**FINAL PROJECT REPORT**

**Project Title:** Utility of rapid tools to assess cleanliness in apple packinghouses


| | | | |
|---|---|---|---|
| **PI**: | Faith Critzer | **Co-PI**: | Ines Hanrahan |
| **Organization**: | Washington State University | **Organization**: | WTFRC |
| **Telephone**: | 509 786 9203 | **Telephone**: | 509 669 0267 |
| **Email**: | faith.critzer@wsu.edu | **Email**: | hanrahan@treefruitresearch.com |


**Total Project Funding:** **Year 1:** 55,956 **Year 2:** 56,525

**WTFRC Budget:**

| Item | 2018 | 2019 |
|---|---|---|
| **Salaries** | 3,900 | 3,978 |
| **Benefits** | 1,287 | 1,313 |
| **Wages** | 3,350 | 3,503 |
| **Benefits** | 1,106 | 1,156 |
| **RCA Room Rental** | | |
| **Shipping** | | |
| **Supplies** | | |
| **Travel** | 500 | 500 |
| **Plot Fees** | | |
| **Miscellaneous** | | |
| **Total** | 10,143 | 10,450 |

**Footnotes:**

Salaries/Benefits: Estimate of percent of time spent for Mendoza (3%) and Hanrahan (2%), a 33% benefit rate and 2% annual increases.

Wages/Benefits: Calculated based on expected staff wage adjustments proportional to the WA state minimum wage increases (2018=$11.50, 2019=$12.00), approx. 250 hours

Travel: In state travel for Hanrahan (lodging in Wenatchee)

**Budget 1**
**Organization Name:** Washington State University
**Contract Administrator:** Samantha Bridger
**Telephone:** (509)786-9204 **Email address:** prosser.grants@wsu.edu

| Item | 2018 | 2019 |
|---|---|---|
| **Salaries** | 32,440 | 34,107 |
| **Benefits** | 2,373 | 2,468 |
| **Wages** | | |
| **Benefits** | | |
| **Equipment** | | |
| **Supplies** | 10,000 | 8,500 |
| **Travel** | 1,000 | 1,000 |
| **Miscellaneous** | | |
| **Plot Fees** | | |
| **Total** | 45,813 | 46,075 |

**Footnotes:**
Salaries: In year 1, $32,440, and year 2, $34,107, is requested for a Graduate Research Assistantship for a PhD student to work on all objectives.

Benefits: $2,373 and $2, 468 are requested for benefits tied to the Graduate Research Assistantship for a PhD student to work on all objectives for years 1 and 2, respectively.

Supplies: Supply costs of $10,000 in year 1 and $8,500 in year 2 are requested to purchase disposable supplies such as swabs, sponges, glassware, microbiological media, Petrifilm, pipettes, and PCR reagents tied to objective 1.

Travel: $1,000 is requested in years 1 and 2 for mileage and associated travel costs at a rate of $0.535/mi and adhering to all university policies for per diem associated with overnight travel.

**Objectives**:

1. Determine the correlation of ATP or carbohydrate swabs to populations of indicator microorganisms (aerobic plate counts, total *Enterobacteriaceae*, coliforms, and *E.* coli) in typical packinghouse settings on zone 1 (food contact) surfaces.
2. Model thresholds for accepting and rejecting a surface cleanliness for ATP and carbohydrate residues and resulting populations of indicator microorganisms based upon material type.

**Significant Findings**

- Rapid tests are not suitable for predicting microbial loads on food contact surfaces.
- Rapid tests are useful to assess residual matter and allow for re-cleaning of equipment.
- Cleaning and sanitation practices should focus on both wet and dry areas of apple packinghouses.
- To validate sanitation practices, traditional microbiological methods are still needed.

**Methods**

***Objective 1.*** Determine the correlation of ATP or carbohydrate swabs to populations of indicator microorganisms (aerobic plate counts, coliforms, and *E.* coli) in typical packinghouse settings on zones 1 and 2.

Packinghouse selection. Commercial apple packinghouses in Washington were recruited into the study. Five packinghouses were enlisted into the study and were sampled once a quarter during packing season (October 2018-August 2019). Table 1 describes the types of surfaces sampled within each unit operation.

**Table 1.** Examples of food contact surfaces tested at each unit operation

| Area | Unit operation | Sample sites (Food contact surfaces) |
|---|---|---|
| Wet | Washing (Dump tank) | Dump tank, rollers, traction belting, brushes under the rot blaster |
| | Washing/Sanitizing/Rinsing (Brush beds) | Brush rollers, bristle rollers, Teflon tapes, plastic flaps |
| | First drying (Fan and/or blower) | Brush rollers, metal dividers, plastic flaps |
| | Wax coating | Brush rollers, rubber flaps |
| | Second drying (Tunnel drier) | Foam rollers, bristle rollers, Teflon tapes, rubber flaps |
| Dry | Sorting | Rollers, foam rollers, bristle rollers, brush rollers, sorter cups, cup-droppers, rubber flaps, interlocking belts, belts, Teflon tapes, guide rails |
| | Packing | Packing tables, belts, rubber flaps, plastic flaps, Teflon tape, guide rails |

Surface sampling methods. Sampling has been coordinated to occur after a sanitation event. For microbiological analysis, a pre-moistened sterile sponge has been utilized to sample a 25 $cm^2$-area. For ATP and carbohydrate swabs, surfaces adjacent to those for microbiological sampling will be used to swab a 25 $cm^2$-area.

ATP determination. An ATP luminometer and accompanying swabs have been utilized to determine the ATP present in the given surface area expressed as reflective light units (RLU).

Glucose and lactose presence. The SpotCheck Plus Glucose and Lactose Residue swab (Hygiena) have been used to determine if there is presence of either of these sugars on the surface. The results will be categorized as pass (no color change=0), moderate fail (light green=1), and severe fail (dark green=2).

Microbiological isolation. Bacteria are eluted in D/E neutralizing buffer and surface plated onto Petrifilm E. coli/Coliform Count Plates (to enumerate *E. coli* and coliforms), Petrifilm Enterobacteriaceae Count Plates (to enumerate total Enterobacteriaceae), Petrifilm Aerobic Count Plates (to enumerate aerobic, mesophilic bacterial counts).

Statistical analysis. Data analysis was carried out using Minitab software (version 19). APC, *Enterobacteriaceae*, coliforms, *E. coli*, and ATP values were normalized using log transformation. To identify the correlation between populations of indicator organisms (APC, *Enterobacteriaceae*, coliforms , and *E. Coli*) with RLU values, Pearson correlation coefficient (r) was determined. A Student's *t* test was performed for pairwise mean comparisons of the different populations of indicator organisms with the scores of Glucose/Lactose residue swabs (Pass or Fail); populations of indicator organisms with the detection of *Listeria* spp. (Positive or Negative), and rapid tests with the detection of *Listeria* spp. (Positive or Negative). Tukey test was used for multiple mean comparisons of populations of indicator organisms (APC, *Enterobacteriaceae*, coliforms and *E. Coli*) and RLU values throughout unit operations with $\alpha = 0.05$.

Alterations to original design of experiments. Due to a high prevalence of Enterococci present on food contact surfaces, it was determined that the methodology for enumerating *Listeria* spp. would always overestimate the population as Enterococci (*Enterococcus faecalis* or *Enterococcus faecium*) cannot be differentiated on selective and differential media. Therefore, enumeration of listeria was abandoned as it is was not going to accurately reflect populations of *Listeria* spp.

***Objective 2.*** Model thresholds for accepting and rejecting a surface cleanliness for ATP and carbohydrate residues and resulting populations of indicator microorganisms based upon material type.

Statistical analysis. Whenever indicators are utilized for making risk-based decisions, many firms wrestle with what thresholds should be established for action (e.g. re-clean surface). Based upon outcomes of objective 1, equations will be evaluated in year two for any moderate to highly correlated indicator to determine the threshold at which the likelihood of having *Listeria* spp. present significantly increases.

Alterations to original design of experiments. Unfortunately, no significant correlations were obtained for any indicator and rapid test, highlighting the fact that rapid tests cannot be utilized to supplant microbiological testing.

**Results and Discussion**

Populations of indicator organisms throughout unit operations. As shown in Table 2, the highest populations recovered were from APC, followed by, in order of population size, *Enterobacteriaceae*, coliforms, and *E. coli*. APC, *Enterobacteriaceae,* and coliforms populations were significantly different at the different unit operations ($p \leq 0.05$). For APC, the wax coating and tunnel drying unit operations showed significantly higher mean values than the washing step. However, regarding *Enterobacteriaceae* and coliform populations, the highest mean populations tended to occur in unit operations associated with

the wet area (Table 2). For all unit operations *E. coli* populations were relatively low (0.2 - 0.3 log CFU/100 $cm^2$) and not significantly different across unit operations ($p>0.05$).

**Table 2.** Mean of populations of indicator organisms at each unit operation

| Unit operation | n[A] | **Mean ± Std Dev of indicator organism populations** (Log CFU/100 $cm^2$) | | | |
|---|---|---|---|---|---|
| | | Aerobic plate count [B] | *Enterobacteriaceae* | Coliforms | *E. coli* |
| Washing | 70 | 2.7 ± 1.2 (b)[C] | 1.7 ± 1.5 (a) | 1.4 ± 1.3 (ab) | 0.2 ± 0.5 (a) |
| Washing/sanitizing /rinsing | 79 | 2.8 ± 1.2 (ab) | 1.6 ± 1.3 (a) | 1.4 ± 1.3 (a) | 0.2 ± 0.4 (a) |
| Fan drying | 75 | 2.9 ± 1.1 (ab) | 1.3 ± 1.2 (ab) | 0.9 ± 1.1 (bcd) | 0.3 ± 0.5 (a) |
| Wax coating | 50 | 3.3 ± 0.9 (a) | 1.3 ± 1.3 (ab) | 1.0 ± 1.1 (abcd) | 0.2 ± 0.4 (a) |
| Tunnel drying | 85 | 3.2 ± 0.8 (a) | 1.5 ± 1.2 (a) | 1.1 ± 1.1 (abc) | 0.2 ± 0.4 (a) |
| Sorting | 302 | 3.0 ± 0.8 (ab) | 1.0 ± 1.0 (b) | 0.6 ± 0.9 (d) | 0.2 ± 0.4 (a) |
| Packing | 80 | 3.0 ± 0.7 (ab) | 1.0 ± 1.0 (b) | 0.8 ± 0.9 (cd) | 0.3 ± 0.6 (a) |

[A] Number of samples

[B] Aerobic plate count (APC) included all the microorganisms that could grow in aerobic conditions and at 35°C

[C] Means within a column that are not followed by the same letter are significantly different ($p\leq0.05$)

Association between RLU values of the ATP test with CFU values of populations of indicator organisms. Table 3 summarizes the Pearson correlation coefficients (r) of RLU values between the different populations of indicator organisms ($r < 0.01$). No statistically significant association was found.

**Table 3.** Pearson coefficient correlation between populations of indicator organisms (Log CFU/100 $cm^2$) with ATP test (Log RLU/100 $cm^2$)

| **Indicator Organism** | **$R^2$ (Pearson coefficient)** | **p-value** |
|---|---|---|
| Aerobic Plate Count | 0.010 | 0.011 |
| *Enterobacteriaceae* | 0.003 | 0.158 |
| Coliforms | 0.001 | 0.373 |
| *E. coli* | 0.011 | 0.009 |

ATP and Glucose/Lactose residue swab readings throughout unit operations. The obtained readings for ATP and glucose/lactose residue swabs on the different food contact surfaces are described by unit operation in Table 4. Concerning the ATP rapid test, the sorting and packing steps, both part of the dry area, showed the lowest and highest RLU mean values respectively. The results for the glucose/lactose residue tests were expressed as percentages of “fail” or “pass” for hygiene surfaces. The unit operations that presented the greatest percentage of “failed” surface hygiene were sorting and packing. Unlike the ATP test, the wet area showed more “pass” results when Glucose/lactose swabs were tested.

**Table 4.** Rapid test readings at each unit operation

| Unit operation | n[A] | ATP test | Glucose/ Lactose residue test | |
|---|---|---|---|---|
| | | Mean ± Std Dev (Log RLU/100 cm$^2$) | % Pass | % Fail |
| Washing | 59 | 2.28 ± 0.83 (ab)[B] | 66.1 | 33.9 |
| Washing/sanitizing /rinsing | 83 | 2.27 ± 0.70 (ab) | 63.9 | 36.1 |
| Fan drying | 75 | 2.09 ± 0.69 (ab) | 60.0 | 40.0 |
| Wax coating | 51 | 2.38 ± 0.81 (ab) | 52.9 | 47.1 |
| Tunnel drying | 78 | 2.19 ± 0.78 (ab) | 38.5 | 61.5 |
| Sorting | 236 | 2.08 ± 0.97 (b) | 22.9 | 77.1 |
| Packing | 77 | 2.48 ± 0.86 (a) | 27.3 | 72.7 |

[A] Number of samples
[B] Means within a column followed by different letters are significantly different ($p \leq 0.05$)

Association of the Glucose/Lactose residue test with different populations of indicator organisms. The APC population was significantly higher when the test for surface hygiene failed. The population dropped significantly to reach a passing level on this test (Table 5). However, the test did not detect significant differences in the populations of *Enterobacteriaceae*, coliforms, and *E. coli* populations with failing and passing scores.

**Table 5.** Association between indicator organism populations with Glucose/Lactose residue test

| Mean ± Std Dev of indicator organism populations (Log CFU/100 cm$^2$) | | | |
|---|---|---|---|
| Indicator organisms | Pass (n=269) | Fail (n=390) | p-value |
| Aerobic Plate Count | 2.91 ± 1.06 | 3.08 ± 0.84 | 0.031* |
| *Enterobacteriaceae* | 1.25 ± 1.26 | 1.13 ± 1.13 | 0.219 |
| Coliforms | 0.98 ± 1.15 | 0.89 ± 1.08 | 0.341 |
| *E. coli* | 0.20 ± 0.42 | 0.19 ± 0.42 | 0.865 |

*Significant difference ($\alpha < 0.05$)

Association between traditional detection of Listeria spp. and rapid tests. Table 6 shows that ATP test readings were not statistically different when comparing both positive and negative detections of *Listeria* spp. ($p > 0.05$). Regarding Glucose/Lactose swabs, the percentage of sites that presented a "pass" result was higher (66.7%) than the percentage of sites with a "failed" result (33.3%), where *Listeria* spp. were detected as positive. However, it is important to consider that the number of positive samples for *Listeria* spp. was low (n=7).

Association between traditional detection of Listeria spp. and populations of indicator organisms. Table 6 also shows that mean populations of APC, *Enterobacteriaceae*, coliforms, and *E. coli*, were not statistically different when comparing both positive and negative detections of *Listeria* spp. ($p > 0.05$). However, it is important to consider that the number of positive samples for *Listeria* spp. was low (n=7).

**Table 6.** Association between indicator organism populations, and rapid tests with the detection of *Listeria* spp.

| | | Detection of *Listeria* spp. | | p-value |
|---|---|---|---|---|
| | | **Positive (n=7)** [A] | **Negative (n=740)** | |
| **Indicator organisms (Log CFU/100 cm²) Mean ± Std Dev** | APC | 3.1 ± 1.4 | 3.0 ± 0.9 | 0.87 |
| | *Enterobacteriaceae* | 1.4 ± 1.4 | 1.2 ± 1.2 | 0.57 |
| | Coliforms | 1.2 ± 1.1 | 0.9 ± 1.1 | 0.47 |
| | *E. coli* | 0.1 ± 0.0 | 0.2 ± 0.4 | 0.44 |
| **Rapid tests** | ATP (Log RLU/100cm²) Mean ± Std Dev | 2.6 ± 0.7 | 2.2 ± 0.9 | 0.22 |
| | Glucose/lactose residue swab | Pass: 66.7% Fail: 33.3% | Pass: 40.2% Fail: 59.8% | ND [B] |

[A] Number of samples
[B] Not determined

**Discussion**

One of the objectives of this study was to evaluate the populations of APC, *Enterobacteriaceae*, coliforms, and *E. coli* at the different unit operations within an apple packinghouse after cleaning and sanitation procedures. For APC populations, means varied from 2.7 to 3.3 log CFU/100 cm$^2$. Unit operations in both wet and dry areas showed significantly higher counts of these indicator organisms. In previous studies, where food contact surfaces were evaluated after cleaning and sanitization procedures, similar values of APC mean populations were found. APC mean counts of 3.4 to 3.5 log CFU/100 cm$^2$ were obtained on food contact surfaces in a facility that processed fresh-cut carrots and lettuce (Lehto et al., 2011), and 2.1 to 4.6 log CFU/100 cm$^2$ on raw vegetable and meat preparation surfaces in a university canteen (Osimani et al., 2014).

The lower mean values obtained after the washing/sanitizing/rinsing step for *Enterobacteriaceae* populations, except for the tunnel drying unit operation, could be explained by the fact that bacteria belonging to the *Enterobacteriaceae* family, which are part of the regular microbiota on apples (Wassermann et al., 2019), are easily inactivated by chemicals used for sanitation purposes (Kornacki et al., 2015). Because coliforms and *E. coli* populations represent sub-populations of the larger *Enterobacteriaceae* family, the total *Enterobacteriaceae* population is expected to be higher than either of the sub-populations (Baylis et al, 2011). Therefore, it was reasonably foreseeable that this relationship was also observed in this study. Other evaluations of *Enterobacteriaceae* populations on food contact surfaces in food manufacturing environments showed higher counts with 3 to 3.3 log CFU/100 cm$^2$ reported in Finnish vegetable processors (Lehto et al., 2011), and 2.1 to 2.5 log CFU/100 cm$^2$ observed in US meat processors (Gómez et al., 2012). However, these results could be explained by the nature of the vegetable and meat product growing/handling environment, in that these commodities are commonly associated with soil and/or fecal contamination, in contrast to the tree fruit packing environment.

Lower coliform populations during sorting and packing (0.6 and 0.8 log CFU/100 cm$^2$, respectively) may be attributed to the removal of potential sources of coliforms that come with the fruit from the orchards within the wet area. Thus, lower carry-over after a sanitation event. In contrast to our findings, Williamson et al., (2018), evaluated automated sorting systems surfaces during peach packing and reported a higher coliform population mean of 2.9 log CFU/100 cm$^2$ after sanitation procedures. According to the authors, this value was expected since it represented natural microbiota present on peach fruits, which was also evaluated. Also, the difference of values could be explained by commodity-specific factors, specifically that unlike peaches, the apple surface is smoother, has a natural wax layer, and is less prone to punctures. Hence, apples may carry a smaller microbial load than peaches. In another study in

bell pepper packinghouses, a similar mean value of coliforms of 0.6 ± 0.2 log CFU/100 $cm^2$ was found on food contact surfaces of equipment such as unloading ramp, roller, conveyor belt and packing bin (Soto-Beltran et al., 2015). Regarding *E. coli,* population means were low throughout all unit operations (0.2 to 0.3 log CFU/100 $cm^2$). *E. coli* is highly related to and used as an indicator for fecal contamination and is regularly employed for water quality standards. In spite of all the tested packinghouses using recirculated water in the dump tank, no higher population was found at this unit operation (the washing step). Indeed, the use of sanitizers, such as chlorine and PAA, in the dump tank could explain this result (Pietrysiak et al., 2019). Similarly, no detectable *E. coli* contamination of the water used for wash produce, was observed by Ailes et al., (2008), who evaluated microbial concentrations on different types of produce during post-harvest processing. Besides, tree fruit traditionally has low populations of *E. coli*. Since fruit is grown on trees above ground, apples are rarely in contact with soil. Therefore, a lower introduction of this microorganism should be seen during tree fruit packing. Duffy et al., (2005), evaluated *E. coli* populations in orange, parsley, and cantaloupe in the field, finding that the only commodity where *E. coli* was not detected was oranges (also a tree fruit).

Moore (2003) did a review from different authors and countries of recommended microbiological limits for acceptable general microbial counts (not a specific type of microorganism) on food contact surfaces. Results for an "appropriate" hygienic surface ranged from < 2.3 to 5 Log CFU/100 $cm^2$ for different types of industries. No specifications for the fresh produce industry were included in this analysis. Additionally, no US regulatory agency currently provides specific standards to define acceptable levels of microbial loads on food contact surfaces. Any such standards should also address differences that may arise given the sampling method employed, surface area sampled, type of product that has been processed, and the processing step at which the samples have been taken. Therefore, it is suggested to use populations of indicator organisms for trend analysis to compare samples that are routinely taken under the same conditions. It is recommended that each facility construct its own thresholds for accepting or rejecting the cleanliness of a surface based upon target standards obtained after a validated sanitation procedure that has been duly and fully performed (Blackburn, 2003; Forsythe, 2000).

The second objective of this research project was to evaluate the association between rapid tests with populations of indicator organisms and the detection of *Listeria* spp. Even though the coefficients of determination ($r^2$) between ATP assay with APC and *E. coli* populations were statistically significant ($p<0.05$), ATP values explained less than 1% of the variance in APC and *E. coli* counts, suggesting that, while a weak positive correlation was found, ATP values alone do not provide significant predictive power for APC and *E. coli* populations. Additionally, no statistically significant correlation was found between the ATP assay and either *Enterobacteriaceae* ($p=0.17$) or coliform ($p=0.38$) populations.

The lack of association observed between the quantification of indicator organisms via the ATP test and the actual populations could be attributed to different factors. ATP is very sensitive to low levels of residual matter on a surface; however, it is not capable of distinguishing if the contamination on the surface originates from microbial or non-microbial sources (Moore, 2003). The amount of ATP varies based upon the type of microorganisms present on the surface. Various studies have shown different amounts of ATP in bacteria, yeast, and fungal spores (Shama and Malik, 2013). Furthermore, ATP tests do not detect whether cells present on the surface are dead or alive (Alfa et al., 2015). Factors such as nutrient level, environmental stress level, and the stage of growth are also known to influence the amount of ATP present (Betts and Blackburn, 2009; Shama and Malik, 2013). Additionally, ATP quantity differs depending on the type of product. Raw fruits and vegetables typically contain a higher amount of ATP compared to dry products (Griffith, 2005). Other factors affecting ATP readings include the use of sanitizers and cleansers (Green et al., 1999), the state of the surface (wet or dry) (Davidson et al., 1999), presence of salts and metal ions that affect the stability of the enzyme luciferase within the reagent of the ATP test (Moore, 2003). In order to establish acceptance limit levels for ATP values, similar factors, as discussed for populations of indicator organisms need to be considered.

Many studies have shown no or low associations between APC populations and ATP quantities, including in retail delis ($r^2=0.10$) (Hammons et al., 2015), milking equipment such as bulk tank ($r^2=0.12$) (Vilar et al., 2008), stainless steel milk contact surfaces (Costa et al., 2006), hospital environments

($r^2$=0.09) (Raia et al., 2018), ($r^2$=0.29) (Amodio et al., 2014), and on hands and surfaces in the home ($r^2$=-0.001, and 0.002 respectively) (Larson et al., 2003).

In contrast, studies have reported strong linear positive correlations between APC populations with ATP, including those on plastic cutting boards ($r^2$=0.97) (Leon and Albrecht, 2007), whole unwashed cantaloupe surfaces ($r^2$=0.995) (Ukuku et al., 2001), and in retail delis (Hammons et al., 2015). However, the detectable sensitivity threshold ranged only from 3.6 to 5.6 log CFU/100 $cm^2$ for the first study, and a minimum detectable level of 6 log CFU/100 $cm^2$ and 3 log CFU/sponge for the second and third study, respectively. These APC populations were significantly higher values than the ones obtained in this study. Also, Ukuku et al., (2001), utilized ATP extractants such as Tris-EDTA rather than commercial ATP swabs. Another study conducted to evaluate the correlation between *E. coli* populations and ATP reported that a minimum concentration of 4 log CFU/100 $cm^2$ of *E. coli* was needed in either wet or dry surfaces to be detectable by an ATP test (Davidson et al., 1999). In addition, one of the limitations of the previous studies (Davidson et al., 1999; Leon and Albrecht, 2007), is that they were performed under laboratory conditions. In real life, situations involving microbial populations at these concentrations are unlikely to occur since microorganisms are not present as pure culture in the environment (Davidson et al., 1999; Turner et al., 2010).

The association between the glucose/residue test swab and APC populations could be explained by the fact that glucose is an energy source and the major nutrient required for microorganism metabolism (Galant et al., 2015). While significantly different APC values (i.e. higher APC counts for a 'failed' hygienic surface), these values, from a practical standpoint, may not represent a numerical difference when establishing thresholds for acceptance or rejection. A study to evaluate cleanliness in cattle barns was conducted using glucose/lactose residues swabs. No difference between outcomes for a 'clean' or 'dirty' surface was found (Kymäläinen and Kuisma, 2016). The authors analyzed different cattle barn soils, which contained different nutrients including sugars such as carrot juice and milk, nevertheless the color of the soil could have interfered with the color detection of the test. Additionally, when assessing this type of rapid test, it is important to note that an absence of detectable sugar residues on a surface does not necessarily mean a clean surface, but rather that the residual contaminants were not present in levels high enough then the detection limit of the test (Schmitt and Moerman, 2016) or the contaminant did not contain sugar residues.

The packing unit operation showed one of the highest readings in both rapid tests: ATP (2.5 log RLU/100 $cm^2$) and glucose/lactose swab (72.7% of "failed" hygienic surface). These values may be due to physical contaminants, such as stickers and labels, that are not easily removed from belts and packing tables, making cleaning procedures harder to perform. Furthermore, the dry area was not cleaned and sanitized as often as the wet area in order to avoid water residues on the dry side of the plant. However, the dry area did not present higher microbial counts of APC, *Enterobacteriaceae*, coliforms, and *E. coli* than the wet side. Thus, it has been hypothesized that since the fruit has already been sanitized within the wet area, less carryover of bacteria was taken to the dry area.

Lastly, the lack of association between both rapid tests and populations of indicator organisms with the positive detection of *Listeria* spp. is supported by previous data. No associations between the detection of *Listeria monocytogenes* with APC (D'Amico et al., 2008; Jackson et al., 2012; Van Kessel et al., 2004), *Enterobacteriaceae* (Jackson et al., 2012), coliforms (Jackson et al., 2012; Martin et al., 2016) and *E. coli* (Jackson et al., 2012) populations have been reported in the dairy industry. APC is not considered an indicator of food safety because it does not specify the presence of any pathogen (Ryser and Schuman, 2015). It has been suggested that the presence of organisms from the *Enterobacteriaceae* family including coliforms and generic *E. coli*, are not suitable to assess the presence of *Listeria* spp. since these species are more resistant to environmental factors than enteric pathogens such as salmonellae, *Shigella dysenteriae*, or pathogenic *E. coli* (Baylis et al., 2011; Tortorello, 2003). However, studies have also observed positive correlations between the growth of *L. monocytogenes* and APC in other environments such as minimally processed fresh endive (Carlin et al., 1995), and retail delis (Hammons et al., 2015), likely due to similar favorable growing conditions for mesophilic bacteria and *L. monocytogenes* (Carlin et al., 1995).

The results of this study suggest that apple packinghouses should use both rapid tests and traditional microbiological methods for indicator organism populations when assessing cleaning and sanitation practices. Rapid tests are valuable for monitoring residual matter on a surface, thus validating the efficacy of cleaning procedures prior to sanitation. However, to validate sanitation practices, traditional microbiological methods are still needed. These findings can help guide packinghouses when establishing microbiological thresholds of indicator organisms (e.g. APC, *Enterobacteriaceae*, coliforms and *E. coli)*. Also, to assess a trend analysis of microbial populations or rapid test readings over a packing season. Future studies should seek to improve dry cleaning and sanitation methods for the dry area. Moreover, it is important to emphasize that a risk of *L. monocytogenes* harborage in apple packinghouses may not be detected when utilizing indicator organisms other than *Listeria* spp. as demonstrated through these findings.

**Acknowledgments**

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**Citations**

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**EXECUTIVE SUMMARY**

**Project Title:** Utility of rapid tools to assess cleanliness in apple packinghouses

**Key words:** ATP, glucose/lactose residue, cleaning, sanitation, apple packing

**Abstract**

The 2014 listeriosis outbreak caused by caramel-coated apples was linked to apples cross-contaminated within an apple packing facility. This outbreak has increased the focus on effective cleaning and sanitation methods that must be validated and monitored during apple packing. Thus, rapid and reliable testing methods are necessary for assessing cleanliness in the apple packing industry. The objectives of this study were to assess the prevalence of common indicator organisms [Aerobic plate count (APC), *Enterobacteriaceae*, coliforms, *Escherichia coli*, and *Listeria* spp.] on food contact surfaces (zone 1) in apple packinghouses and to evaluate the utility and accuracy of currently used rapid tests (ATP and glucose/lactose residue swabs). Food contact surfaces were sampled over a 100 $cm^2$ area in five commercial apple packinghouses to evaluate populations of indicator organisms APC, *Enterobacteriaceae*, coliforms, *E. coli* (n=741), and rapid test readings (n=659). Petrifilm plates were used for the quantification of APC, *Enterobacteriaceae*, and coliform/*E. coli*. Rapid tests [ATP swabs (UltraSnap) and glucose/lactose residue swabs (SpotCheck Plus)] were processed on-site. A larger area (0.93 $m^2$) was sampled for the detection of *Listeria* spp. (n=747), following a modified protocol of the FDA's Bacteriological Analytical Manual method, and confirmed with PCR and gel electrophoresis via the *iap* gene. No significant association was found between either rapid test and populations of APC, *Enterobacteriaceae*, coliforms, *E. coli*, and *Listeria* spp. detection. However, recovery of APC (log CFU/100$cm^2$) was higher with a failed glucose/lactose residue swab surface hygiene result (3.1) than a passed result (2.9) ($p=0.03$).

Populations of APC, *Enterobacteriaceae*, and coliforms were significantly different at each unit operation during the packing process ($p \leq 0.05$). This study concluded that ATP and glucose/lactose residue rapid tests were poorly suited for determining microbial load since they were not related to populations of any common indicator organisms or the detection of *Listeria* spp. These findings emphasize the need to utilize a rapid test, which can be a good indicator of residual matter on a surface, along with traditional microbiological methods to assess cleaning and sanitation practices in apple packinghouses.