FINAL REPORT

Project Title: Understanding and managing the food safety risk of packline brushbeds

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Cooperators: Eight packing facilities in Washington

Total Project Request: **Year 1:** $51,966

WTFRC Collaborative expenses:

Item	2017
Salaries & Benefits	$2,736
Wages	$3,472
Benefits	$729
Travel	$4,180
Total	**$11,117**

Footnotes: Salary and benefits for Ines Hanrahan; Wages and benefits for intern.

Budget 1
Organization Name: WSU
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Contract Administrator: Katy Roberts
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Item	2017
Wages	$6,256
Benefits	$1,314
Equipment	$11,000
Supplies	$16,707
Travel	$5,574
Total	**$40,850**

Footnotes: Wages and benefits for technical assistant; Equipment is incubator ovens, plate counter and pipettes; Supplies for microbial test plates and sponges.

1. Objectives

1. Compare current brush cleaning and sanitation procedures in eight apple packing houses in Washington to determine the effectiveness of these procedures.
2. Determine if fruit sanitation practices are adequate to reduce the risk of cross-contamination from wax brushes during a production shift.
3. Determine if wax brushes are a commercially significant source of spoilage organisms (yeasts and molds)
4. Determine if there is a difference between the packing organic and conventional fruit in the above objectives.
5. Conduct appropriate food safety extension outreach with the apple packing industry

2. Significant Findings

- Aerobic plate counts were not correlated with populations of coliforms and *E. coli.* r^2=0.17 and 0.018, respectively. This indicates that there is very little utility in testing for APCs on food contact surfaces as they are readily in the environment and of no connection to indicators which are more indicative of sanitary concerns (*E. coli* or coliforms).
- Clean out of place (COP) steam cleaning was very effective in reducing microbial counts on packing line brushes – aerobic colony counts were 1700 times lower than the average of the other five packing facilities.
- Extremely low populations of *E. coli* were found in all facilities across both years, indicating that current sanitation practices are significantly reducing the risk of harborage. Setting sanitary performance metrics for food contact surfaces (zone 1) based upon *E. coli* or coliform populations would be recommended for tree fruit packers.
- False positives for *Listeria* spp. are common when relying solely on selective and differential media. Subsequent steps should involve conformation utilizing standardized methods, such as PCR. No samples were confirmed positive for *Listeria* spp. in this study.

3. Methods

Six representative apple packing facilities in Washington were selected for this project in 2017, partly based on responses from a project pre-survey. Packing facilities are numbered to maintain confidentiality (Table 1). Brushes and other packing line surfaces (oven rollers, drying oven walls, belts, curtains and transfer rubbers) were swabbed (3M™ Quick Swab) both before and after a production shift. The focus of the study was drying and wax brushes, but also included other brushes and surfaces in the wet area of the packing line.

Fruit samples were taken off the line before and after the brushbed at the start and end of the production shift; 10 fruit were taken at each location. Swabs and fruit were stored in a cooler box with ice packs during transportation from Yakima or Wenatchee, stored in a refrigerator overnight, and plated the following morning at WSU IAREC in Prosser. Fruit were placed in buffered peptone water incubation pouches for 1 h before plating.

The following microbial tests were conducted on swabs using 3M Petrifilm™ plates: aerobic colony count, coliform/*E.coli*, environmental *Listeria*, and yeasts and molds following the 3M Petrifilm methods for each test. The same tests were conducted on fruit samples, except that environmental *Listeria* and coliforms/*E.coli* testing were omitted. Enumeration was done using a 3M

Petrifilm Plate Reader. Samples were diluted 1:10 using Butterfields solution for APC and yeast and molds if high microbial loads were anticipated.

In 2018, the project was continued with four facilities (two from 2017 and two which were new to the study) to collect more data on microbial populations of food contact surfaces (Table 2). The methodology was modified slightly for inclusion of sponge swabs with Dey Engley (D/E) neutralizing buffer, increasing the surface area to $1ft^2$ for *Listeria* spp., and identification of *Listeria* species through selective enrichment with PCR confirmation targeting the *iap* gene.

Table 1: Packing facility numbers and description sampled in 2017

	Packing Facility Number					
	1	2	3	4	5	6
Relative Age of Line	Newer	Older	Newer	Newer	Older	Newer
Wet/Dry Separation	Yes	No	Yes	No	No	Yes
Hygiene Monitoring	Yes	No	Yes	Yes	Yes	Yes
Brush CIP/COP	CIP & COP	CIP	CIP & COP	CIP	CIP	COP
Brush Cleaning Method	Chlorine foam	Chlorine foam	Chlorine foam	Chlorine foam	Chlorine foam	Steam
Sanitizer during Production	Ozone, PAA, ClO_2	PAA	Ozone, PAA, ClO_2	PAA	Ozone	PAA

CIP, Clean in Place; COP, Clean out of Place; PAA, peracetic acid; ClO_2 chlorine dioxide.
Newer lines < 5 years old; Older lines >15 years old

Table 2: Packing facility numbers and description sampled in 2018

	1	2	3	4
Relative Age of Line	Newer	Older	Newer	Older
Wet/Dry Separation	Yes	No	No	No
Hygiene Monitoring	Yes	Yes	Yes	Yes
Brush CIP/COP	CIP & COP	CIP	CIP & COP	CIP
Brush Cleaning Method	Chlorine foam	Chlorine foam	Chlorine foam	Chlorine foam
Sanitizer during Production	Ozone, PAA, ClO_2	Chlorine, Ozone, PAA	Chlorine, PAA	Chlorine, PAA

CIP, Clean in Place; COP, Clean out of Place; PAA,peracetic acid; ClO_2 chlorine dioxide.
Newer lines < 5 years old; Older lines >15 years old

4. Results & Discussion

4.1. Environmental Listeria

It should be noted when relying upon differential and selective media, as was used in this study, there are frequently false positive isolates identified that when confirming with secondary tests like PCR, are actually not *Listeria* spp. but rather Enterococci, such as *Enterococcus faecium* or *Enterococcus faecalis*. Therefore, we cannot definitively state if the isolates from 2017 were in fact *Listeria* spp. Only facility 5 (older line) had environmental *Listeria* detections in 2017. These detections were on:

- a transfer rubber at the end of shift (10/2),
- soap brushes and a felt fabric transfer curtain at the start of shift (10/30), and
- a wax brush at end of shift on 10/30.

This facility had high aerobic colony counts at the start and end of shifts (Figure 2).

Of the four facilities which were sampled in 2018, none were positive for environmental *Listeria* spp. after sanitation and with 4 hrs of production startup (n=156). Brushes and transfer points were identified as common targets for sampling, but were not observed to be harborage points.

4.2. Coliforms & *E.coli*

Coliforms were detected at least once at all packing facilities at the start of the production shift (Figure 1, 2017 data). Areas that regularly tested positive for coliforms at the start of the production shift were:

- Wax brushes
- Transfer brushes after the drying oven
- Bin filler brushes
- Transfer brushes in general

Coliforms were also detected in all of the facilities during 2018 sampling, but no significant associations were made by material or unit operation with populations ranging from 14-700 CFU per 25 cm^2.

There were four *E.coli* detections in 2017:

- Facility 1 on a wax brush under the wax applicator at the start of the shift.
- Facility 2 on repair tape on a spacer bar.
- Facility 5 on a transfer rubber – the same date (10/2) and location where environmental *Listeria* was detected (see above) – and one fruit sample at the start of shift after going over the brushbed.

These three facilities had the highest average aerobic colony counts (Figure 2).

Three of the four facilities in 2018 had sites which were positive for *E. coli* in 2018.

- Twenty-two of 156 sites tested positive for *E. coli* in 2018 with populations remaining very low (1-5 CFU/25cm^2)
- Sites were evenly divided between dry (sorter, oven rollers, packing tables) and wet (spray bars, dump tank) areas of the packing line.

4.3. Aerobic Bacteria

Aerobic plate counts (APCs) are considered of very little utility for monitoring cleanliness within the food industry given that they have no linkage to food safety and many times encompass bacterial populations which may be more resistant to sanitizers and heat than target organisms of

quality or safety concerns. There were no significant correlations found between APCs and *E. coli* or coliforms. If using APC as a sanitation performance metric, it is important to set baseline populations for each surface. When evaluating 2017 data, Facility 6 had aerobic colony counts 3 orders of magnitude lower at the start of shift and 2 orders of magnitude lower at the end of shift than the other five facilities – the APCs at the end of the production shift at facility 6 were often lower than the APCs at the start of the shift at other facilities. Their success demonstrates that it is possible to clean a packing line to very low counts, and reduce these by 2-3 $\log_{10}$ values with COP steam cleaning and a multi-hurdle approach during a production shift.

Facilities 3 and 6 (2017) had the lowest average aerobic colony counts on the packing line (Figure 2), and also had the lowest aerobic colony counts on fruit (Figure 4). Facility 5 had high counts throughout the line and consequently the fruit from that facility had the highest counts. General comments regarding specific areas on packing lines are given below in Table 2.

When evaluating data for 2018, recovery of total aerobic bacteria was significantly different at sites along the processing line (p=0.0179). A post-hoc analysis revealed that recovery was higher at spray bar sites (Mean=3,255) than at dryer (Mean=999), dump tank (Mean=718), or sorter (Mean=484) sites, but indistinguishable from packaging (Mean=1,735) or wax bar (Mean=1,227) sites (LSD test). No significant differences were found between the four facilities.

Table 2: Comments on cleaning and sanitation procedures for zones 1 and 2 areas on apple packing facilities.

Area	General Comments
Soap and sanitizer brushes	Need attention during cleaning
Drying brushes	ATP swab first brushes; highest APC there, decreasing down bed
Wax brushes	All CIP lines have high APCs, especially under the wax applicator. ATP swab brushes under the wax applicator.
Oven rollers	Lower concern, but high residues indicate higher APCs
Post-oven transfer brushes	Can have high loads, need more attention during cleaning and sanitation
Alignment brushes	Lower concern
Bin filler brushes	Some concern, need more attention during cleaning and sanitation
Transfer brushes	Some concern, need more attention during cleaning and sanitation
Other surfaces	Other surfaces, like fruit pushers, oven walls, etc. require attention during cleaning and sanitation in the worse performing packing facilities. Surfaces like tape, foam, cloth and rubber should ideally be removed from the line because they are potential harborage sites for food pathogens.

APC, aerobic plate count; CIP, Clean in Place

4.4. Molds

The mold counts on the packing lines generally increased during the production shift and correlated with the aerobic colony counts. Facilities 3 and 6 having lower mold counts and facilities 1, 2, and 5 having higher loads (Figure 5 and Figure 6). This will vary by lot and storage duration, however, and requires longer term monitoring. Facility 1 did not have good mold control over the brushbed and

consequently through the shift, with both mold and aerobic counts increasing over time on fruit (Figure 6). Yeast counts followed a similar trend to molds so data were not presented for brevity. Good cleaning and sanitation practices not only reduce food safety risks, but may improve returns by reducing rejections of packed fruit with an extended storage period – such as exports or in a high production season.

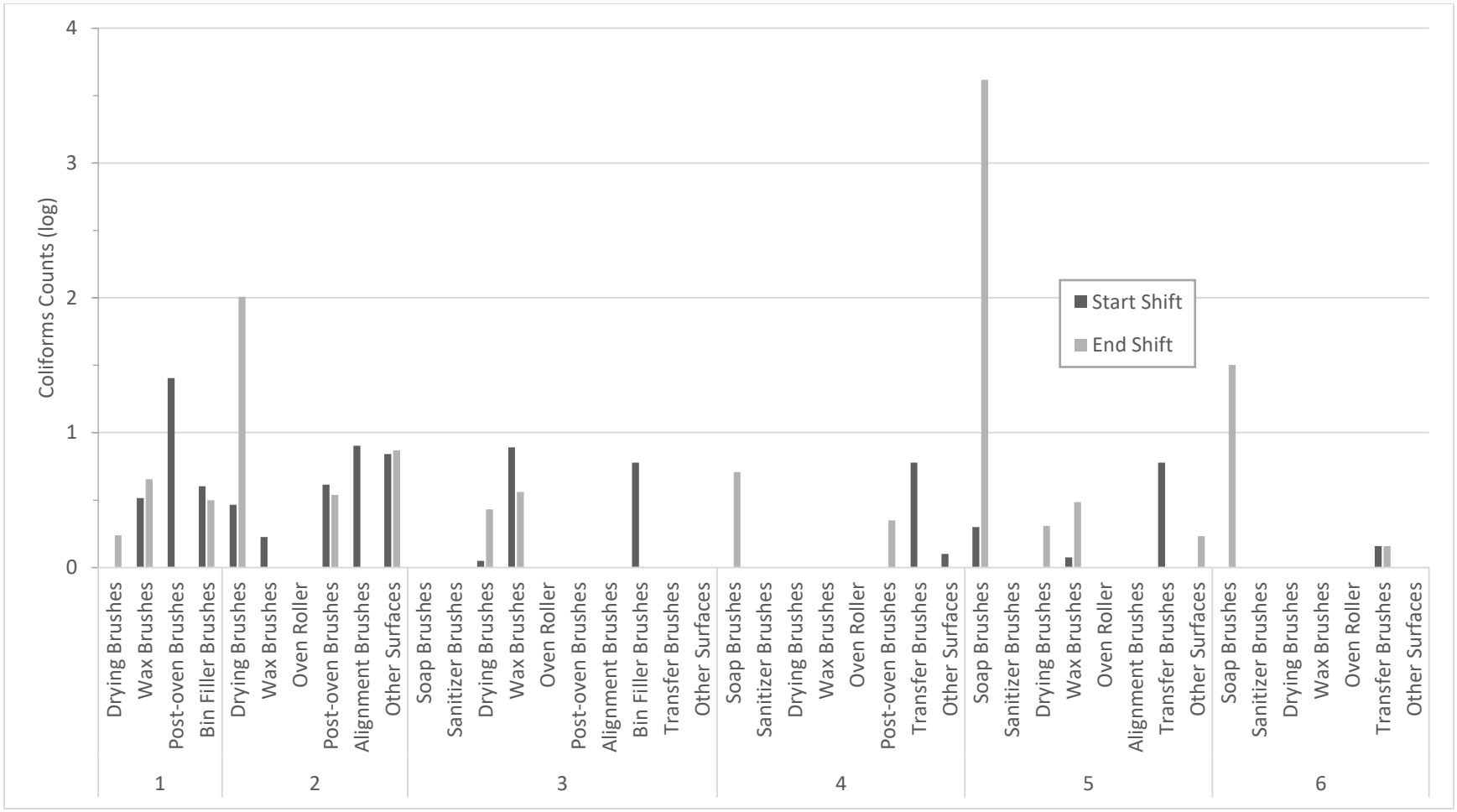

Figure 1: Coliform counts on the packing lines of six particpating packing facilities (2017).

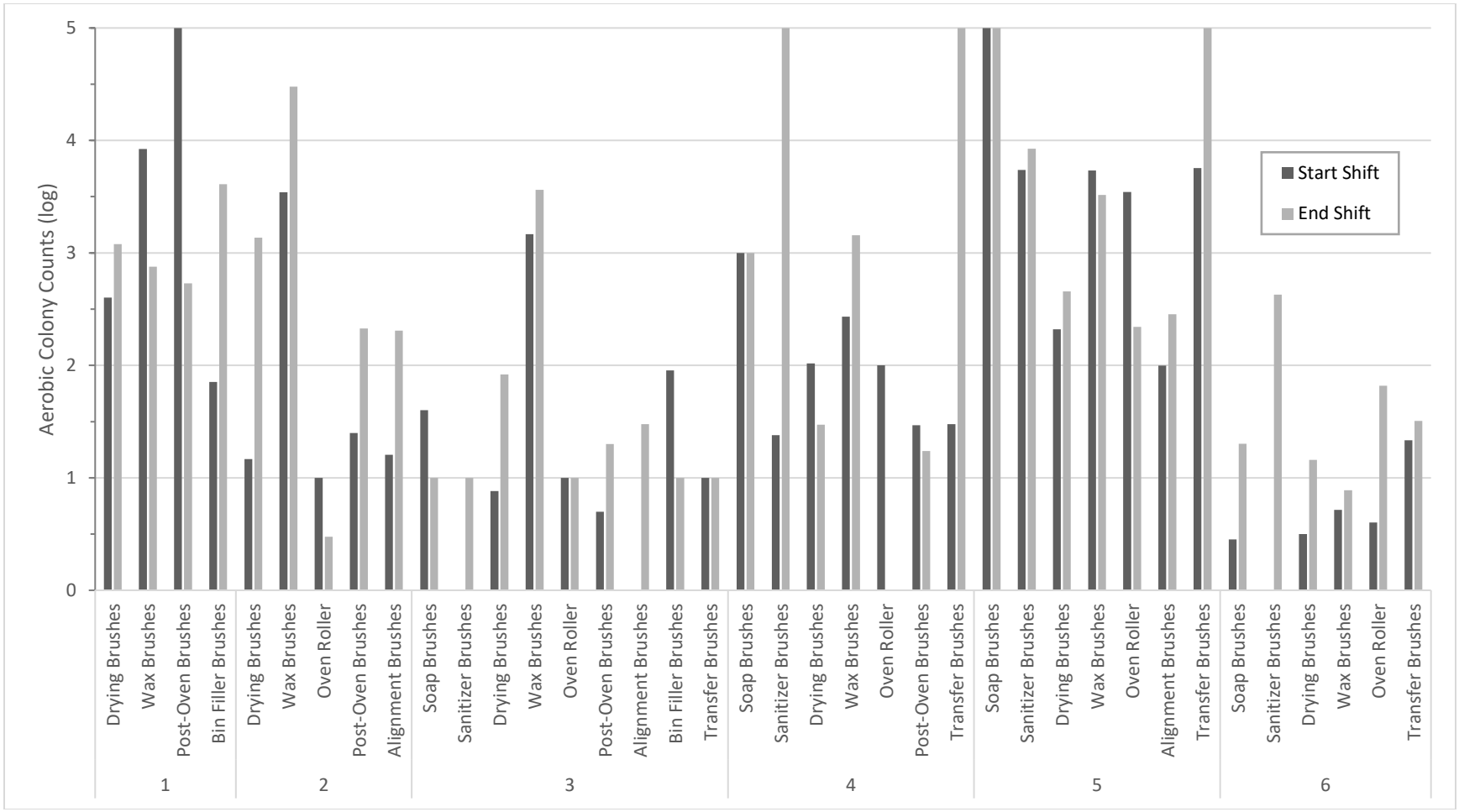

Figure 2: Aerobic colony counts on the packing lines of six particpating packing facilities (2017).

Figure 3: Aerobic colony counts on selected packing line surfaces at four of the participating packing facilities (2017).

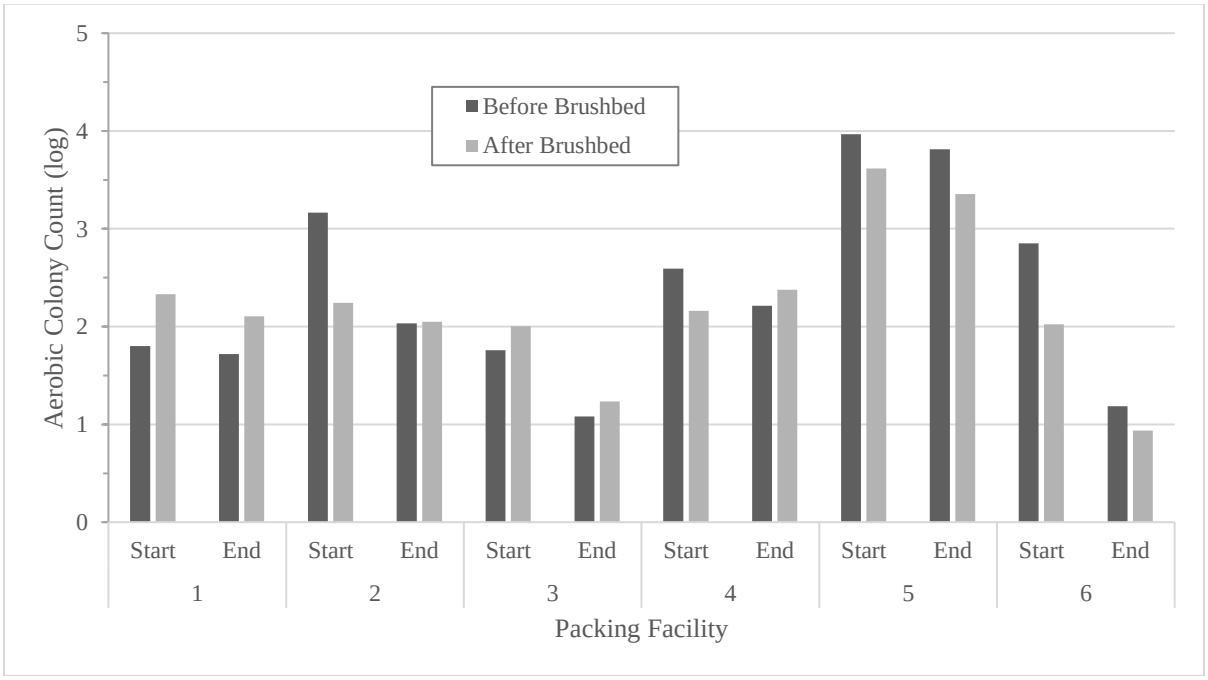

Figure 4: Aerobic colony counts on apple fruit at the start and end of shift, sampled before and after the brushbeds of the six participating packing facilities (2017).

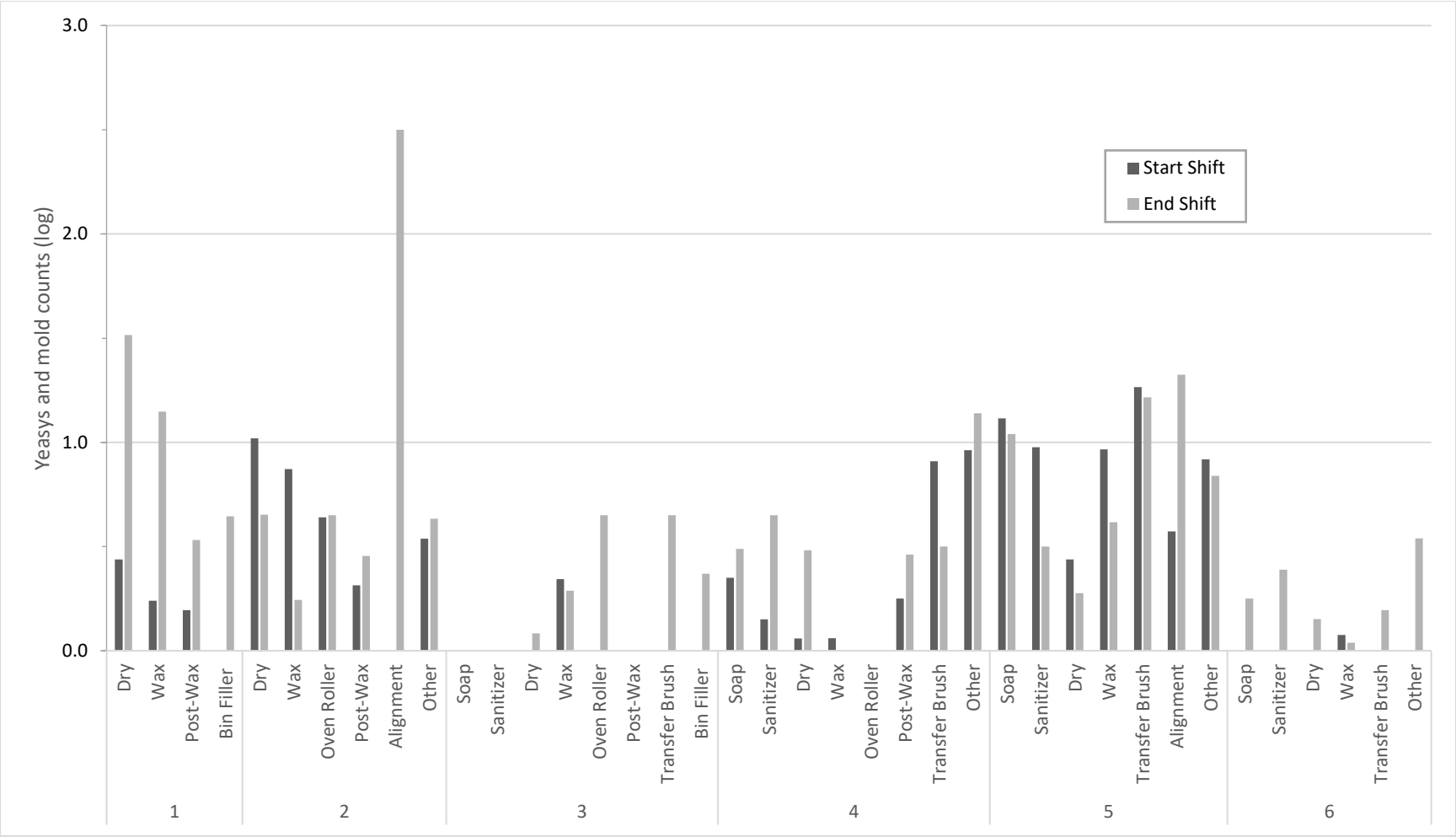

Figure 5: Mold counts on the brushes and other surfaces of packing lines of six particpating packing facilities (2017).

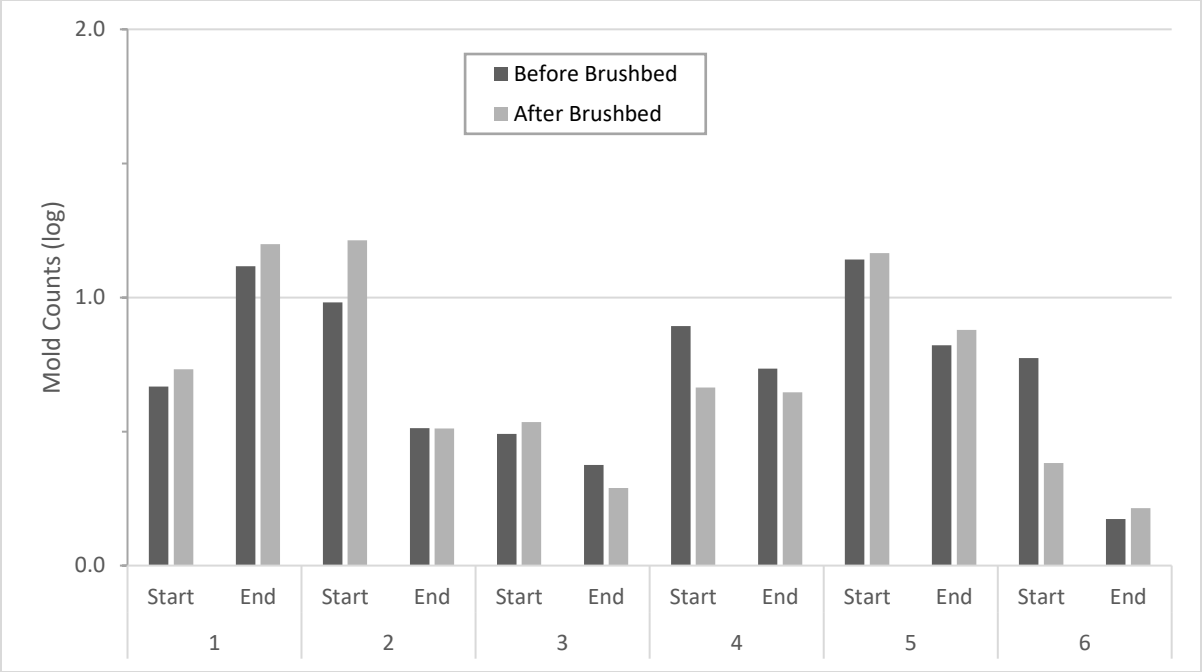

Figure 6: Mold counts on fruit at the start and end of shift, taken before and after the brushbeds of the six participating packing facilities (2017).

5. Conclusion

Generally speaking, there is little value in testing surfaces for aerobic mesophilic bacteria (APC) as high populations are regularly recovered and are not correlated with populations of indicators more commonly employed for food safety (generic *E. coli* or coliforms). Cleaning practices dictated recovery of microbial populations to a greater extent than the age of the facility. This is encouraging as capital outlay for new facilities and packing lines are significant and not an option for many operations. COP steam cleaning of brushes resulted in a significant reduction in microbial counts, but has been noted to reduce the life of brushes. Some key points to improving hygiene levels in packing facility, and reducing food safety risk are: a motivated, properly equipped sanitation crew with attention to detail and sufficient time to clean and sanitize the packing facility, a validated hygiene monitoring system, an appropriate sanitizer monitoring system and protocol, and leadership from management to continually improve hygiene levels in a facility.

These results only provide a snap shot at each packing facility. To be effective, a food safety program requires daily attention, and long term planning for continual improvement. These assays, excluding the environmental Listeria test, can be done easily at a packing facility and the results used to improve cleaning and sanitation procedures at the facility.

6. Executive Summary

Aerobic plate counts (APCs), coliforms and *E.coli*, environmental *Listeria*, and yeasts and molds samples were taken at six apple packing facilities in Yakima and Wenatchee between August and October 2017. These facilities were representative of the types of packing lines currently in Washington. The brushbed was swabbed before and after a production shift. Fruit samples were also taken at the same times, before and after going over the brushbed. Microbial tests were performed using 3M Petrifilm™ plates. In 2018, four facilities were sampled (two from 2017 and two new facilities) with sampling of food contact surfaces (zone 1) to determine populations of APC, *E.coli*, coliforms and *Listeria* spp..

APCs were not correlated with populations of more common food safety indicator organisms (*E. coli*, coliforms and *Listeria* spp.). However, APCs may be a cost effective means of measuring cleaning and sanitation efficacy, with appropriate baseline establishment. When evaluating data from 2017 data, **APCs** at the start of production were lower in the three newest lines, but results show that it is possible to clean older facilities to levels comparable to those of newer lines. **Yeasts and molds** correlated with APCs, suggesting that evaluation of these populations may also help decrease post-packing decay – particularly on fruit with an extended post-packing storage duration. **Coliforms** were detected on all the packing lines in 2017 and 2018. ***E.coli*** was sporadically detected at the three facilities in 2017 and all facilities in 2018. There may be better utility in testing zone one surfaces for these organisms compared to APC as an indication of sanitation efficacy. This is due to the fact that target foodborne pathogens share similar inactivation behavior to these organisms, whereas APCs will detect many bacteria which are not a concern for quality or safety and may be more resistant to our cleaning and sanitizing practices. **Environmental *Listeria*** was only detected on one older line in 2017, however these positives were not confirmed. No *Listeria* spp. were detected in 2018 demonstrating efficacy of current practices used in facilities of all ages employing many different sanitation practices.

It is clear from this study that detailed attention and evaluation of cleaning and sanitizing efficacy should be conducted by all facilities. Regardless of sanitizers used or age of a facility, very low populations of common food safety indicators (coliform, generic *E. coli*, and *Listeria* spp.) were observed amongst eight facilties. It is key that facilities continually evaluate their sanitation programs and inclusion of testing for indicators associated with foodborne pathogens will help to mitigate risks and identify areas which may need more frequent or intensive sanitation. Coliform or *E. coli* populations could be determined through in-house testing and may be the best organisms to indicate the efficacy of a sanitation program from a food safety perspective. APCs will enumerate any organism which can grow at 98°F and is tolerant to air. There are many organisms which will be enumerated on APC which are not a food safety or quality risk. However, higher yeast and mold counts did align with higher APCs and may assist when trying to control for cross-contamination onto fruit.