Evaluation of multiple quantification methods for verification of cleaning and sanitation of produce harvest bins

INTRODUCTION

Food contact equipment presents an opportunity for cross-contamination from microbial pathogens if surfaces are not adequately cleaned and sanitized. Harborage points on equipment have historically been identified as a root cause during past produce outbreak investigations, emphasizing the critical importance of designing systems to clean, assess, and monitor the effectiveness of sanitation practices. While equipment cleaning is critical in all food segments, an added challenge in the fresh produce industry is that products are produced, harvested, and packaged/processed in often uncontrollable agricultural environments susceptible to soil transfer, wind, wildlife, and weather. Despite the added challenges, these agricultural food contact surfaces must aim to be controlled to minimize risks of cross-contamination and safety for consumers.

Combining unpredictable processing and options in open agricultural fields with the short shelf life of the products creates further challenges for growers to assess real-world potential risks and practices for microbial safety. Currently, there are few technologies that are field deployable to provide valuable feedback about contamination and/or effectiveness of microbial treatments and sanitation during production, harvest, and/or packing/processing. Given the potential risks for food safety, there is high demand and interest in the produce industry for these types of tools and technologies since they would offer real-time feedback and better risk-based management. Today, there are limited tools for growers to assess efficacy of cleaning and sanitation in the field, with microbial indicator tools such as Adenosine Triphosphate (ATP) being used most frequently. Monitoring ATP is convenient, economical, and proven to be a good indicator of surface cleanliness in some food safety monitoring applications. However, despite its effectiveness in some scenarios, ATP is the energy currency in all living cells (not limited to bacterial cells) and has a more complicated meaning when observed in agricultural environments since it is also abundant in soil, vegetable/fruit tissue, etc. With ATP being so ubiquitous in agricultural environments, it renders the molecule less effective as an indicator of microbial cleanliness and control in these settings. If knowledge on microbial state is desired, growers must rely on most costly traditional quantitative microbial testing, and these results are generally slow (1-2 days), further limiting the utility of the test since results cannot inform behavior-change during production/harvest.

To evaluate new options for field assessment of cleaning and sanitization of food contact surfaces, a study was performed on a new field deployable flow cytometer marketed to be able to quantify viable bacterial cells between 30 to 120 seconds. This trial was completed in partnership with a leafy green processer under real-world production settings, and flow cytometer samples were obtained in parallel with traditional microbial quantification and ATP measurements. The technology was evaluated against routinely used microbial monitoring tools (ATP, microbial plating) to understand potential correlations, and the study incorporated an assessment on the practical performance and utility of the instrument within agricultural produce settings.

METHODS

Harvest Bins and Conditions

This study focused on conducting an evaluation and comparative analysis of different quantification methods for cleaning and sanitation of harvesting bins. 20 harvest bins were sampled during the experiment. During the sampling day the harvesting bins were used for transportation of spinach from a local field to a processor in Monterey County, California. The harvest bins evaluated in this process were collapsible harvest bins from supplier, Denham Plastics. **Figure 1.**

Figure 1. Harvest Bins

Sampling Time Points

For each sampling event, the sample team collected samples at two different time points:

- Pre cleaning and Sanitizing (Pre CS): when the harvesting bins had arrived from the field, and the product was transferred out of the bins for packing
- Post cleaning and sanitizing (Post CS): immediately after cleaning and sanitizing.

Cleaning Procedure

Briefly, the collapsible bins were staged to be cleaned up to eight bins at a time. Excess product and debris were first rinsed using a pressure washer and potable water to remove leaf tissue, residues, and aimed to be visually clean of dirt and debris. The industry partner's regular sanitation personnel were used for performing the cleaning, preparing cleaning/sanitation materials, and ensured that standard operating procedures were followed per their routine policies.

Following high-pressure washing, sanitation personnel sprayed a solution of MG Chlorsan maintained at 100-200 ppm chlorine concentration at a pH of 6.5-7.8 to cover the bins. Bins were left to drip- and air-dry prior to post clean testing.

Sample Locations

For each individual harvest bin, the team collected swab samples from three individual locations (A, B, and C), **Figure 2**. Each sampling location and swabbing methodology was standardized and performed by one person across all samples.

A. Inside bottom corner

B. Inside middle

C. Outside bottom corner

Figure 2: Sample Locations

Sample Types

The team collected samples to assess the concentration of a variety of indicator measurements including:

- Adenosine Triphosphate (ATP) - Charm FieldSwab with NovaLUM ATP reader
- Flow cytometry (CytoQuant®)
- Microbiological measurements

- Aerobic Plate Count (APC) - AOAC 990.12
- Total Coliform Counts (TCC) - AOAC 991.14
- Enterobacteriaceae - AOAC 2003.01
- Generic E. coli - AOAC 991.14

For the ATP testing Charm FieldSwab ATP swabs were used for all ATP assessments in the field.

Figure 3. Charm FieldSwab and NovaLUM ATP reader

For the flow cytometry measurements, a CytoQuant® flow cytometer by Romer Labs was used. Using their CytoQuant® swab vial.

Figure 4. CytoQuant® flow cytometer by Romer Labs

The microbiological swabs were submitted to Eurofins Microbiological Laboratories in Salinas, CA. The methods used were APC: AOAC 990.12, TCC/ *E. coli*: AOAC 991.14, *Enterobacteriaceae*: AOAC 2003.01. Using Gold Standard Diagnostic swabs in Hi-Cap™ Neutralizing Broth solution.

RESULTS AND DISCUSSION

CytoQuant® Results and Experience

Electrical impedance flow cytometry was performed using a CytoQuant® flow cytometer. This piece of equipment is a compact and high-throughput instrument designed for quantification of viable cells in swab samples. Unlike culture-based methods, this technology enables real-time analysis based on recognizing the electrical properties of intact bacterial cells in an electrolyte solution as opposed to particles and/or dead bacterial cells (Romer Labs). In addition to viable cells, the flow cytometer also quantifies the total particles on a swab, capturing objects beyond cells (e.g. cell debris, dust, soil, etc.).

During the study, it was observed that a notable hurdle with the CytoQuant® was the ability of the product to provide viable cells in scenarios with high levels of particles. In this scenario, the device gave a notice that read "Too many particles to count the intact cells", this appeared to occur at times when the particle count exceeded the 5,000,000-particle threshold. Table 1 denotes the percentage of samples that were countable.

Table 1. The percentage of Flow cytometry results did not result in a reading error due to high levels (>5,000,000) of particle matter.

SAMPLING AREA	SAMPLING STAGE		
	PRE-CS	POST-CS	OVERALL
A (Inside - Bottom)	1/20 (5%)	3/20 (15%)	**4/40 (10%)**
B (Inside Middle)	13/20 (65%)	9/20 (45%)	**22/40 (55%)**
C (Bottom)	3/20 (15%)	6/20 (30%)	**9/40 (22.5%)**
Overall	**17/60 (28%)**	**18/60 (30%)**	**35/120 (29%)**

Across all sampling sites, CytoQuant® readings showed a consistent reduction following the cleaning process. On average, pre-clean samples across all sites had a mean of 6.53 $\log_{10}$ CFU/swab (SD = 0.30), which declined to 6.22 $\log_{10}$ CFU/swab (SD = 0.37) post-cleaning.

At Site A, the mean CytoQuant® value prior to cleaning was 6.91 $\log_{10}$ CFU/swab, with no standard deviation available (due to a single or highly consistent result), while the post-clean value dropped to 6.24 $\log_{10}$ CFU/swab (SD = 0.65). Site B showed a reduction from 6.47 $\log_{10}$ CFU/swab (SD = 0.31) before cleaning to 6.20 $\log_{10}$ CFU/swab (SD = 0.32) after cleaning. Similarly, Site C had a mean of 6.65 $\log_{10}$ CFU/swab (SD = 0.22) pre-clean, which decreased to 6.24 $\log_{10}$ CFU/swab (SD = 0.36) post-cleaning.

These results suggest that the cleaning process consistently reduces overall microbial load. The observed deductions reflect meaningful shifts in microbial load and support the utility of these methods, whenever not limited by high particulate matter on surfaces to me analyzed, as a verification tool.

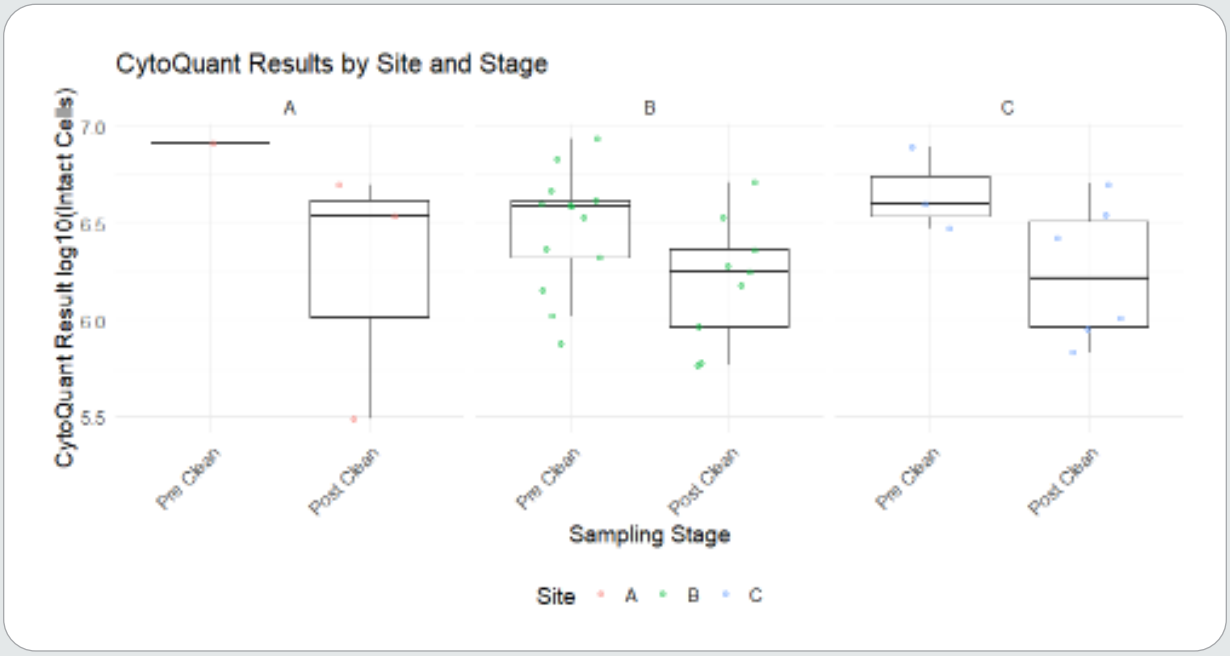

Figure 5. Results observed for the CytoQuant Results with countable results.

CytoQuant® Statistical significance: An ANOVA model with a post-hoc Tuckey HSD was performed on the CytoQuant® results. The models show the difference between pre-clean and post-clean was statistically significant ($p = 0.013$). There were no significant differences between areas (A, B, or C), and there were no significant differences between stage and areas.

The results suggest that the cleaning process consistently reduced microbial presence across all areas. No statistical differences were observed between pre-clean sites suggesting that all sites had relatively the same level of intact cells at all stages. It is worth mentioning that the sites have limited number of observations pre-clean due to a high number of samples being impacted with high particle readings.

ATP Results

Adenosine triphosphate ATP levels were assessed using the Charm NovaLum ATP luminometer to evaluate the effectiveness of cleaning and sanitizing across our different sites. ATP readers detect ATP through bioluminescence, and results are represented in Relative Light Units (RLU) (Charm Sciences Inc.). ATP is the go-to rapid, on-site method for verifying surface cleanliness.

However, ATP testing does have limitations, as it does not differentiate between microbial and residual plant/soil ATP (Bakke 2022), which may lead to elevated readings even when microbial risk are not present. Despite this, ATP remains a valuable verification tool when used correctly and interpreted against a specific system under specific conditions.

All ATP results have countable results. The mean ATP levels were higher in post-clean samples compared to pre-clean samples, averaging 910,000 ± 1.0 (standard deviation) million RLU and 639,000 ± 598,000 RLU, respectively. When stratified by sampling site, pre-clean mean ATP levels were Site A, 758,000 ± 711,000 RLU; Site B, 881,000 ± 841,000 RLU; and Site C, 279,000 ± 242,000 RLU. Post-clean mean ATP levels were Site A, 711,000 ± 587,000 RLU; Site B, 1.7 million ± 2.0 million RLU; and Site C, 282,000 ± 396,000 RLU. **Figure 6** shows a boxplot of the ATP results for the different stages and areas.

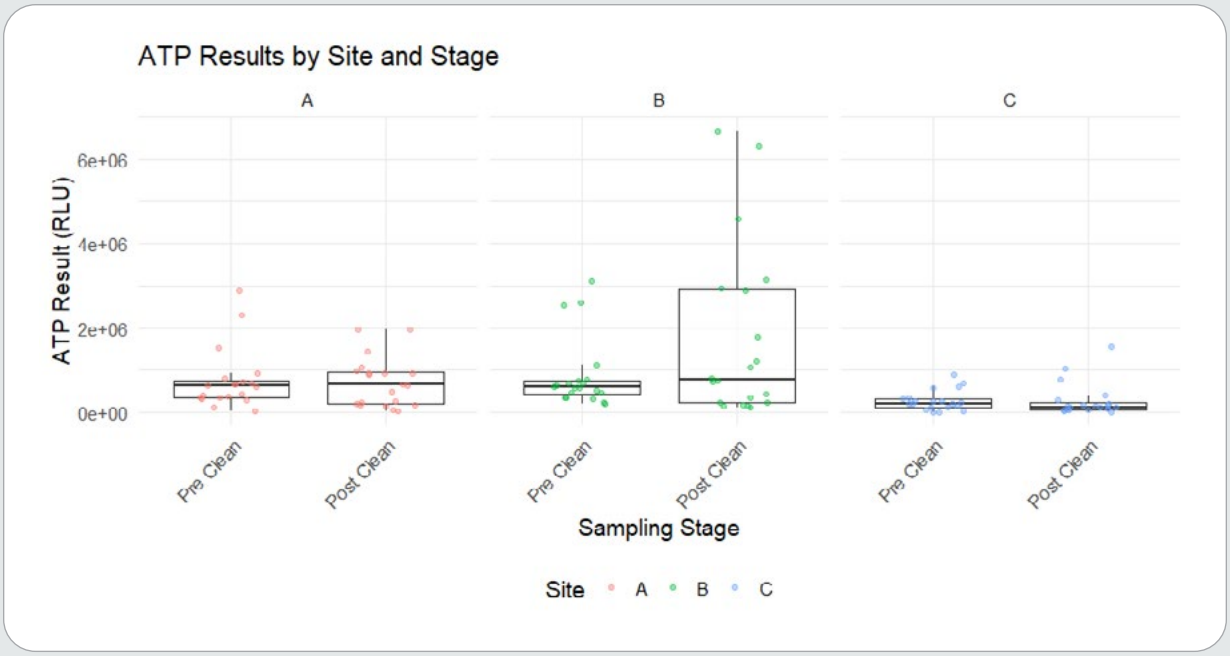

Figure 6: Comparison of ATP results for the different stages and areas.

ATP Statistical Analysis: An ANOVA model with a post-hoc Tuckey HSD was performed on the ATP results. Stage differences alone (Pre- vs Post-Clean) were not significant when collapsing across sites, suggesting site-specific effects matter more ($p = 0.14$). Site B consistently showed higher ATP levels, particularly after cleaning, compared to both Sites A and C ($p = 0.018$, and <0.001), respectively.

The results indicate the ATP differences between stages were not observed, which is not consistent with the CytoQuant® and microbiological results. While, location differences were observed, these may be a result of the very high ATP counts observed in site B post-clean. No statistical difference was observed between sites pre-clean, suggesting that from an ATP standpoint, all sites showed similar ATP loads.

Microbiological Results

For this study, microbiological verification methods included: Aerobic Plate Count (APC), Total Coliform Counts (TCC), Enterobacteriaceae, and Generic E. coli. These methods were chosen to provide a comprehensive overview of both the general microbial load and indicators of potential contamination. Each serves a specific role in verifying the effectiveness of sanitation practices and identifying potential food safety risks. APC is a broad indicator of the total viable aerobic bacterial population in a sample. While not specific to pathogenic organisms, APC results reflect the overall microbial load and can signal deficiencies in general sanitation or post-harvest handling practices. Total Coliform Counts serve as a general indicator of sanitary conditions and, in some scenarios, may indicate the possible presence of fecal material, though environmental presence is common outside of direct fecal contamination. Enumeration of *Enterobacteriaceae* is a broad family of gram-negative microorganisms that are routinely used as indicators of quality and sanitation in the food industry. This bacterial family includes both harmless and pathogenic organisms (*e.g., Salmonella, Shigella, E. coli*). Finally, *E. coli* is a species within the Coliform group and generally used in food and water testing as an indicator organism associated with fecal material, insanitary conditions, and is also routinely found within the agricultural environment.

Aerobic Plate Count APC: The limit of quantification of the APC test was <10 CFU/swab. The results indicate that all (n = 60) pre-cleaning swabs were above the limit of quantification while for the post-cleaning tests (n = 13, 21%) were below the limit of quantification.

For the results that were quantifiable, across all sites, the mean APC decreased from 3.93 $\log_{10}$ CFU/swab (SD = 0.52) in pre-clean samples to 2.29 $\log_{10}$ CFU/swab (SD = 0.71) post-cleaning.

Site A showed a reduction from 4.14 $\log_{10}$ CFU/swab (SD = 0.42) to 2.51 $\log_{10}$ CFU/swab (SD = 0.42). Site B had a pre-clean mean of 3.63 $\log_{10}$ CFU/swab (SD = 0.59), which declined to 2.44 $\log_{10}$ CFU/swab (SD = 0.75). Site C showed the most pronounced decline, from 4.01 $\log_{10}$ CFU/swab (SD = 0.43) to 1.37 $\log_{10}$ CFU/swab (SD = 0.41).

These results strongly indicate that the cleaning process consistently and substantially reduced aerobic bacterial contamination on the surfaces tested.

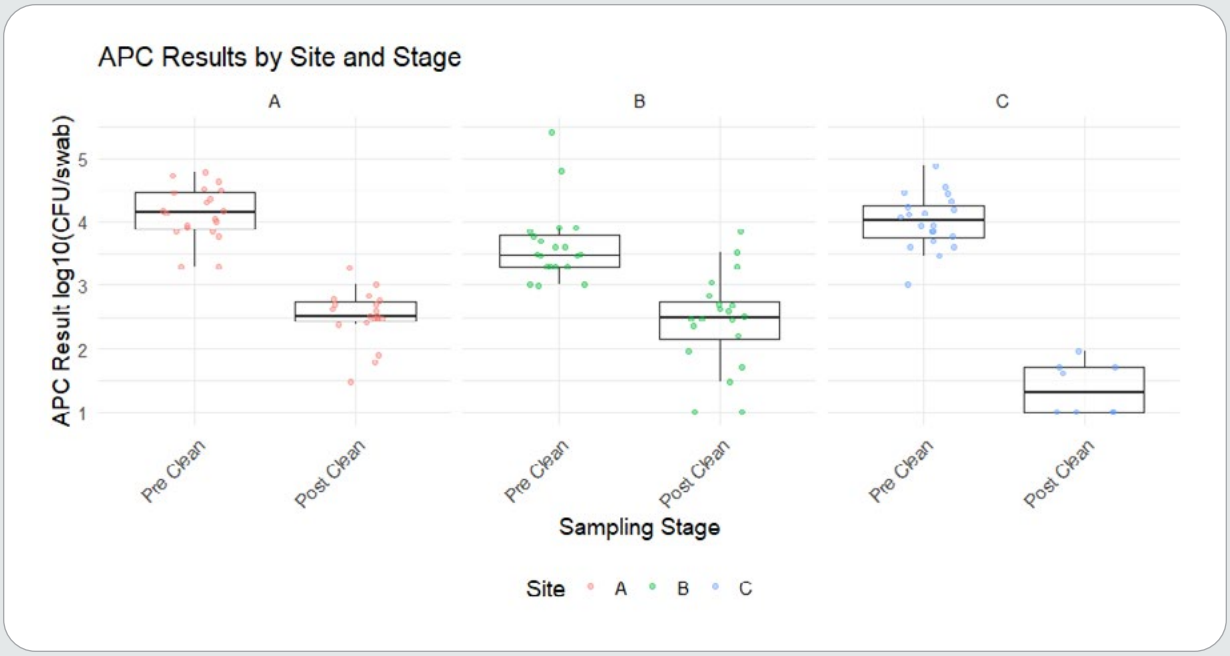

Figure 7: Comparison of APC results for the different stages and areas.

APC Statistical Significance: The ANOVA and Tukey HSD post-hoc comparisons for aerobic plate count (APC) showed several statistical differences. Post-Clean samples had significantly lower APC values compared to pre-clean samples (mean difference = –1.63 $\log_{10}$ CFU, $p < 0.001$), demonstrating a strong overall cleaning effect.

Interaction comparisons further emphasized these differences. Post-clean reductions were significant at all sites ($p < 0.001$, for all sites). Among pre-clean comparisons, Site B had significantly lower APC than Site A (–0.51 $\log_{10}$ CFU, $p = 0.036$). Notably, Site C's post-clean APC remained significantly lower than both Site A (–1.14 $\log_{10}$ CFU, $p < 0.001$) and Site B (–1.07 $\log_{10}$ CFU, $p < 0.001$) post-clean, suggesting the most effective microbial reduction occurred at Site C.

The results suggest strong cleaning effect among all sites, and significant differences between sites B and C and B and A pre-clean show that initial contamination is higher inside bottom and outside bottom the bin, compared to the middle of the bin.

***Enterobacteriaceae*:** A second microbial quantitative test performed was for *Enterobacteriaceae*. The goal was to test an indicator that was broader than total coliforms. The tests have a limit of 10 CFU/swab or 100 CFU/swab for Post clean and pre-clean tests respectively. For the pre-clean test 54 out of 60 tests (90%) were below the limit of quantification, while for the post-clean tests, 58 out of 60 (97%) were below the limit of quantification. Considering the lower limit of quantification and larger percentage on non-quantifiable tests for post-clean tests, the results show that there was a significant reduction by cleaning and sanitation process in the *Enterobacteriaceae* population. **Table 2**. Similarly for the few quantifiable tests, those bins pre-clean show to have higher levels than those post clean.

Table 2. Result summary for *Enterobacteriaceae* tests

Stage	Area	Mean CFU/ Swab	SD	N (quantifiable)	N < 100 CFU/swab	N < 10 CFU/swab
Pre Clean	A	200	0.0	2/20	18/20	–
Pre Clean	B	450	353.6	2/20	18/20	–
Pre Clean	C	1000	1272.8	2/20	18/20	–
Post Clean	A	40.0	–	1/20	–	19/20
Post Clean	B	10.0	–	1/20	–	19/20
Post Clean	C	–	–	0/20	–	20/20

Total Coliforms: The third microbial test was performed for total coliforms, total coliforms are indicators that are more specific than *Enterobacteriaceae*, but broader than generic *E. coli*. The limit of quantification for all total coliform tests was 10 CFU/swab. For the pre-clean tests, 52 out of 60 samples (87%) were below the limit of quantification. For the post-clean tests, 59 out of 60 samples (98%) were below the limit. This suggests that the majority of coliform detections, particularly after cleaning and sanitation, fell below quantifiable levels.

Considering the high proportion of non-quantifiable samples in the post-clean stage and the consistent trend of lower detected levels, these results support a significant reduction in coliform presence due to the cleaning and sanitation process. As shown in **Table 3**, the few quantifiable results observed in the pre-clean stage exhibited higher levels than the one detected in the post-clean stage, further reinforcing the effectiveness of sanitation.

Table 3. Result summary for total coliform counts

Stage	Area	Mean CFU/ Swab	SD	N (quantifiable)	N < 10 CFU/swab
Pre Clean	A	75.0	77.8	2/20	18/20
Pre Clean	B	510.0	523.3	2/20	18/20
Pre Clean	C	237.5	360.4	4/20	16/20
Post Clean	A	50.0	–	1/20	19/20
Post Clean	B	–	–	0/20	20/20
Post Clean	C	–	–	0/20	20/20

Generic *E. coli*: The limit of quantification of all generic *E. coli* samples was 10 CFU /swab, all results were below the limit of quantification.

Comparing Quantitative Methods

A second goal of this experiment was to evaluate how well the CytoQuant® flow cytometer performed compared to APC and ATP reading.

CytoQuant® flow cytometer and ATP readings: as mentioned in the result section not all CytoQuant® results were quantified because of excess particles impeding a usable result. Meanwhile, all ATP test results were quantifiable. For this analysis we removed those paired observations that were not quantifiable n = 85. To evaluate the correlation between CytoQuant® results (intact cells) and ATP results (RLU), we conducted a spearman rank correlation coefficient for the tests pre-clean and post-clean.

Pre-Clean: The correlation was 0.25, indicating a weak positive monotonic association between CytoQuant® and ATP values prior to cleaning. This suggests that as one measure increases, the other tends to increase slightly, but the relationship is not strong.

Post-Clean: The correlation dropped to 0.20, also reflecting a weak positive association after cleaning. The lower correlation suggests that cleaning may reduce biological signal levels, making the relationship between the two measures even weaker.

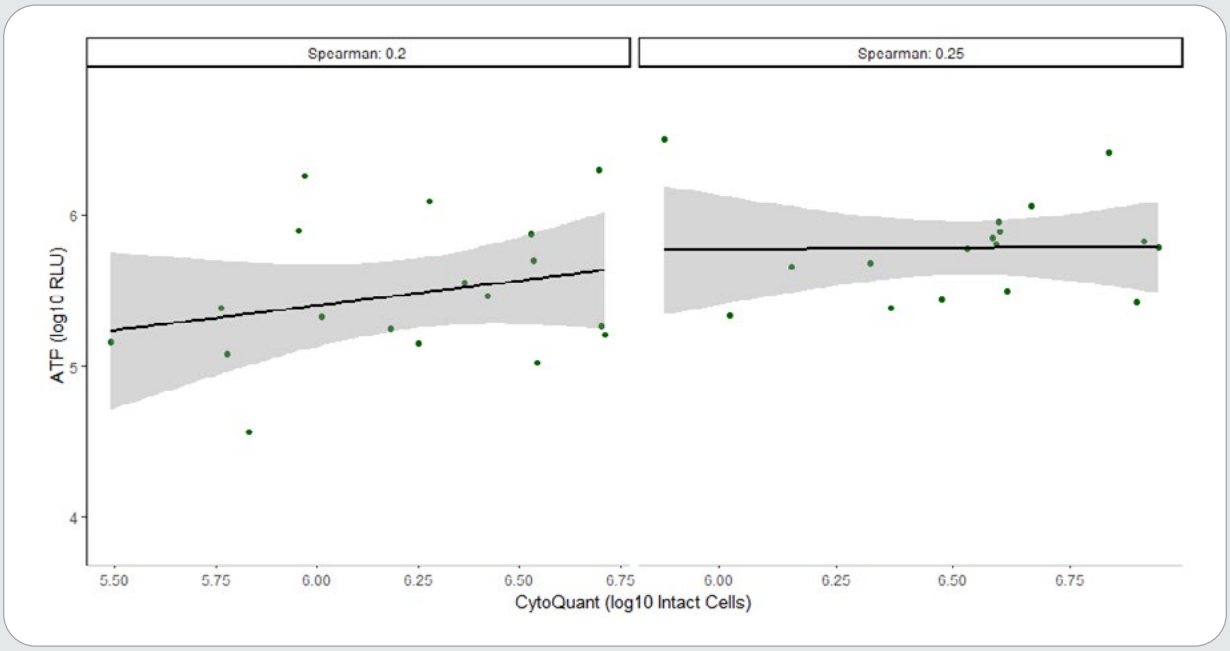

Figure 8: Correlation between ATP and CytoQuant

Regression: A linear regression model assessing the relationship between $\log_{10}$ ATP and CytoQuant® values showed that a 1-$\log_{10}$ increase in CytoQuant® was associated with an average 0.35-$\log_{10}$ increase in ATP (p-value = 0.079). On the original scale, this suggests that a 10-fold increase in CytoQuant® levels corresponds to approximately a 2.24-fold increase in ATP ($100.3485 \approx 2.24$), though this relationship was not statistically significant at the 0.05 level. The model explained 9.1% of the variance in ATP levels ($R^2 = 0.091$), indicating a weak linear relationship between the two measures across the dataset.

An interaction linear regression model was used to examine whether the relationship between $\log_{10}$-transformed CytoQuant® and ATP levels differed by cleaning stage. In the pre-clean condition, the slope was nearly flat ($\beta = 0.02$, $p = 0.95$), indicating no detectable association between the two measures. In the post-clean condition, the slope increased modestly ($\beta = 0.33$), suggesting that higher CytoQuant® levels may correspond to slightly higher ATP levels, though this effect was not statistically significant ($p = 0.48$). The interaction term and overall model fit ($R^2 = 0.09$, $p = 0.11$) indicate that the predictive power of CytoQuant® for ATP is limited and highly variable, especially across cleaning stages.

CytoQuant® flow cytometer and APC readings: As performed above non-quantifiable results were removed from this analysis n = 90. Spearman correlation coefficients were calculated separately for each cleaning stage. The results indicate that for pre-clean samples, the correlation was 0.24, indicating a weak positive monotonic relationship between CytoQuant® and APC prior to cleaning. This suggests that while increases in CytoQuant® tend to correspond to increases in APC, the association is not strong. Post-clean, the correlation increased to 0.51, suggesting a moderate positive association between CytoQuant® and APC after cleaning. This stronger relationship post-cleaning may reflect a more consistent or proportional reduction in both intact cell counts and viable bacterial loads at lower contamination levels.

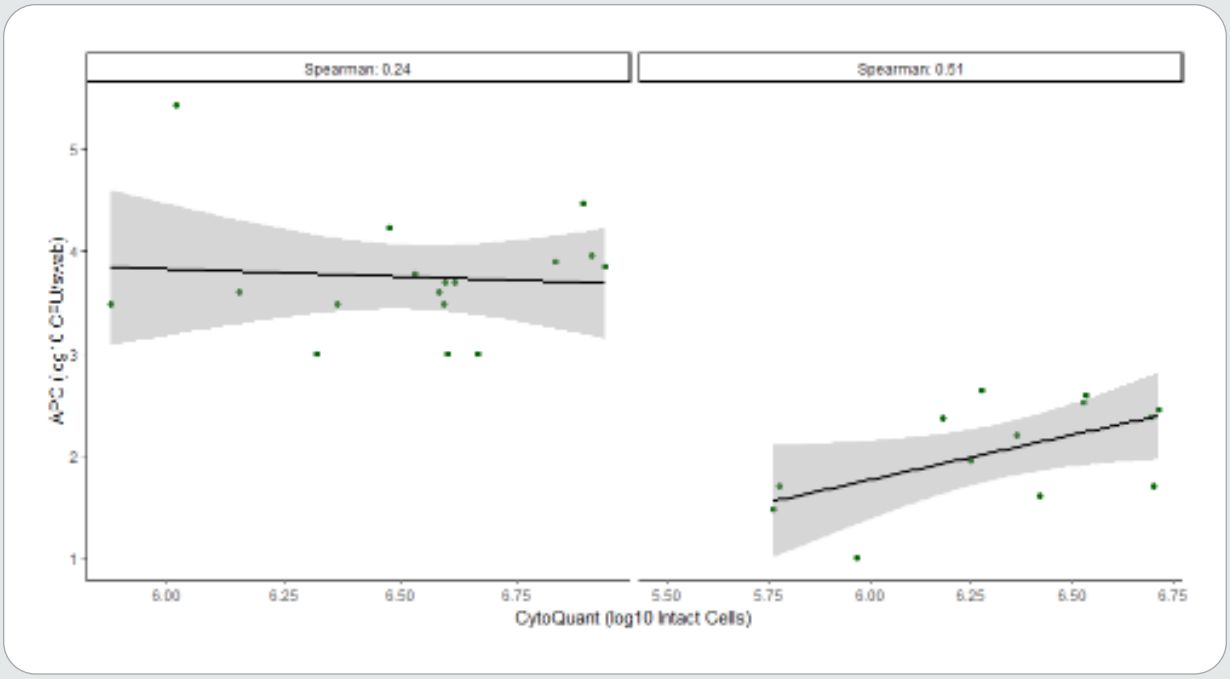

Figure 9: Correlation between APC and CytoQuant®

Regression: A log-log linear regression showed a significant positive association between CytoQuant® and APC levels ($p = 0.047$). Specifically, a 10-fold increase in CytoQuant® levels was associated with an approximate 13.8-fold increase in APC counts (since $10^{1.14} \approx 13.8$). The model explained 13.4% of the variation in APC values ($R^2 = 0.13$), suggesting that while CytoQuant® provides some predictive value for APC, substantial variability remains unaccounted for.

Magnitude Comparison: Across both cleaning stages, CytoQuant® results were consistently higher in magnitude than APC, reflecting the broader detection capability of CytoQuant® (intact cells) compared to APC (culturable cells only).

Pre-clean, the median CytoQuant® value was 6.59 $\log_{10}$ cells/swab, while APC was 3.90 $\log_{10}$ CFU/swab, indicating a typical ~700-fold difference in magnitude. Post-clean, the median CytoQuant® level remained high at 6.26 $\log_{10}$ cells/swab, while APC dropped to 2.48 $\log_{10}$ CFU/swab — a ~7500-fold difference, showing that CytoQuant® continues to detect microbial signals even when viable counts are low. See **Figure 10** for comparison. Overall, both methods showed a reduction between pre- and post-cleaning, however, the CytoQuant® reduction was lower than the one observed from APC.

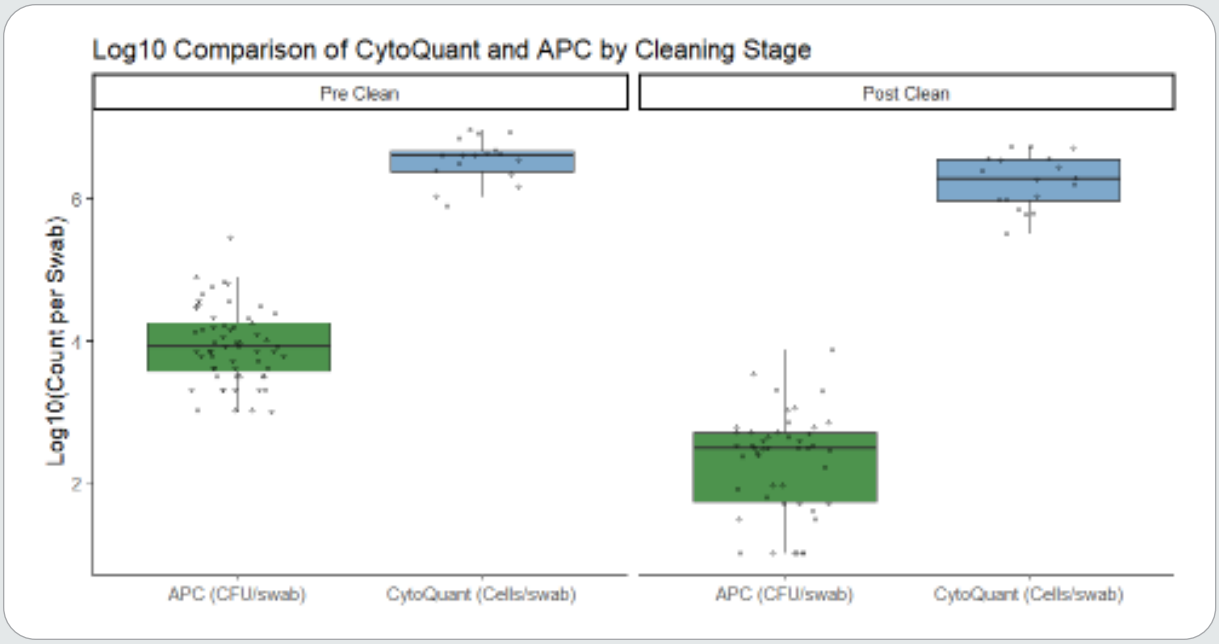

Figure 10: Magnitude comparison between CytoQuant and APC

Applicability of the methods tested and experience

The CytoQuant® during this study has shown to be a potential tool for verification of cleaning and sanitation of harvest bins. Overall, the results showed that pre-clean results were higher than post-clean results, which aligns with the results observed with the APC, EB, and TC microbiological tests. This observation indicates that the CytoQuant® may be a superior tool than ATP to be able to understand and observe in real-time whether cleaning and sanitation had the desired effect. However, the magnitude of results from the CytoQuant® raises questions related to its accuracy at high levels of bacteria and/or in conditions with high levels of soil and debris. It is unknown from this study whether this observation is related to the high background from debris and soil, and future studies in conditions of low background soil and debris are needed to better understand the quantitative results from the instrument. Despite this noted quantification observation, the CytoQuant® was found to be a useful real-time tool to inform about overall trends resulting from cleaning and sanitation practices. The other rapid tool evaluated which is often used in industry, ATP monitoring, was unable to capture any effect from the sanitation process.

Discussion

One of the greatest challenges for the fresh produce industry is being able to understand in the field whether cleaning and sanitation efforts on food contact equipment have been effective. Tools, harvest equipment, and product containers represent recognized cross-contamination risk from microbial pathogens, but thus far, the abilities to clean and sanitize are difficult to conduct

and evaluate in agricultural environments demanding fast turnaround. Technologies that offer real-time insight into the microbiological quality of surfaces pre- and post-clean introduce a valuable opportunity for growers to be better able to assess effectiveness of cleaning, sanitation, and microbiological state of food contact equipment. Additionally, with real-time feedback, growers can transition from visual checks of cleanliness to a more accurate assessment of microbiological risk. More critically, these real-time tools offer risk identification and a real-time opportunity to implement mitigations and corrective actions.

In the current evaluation, the CytoQuant® was successful in providing a tool that observed the overall microbiological trend measured in 30 seconds compared with traditional microbial quantification that took 2 days for results. While the microbiological relationship between the CytoQuant® and the microbiology plating was consistent (i.e., each capturing a reduction in microbial counts post cleaning), the observed microbial counts significantly differed. ATP, on the other hand, returned high levels irrespective of when sampling occurred, and was unable to discern any impact on microbial quantity from the cleaning and sanitation process. For this, the CytoQuant® proved to be a better evaluation tool to instantly inform of whether sanitation and cleaning had the desired effect. The ATP findings align with prior studies indicating lower value of using ATP in produce environments where debris/dirt is found frequently since ATP is generally present, and studies that highlight that it is critical to consider residual sanitizer and ATP swab buffers if evaluation post-sanitation are to be performed.

From a practical assessment of the CytoQuant® tool for Produce industry function, it is a mobile device that is straightforward to use and calibrate. Prior to use, and monthly thereafter (depending on use), a calibration kit is used, and daily and weekly cleaning vials to ensure the instrument's readings are as expected. The instrument requires a full charge (overnight) to be set up and was found to be very limited in battery power. During the study, its battery lasted only a few hours while also running a relatively small number of swabs (60 swabs). From a functional perspective, the battery was the most noted limitation of the CytoQuant® instrument, especially considering that the ATP instrument used was reported by the industry participant to generally have about a week's worth of battery life on one charge. Despite this, the CytoQuant® battery limitation can be accommodated with planning in most field settings and could be addressed if used at processing facilities with steady access to electrical outlets. If desired to be used on harvest and/or field equipment, this device would require consistent access to mobile battery power and/or generators available on field equipment and vehicles, and users should be aware of using the tool on surfaces with debris and soil. If used within processing and packing facilities, the CytoQuant® battery is less of a concern and there are other potential utilities such as monitoring processing equipment and wash water quality. While not studied in this trial, the CytoQuant® also has the ability to monitor the microbiological quality of water, and future studies using the CytoQuant® are of interest to be evaluated and may be of high utility in the industry.