This study can be used as guidance to help other food manufacturers achieve similar results.

Introduction

Food manufacturing facilities, regardless of size or product produced, must comply with established regulations for food safety and quality. Regional and local regulations can require control of biological and chemical hazards to prevent product contamination caused in production environments. For example, in the United States, food manufacturing surfaces are required to be cleaned and sanitized as frequently as necessary to prevent contamination of products.^{1,2}

Meeting these requirements can be accomplished through a robust environmental monitoring program (EMP) that utilizes both adenosine triphosphate (ATP) bioluminescence testing and microbiological indicator testing. Hygiene monitoring can evaluate the effectiveness of cleaning and sanitation procedures. ATP bioluminescence testing is a widely accepted method of hygiene monitoring that can indicate in real time whether cleaning and sanitation have been effective. In addition, microbiological indicator testing provides results to verify sanitation status.

Using ATP bioluminescence testing and microbiological indicator testing, in combination, can provide an assessment of cleaning and sanitation operations. However, to utilize these tools most effectively, a systematic framework for the introduction and application of these tools is also needed.

The Cornell University Department of Food Science and Neogen conducted a multi-phase study³ in a ready-to-eat (RTE) food manufacturing facility to evaluate the effectiveness of using ATP bioluminescence testing and microbiological indicator testing in a framework and their combined impact on cleaning and sanitation efficacy, cleanliness of the processing environment and microbiological product quality.

Method

As the three-phase study began, 30 sites were identified for ATP and microbiological testing, based on relative difficulty of cleaning and sanitation, with a preference for sites considered harder to clean.

During Phase 1, ATP testing of the 30 sites per day for 3 weeks using the Clean-Trace Hygiene Monitoring and Management System verified cleaning and sanitation procedures and established baseline results. Microbiological testing of the environment was performed, in parallel, for yeast and mold, lactic acid bacteria and aerobic microorganisms. Environmental samples were collected from adjacent surfaces using Neogen® Quick Swabs and tested using Petrifilm Rapid Yeast and Mold Count Plates, Petrifilm Lactic Acid Bacteria Count Plates and Petrifilm Rapid Aerobic Count Plates.

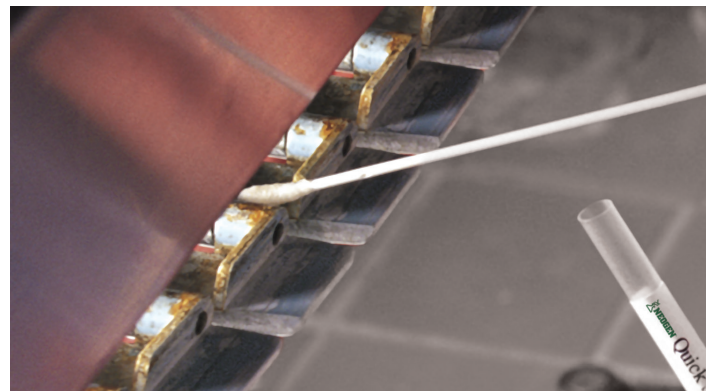
During Phase 2, results from Phase 1 were used to identify and target sites that needed enhanced cleaning. Enhanced cleaning included increased time spent cleaning and some disassembly of equipment to access hard-to-clean areas. ATP and microbiological testing were maintained for 30 sites per day for 6 weeks.

In Phase 3, the Clean-Trace Hygiene Monitoring Software was used to randomize sites and optimize sampling. ATP testing and microbiological testing were conducted on 18, rather than 30, sites per day for 16 weeks, while maintaining the modified cleaning practices.

In Phases 1 and 3, food product samples were collected during production and evaluated using Petrifilm Rapid Yeast and Mold Count Plates, Petrifilm Lactic Acid Bacteria Count Plates and Petrifilm Rapid Aerobic Count Plates to determine the impact of targeted cleaning on the microbial quality of products.



ATP test swab with Clean-Trace Hygiene Monitoring System



Sample collection using Quick Swab



Inoculating Petrifilm Plate using Quick Swab

Results and Discussion

Environmental Quality

During study phases 1, 2 and 3, 960 environmental samples were evaluated with both the Clean-Trace Hygiene Monitoring and Management System and Petrifilm Plates.

In the ATP testing results, the proportion of sites that failed to meet the minimum sanitary requirements day-to-day was highest during Phase 1 but steadily decreased during Phase 2 before leveling off in Phase 3 (Figure 1, next page). ATP swab failures decreased by 26.5% for Zone 1 (food contact surface) sites and by 51.0% for Zone 2 (non-food contact surface) sites from Phase 1 to Phase 3 (Figure 2, page 6).

Microbiological testing results for aerobic count and lactic acid bacteria from complementary surface sampling correlated with the ATP testing results, showing a significant reduction in failures from Phase 1 to Phase 3 ($p < 0.001$; Figure 2, page 6). Aerobic count failures decreased by 21.8% for Zone 1 sites and by 26.8% for Zone 2 sites. Lactic acid bacteria failures decreased by 9.7% for Zone 1 sites and by 14.1% for Zone 2 sites. Targeted cleaning did not significantly change the proportion of swabs that failed to meet the minimum sanitary requirement for yeast and mold in either Zone 1 or Zone 2 ($p < 0.05$).

Microbiological Product Quality

In this food manufacturing process, the product underwent two thermal processing steps: an initial heat-treatment of 88 °C to reduce microbial load in raw materials, followed by an in-package pasteurization. After implementation of targeted cleaning, quantitative data from packaged, pre-pasteurized food products indicated a significant reduction in the load of microorganisms from Phase 1 to Phase 3 (Figure 3, page 6).

In total, 175 samples were collected before in-package pasteurization (pre-pasteurization) and after in-package pasteurization (post-pasteurization) in Phase 1 ($n=87$) and Phase 3 ($n=88$). Among pre-pasteurized products, the populations of yeast and mold, lactic acid bacteria and aerobic counts were significantly different ($p < 0.05$) when each of the indicator groups was compared between Phase 1 and Phase 3. This demonstrated a decrease in microbial load and an improvement in microbiological quality.

Initial processing of the raw materials involves heating at 88°C, which significantly reduces microbial load. After heating, product is pressed, cut and packaged before pasteurization. Therefore, the microbial load found in pre-pasteurized product is largely associated with post-process contamination, including that from contact with equipment surfaces. These results demonstrated that targeted cleaning monitored by both ATP and microbiological indicator testing may improve the microbiological quality of products.



The combination of Neogen's Clean Trace System and Petrifilm Plates have helped us to significantly improve our overall plant sanitation. We are able to quickly identify areas that need more thorough cleaning and take corrective action.

Co-Proprietor

RTE Food Manufacturing Company

Figure 2: Reduction in environmental sample failure rates

	Zone 1	Zone 2
Clean-Trace Surface ATP Test		
ATP ²	26.5%	51%
Petrifilm Plates		
Rapid Aerobic Count ²	21.8%	26.8%
Lactic Acid Bacteria ²	9.7%	14.1%
Rapid Yeast and Mold ³	**	**

² There was a significant difference in the % of failure rate decrease between Phase 1 and Phase 3 (p<0.05)
³ No significant change was determined (p>0.05)

Figure 2. Reduction in environmental sample failure rates for ATP and microbiological indicator tests, before and after implementation of targeted cleaning. Failure rate is represented as a % decrease from Phase 1 (before implementation of targeted cleaning) to Phase 3 (after implementation of targeted cleaning). Test results are grouped by environmental sampling zone.

Figure 3: Improvement in microbiological quality of products

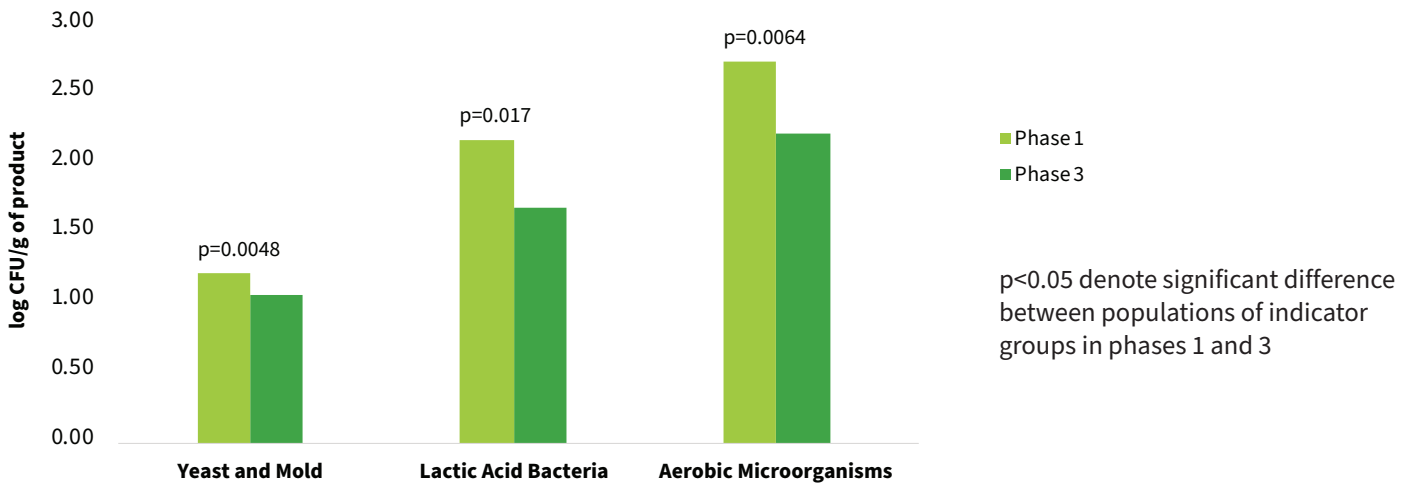


Figure 3. Microbial load of yeast and mold, lactic acid bacteria, and aerobic microorganisms in packaged pre-pasteurized products. Samples were collected in Phase 1 (before implementation of targeted cleaning) and in Phase 3 (after implementation of targeted cleaning).

Implications for Food and Beverage Manufacturers

During the study, the combined use of Clean-Trace Hygiene Monitoring and Management System and Petrifilm Plates enabled optimization of cleaning and sanitation. This resulted in improvements that were observed in the manufacturing facility's environmental hygiene and the microbiological quality of food produced.

As the study progressed and the facility became cleaner, fewer failures in testing occurred (Figures 1, 2, and 3). These results enabled the team to confidently decrease the number of test sites yet control the cleaning and sanitation process to ensure it delivered the desired results.

In addition, the study established a framework so that in the future, when the facility encounters cleanliness problems or observes unwanted trends in swab failures, they could take appropriate actions to reestablish control of their cleaning and sanitation.

The Clean-Trace Hygiene Monitoring Software also played a critical role in the process through data collection and analysis of performance. Data and data analysis by the software helped identify and justify opportunities for improvements in cleaning and sanitation.

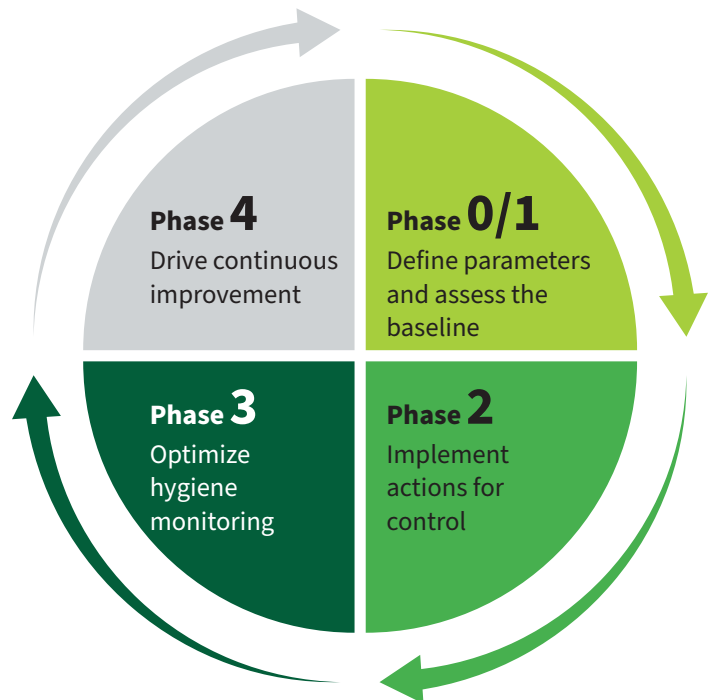
A Framework for Implementing Hygiene Monitoring

Developing and implementing a hygiene monitoring program, as described in the Neogen Cornell Environmental Monitoring Handbook for the Food and Beverage Industries,⁴ involves a number of considerations including frequency of testing, location of test sites and acceptable limits for tests. These considerations are product- and process-specific but require a systematic framework to implement.

Selecting sampling sites may require mapping the complete facility and production process, dividing the facility into zones based on microbiological risk to the product, and completing an assessment of the most appropriate test sites. Test sites should be selected after conducting an appropriate risk analysis to understand the risks associated with sites given the processing stage, proximity to food, potential for cross-contamination, ease of cleaning and condition of the surface being tested.

A conceptual overview of a framework to implement a hygiene monitoring program is presented in Figure 4 (page 7) and can be summarized as follows:

Figure 4: Framework to implement hygiene monitoring



Phase 0: Define Parameters

- Identify test sites and test targets using a risk-based approach analysis.
- Identify appropriate test methods (e.g., Clean-Trace Hygiene Monitoring and Management System or Petrifilm Plates).
- Define initial acceptance criteria (e.g., pass/fail limits) for test targets.
- Determine action plans and corrective actions when failures occur.

Phase 1: Assess the Baseline

- Start testing at a high frequency for a determined period (e.g., a minimum of 2–3 weeks) while maintaining normal cleaning and sanitation procedures.
- Track and trend the testing results. This may be done using a software system (e.g., Clean-Trace Hygiene Monitoring Software or Petrifilm Plate Manager Software). Accept or adjust acceptance criteria defined in Phase 1 to determine a baseline for each test target.
- Identify problem sites that will require targeted cleaning and sanitation.

Phase 2: Implement Actions for Control

- Maintain testing at a high frequency for a determined period (e.g., 4–6 weeks).
- Continue tracking and trending testing results.
- Target problem sites identified in Phase 1 and implement modified cleaning and sanitation procedures (e.g., targeted cleaning and sanitation).
- Determine if baseline(s) determined in Phase 1 require adjustment.

Phase 3: Optimize Hygiene Monitoring

- Randomize test sites. This will reduce testing frequency while ensuring all identified test points are evaluated over a defined period. A software system can randomize a sample plan while maintaining critical test points and ensure proper verification of cleaning and sanitation (e.g., Clean-Trace Hygiene Monitoring Software).
- Continue tracking and trending testing results.
- Continue modified cleaning and sanitation procedures (e.g., targeted cleaning and sanitation).
- When failures occur, immediately implement action plan and corrective actions
- Maintain attention to previously identified problem sites. A software system may increase focus on testing sites and identification of failures. If failures reoccur at these sites, repeat procedures from Phase 1 and Phase 2.

Phase 4: Drive Continuous Improvement

- Continue tracking and trending testing results to enable identification of potential problems before they impact the quality or safety of an operation.
- Review acceptance criteria on an ongoing basis. For example, if an ATP test point has never failed for many measurements over an extended period, the pass/fail limit may be reduced to **pursue continuous improvement**.
- Determine if other elements may be added to current hygiene monitoring program. If new elements are added, repeat Phase 1 and Phase 2 with optimized testing.



This study demonstrates the power of a robust hygiene monitoring program — combining ATP testing with microbiological indicator testing — to strengthen current sanitation programs and ultimately improve the safety and quality of food for consumers. In addition, hygiene monitoring results can be used to develop a data-driven cleaning and sanitation program that is effective and efficient, which can help reduce costs. As a standard, the industry should be using this method to verify cleaning and sanitation programs. It's key.

– **Randy Worobo, Ph.D.**,
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Department of Food Science

Considerations for Implementation in Your Facility

In this study, ATP bioluminescence testing and microbiological indicator testing were demonstrated to be complementary tools for assessing and improving the cleaning and sanitation status of a food manufacturing environment. The results showed an improvement in microbiological quality of the product following targeted cleaning.

This study also describes a framework that utilizes the Clean-Trace Hygiene Monitoring and Management System and Petrifilm Plates, which food manufacturers can use to implement hygiene monitoring and microbiological testing as part of a robust environmental monitoring program.

As you implement or expand your environmental monitoring program in your facility, here are several questions to consider:

- 1 How did you select your test sites?
- 2 Do you know how the pass and fail limits were established?
- 3 Can you quantitatively confirm that your cleaning and sanitation process is in control?



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4. Neogen. 2019. Neogen Cornell Environmental Monitoring Handbook for the Food and Beverage Industries.

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