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Fibre Box Association
25 Northwest Point Blvd.
Suite 510
Elk Grove, Illinois 60007

Attention: Mr. Dennis Colley
President/CEO

Subject: Microbiological Status of Corrugated Containers – 2017 Annual Review Study

Dear Mr. Colley,

Fresh produce has been documented by the United States (US) Center for Disease Control and Prevention (CDC) as a leading source of food-borne illness (CDC, 2014). With the passage of the US Food Safety Modernization Act (FSMA) in 2011, the supply chain has become an even greater source of regulatory scrutiny for growers, shippers and even retailers. FSMA now requires US entities take a proactive rather than reactive approach to food safety (US FDA, undated). Although food-borne illness has not been directly associated with shipping and transport containers, the potential for containers to harbor and transfer microbial loads to the fresh produce placed in those containers has been documented (Danyluk, 2010; Sanders, 2015a; Warriner, 2013).

To confirm the continued due diligence by corrugated manufactures to provide clean containers to the fresh produce industry, an annual sampling regimen has been established by the FBA and its member companies. This report summarizes the results of the 2017 annual testing.

Project Background

The Fibre Box Association (FBA) has conducted multiple evaluations of the microbial cleanliness of corrugated containers from its member companies across various regions in the US since 2014. This current report, sponsored by the FBA, summarizes the results of a recent survey (2017 annual review) that assesses the microbial status of corrugated containers currently being used for the storage and transport of fresh produce. The FBA and its member companies are committed to this self-evaluation program to confirm that the microbial loads on corrugated containers currently being used by the fresh produce industry continue to meet acceptable limits.

Acceptable microbial levels for produce storage and transport containers are not currently defined by any regulatory agencies in the US. A European Union (EU) Commission Decision (2001/471/EC) states that the total viable microorganism count on containers for transport of fresh meat or poultry should not exceed 10 colony forming units (CFU)/cm², while the value of *Enterobacteriaceae* should not exceed 1 CFU/cm² (European Commission, 2011). These limits have been subsequently employed as a benchmark level by the Ireland Food Authority and the New South Wales Food Safety Authority for clean and sanitized food contact surfaces (Ireland Food Authority, 2006; New South Wales Food Safety Authority, 2013).

In a publicly available, peer-reviewed study, Cunningham defined the acceptable levels of aerobic microorganisms on food contact surfaces as 125 CFU/50 cm² (equal to 103.4 CFU/930 cm²) as the upper limit for a clean and sanitized food contact surface (Cunningham et al., 2011). Dr. Keith Warriner of the University of Guelph, in his evaluation of containers used for the transport of fresh produce specified that less than 10³ CFU *Enterobacteriaceae*¹ or thermotolerant coliforms²/container would be representative of sanitary conditions and be deemed acceptable (Warriner, 2013).

In a 2013 study of RPCs used for the shipping and transport of fresh produce, Dr. Keith Warriner of the University of Guelph, specified that *Enterobacteriaceae*³ or thermotolerant coliforms⁴ levels less than 10³ CFU/container would be representative of sanitary conditions and deemed acceptable (Warriner, 2013).⁵ This acceptance criteria, established by Warriner, was used to evaluate data from a previous field studies on the cleanliness of both corrugated containers from multiple manufacturers and reusable plastic containers (RPCs) across the US and Canada (Sanders, 2015b). In this review, all corrugated containers tested (N=360) had microbial loads below 1,000 CFU/container (Sanders, 2015a).

Project Methodology

Corrugated containers collected at grower/shipper locations in both Florida or California were sampled and tested using the attached protocol (Appendix A: Corrugated Container Sampling and Testing Protocol). This protocol was developed based on the prior sampling protocol with the final review and approval by Dr. Trevor Suslow of the University of California, Davis. Testing included the microbial evaluation of corrugated containers for thermotolerant coliforms and *Enterobacteriaceae*; a total of 288 samples from 144 containers were sampled, (96 samples from 48 containers at each location).

¹ *Enterobacteriaceae* are often evaluated as an indicator for *Salmonella* spp.

² Thermotolerant Coliforms are often evaluated as an indicator organism for *Escherichia coli* (*E.coli*).

³ *Enterobacteriaceae* are often evaluated as an indicator for *Salmonella* spp.

⁴ Thermotolerant Coliforms are often evaluated as an indicator organism for *Escherichia coli* (*E.coli*).

⁵ 10³ can also be expressed as 1,000 or log 3.

Results

The microbial results based on the sampling of the interior surfaces of the containers were reviewed to assess the presence of microorganisms per container and per sponge sample. The distribution of the microbial load found on the containers is summarized on both a container and sponge sample basis in the Table 1 and Table 2.

Table 1: Organisms per Container (Thermotolerant Coliforms and *Enterobacteriaceae*)⁶

Sampling Location	# of Containers	Containers with ≤10 CFU	Containers with >10-≤100 CFU	Containers with >100-≤1000 CFU	Containers with >1000-≤10,000 CFU
Florida 1	48	41	6	0	1
California	48	42	5	0	1
Florida 2	48	45	3	0	0
Total	144	128	14	0	2

Table 2: Organisms per Swab Sample (Thermotolerant Coliforms and *Enterobacteriaceae*)⁷

Sampling Location	# of Swab Samples	Swab Samples with ≤10 CFU	Swab Samples with >10-≤100 CFU	Swab Samples with >100-≤1000 CFU	Swab Samples with >1000-≤10,000 CFU
Florida 1	96	88	7	0	1
California	96	90	5	0	1
Florida 2	96	93	3	0	0
Total	288	271	15	0	2

Ninety-nine percent (99%) of all containers sampled had microbial loads below 100 CFU per container, well below the acceptable limits defined by Warriner. With only two samples across the 288 samples taken showing microbial levels above 100 CFU/swab.

- A single sample from a single container at the Florida 1 location showed 3.7 log of *Enterobacteriaceae*, but no thermotolerant coliforms.⁷
- A single sample from a single container at the California sampling location showed 3.4 log of thermotolerant coliforms, but no *Enterobacteriaceae*.⁸

⁶ The data used to generate these tabular data can be found in Appendix B.

⁷ The data used to generate these tabular data can be found in Appendix B.

⁸ It is important to note that the presence of these indicator organisms does not denote the presence of pathogenic organisms.

Conclusion

This study was performed as a follow up to prior industry-wide corrugated container cleanliness studies performed in 2014 (initial) and 2016 (annual). In these studies, corrugated containers from various manufacturers showed an overall lack of the presence of two pathogenic indicator organisms (Enterobacteriaceae and thermotolerant coliforms). (Sanders, 2015a, and Sanders, 2016).

The results of this current 2017 annual study indicate that 99% of the corrugated containers sampled had Enterobacteriaceae and thermotolerant coliform loads below acceptable limits. These data show the continued due diligence on the part of individual manufacturers and the corrugated industry to mitigate potential sources of contamination and provide clean containers to grower/shippers.

Sincerely yours,
HALEY & ALDRICH, INC.



Nagi Alla
Technical Specialist



Maryann Sanders
Product Stewardship Leader
Microbiologist

Attachments:

Appendix A: Corrugated Container Sampling and Testing Protocol

Appendix B: 2017 Annual Corrugated Test Data Summary

References

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APPENDIX A

Corrugated Container Sampling and Testing Protocol

Corrugated Container Sampling and Testing Protocol (final)

This protocol provides information and methodologies for the follow-up microbial assessment / cleanliness evaluation of corrugated containers used for the storage and transport of fresh produce. Container sampling will occur at one or more field sites across the United States with laboratory analyses being performed at Primus Laboratories Santa Maria, CA location. The protocol was developed by Haley & Aldrich and Primus Laboratories with input from Trevor Suslow and the Fibre Box Association.

1. Sampling sites

Corrugated containers will be sampled at grower/shipper locations in the United States.

2. Sampling date

Sampling will occur on a single day per site

3. The container selection and sampling process will be conducted following Good Laboratory Practices (GLP). The containers chosen for sampling as well as actual sponge samples will be handled according to standard GLP chain of custody technique to ensure sample integrity and identity.

4. Container selection:

- a. Containers will be selected for sampling from two different corrugated manufacturers (if available). If not available, pallets from a single manufacturer may be sampled. Any pallet wrappings will be inspected for evidence of substantial soil/dust deposits or other foreign materials. If deposits are observed alternative pallets will be selected. If all pallets have external deposits on pallet wrappings a dry-brush procedure will be used to exclude as much as practical before removing the wrapping. Regardless of final condition, wrappings will be removed by technical staff wearing sterile disposable gloves and pulled outward and down from the top rather than lifting over the palletized stack.

After the pallet wrapping is removed, individual containers will be removed from the pallet for microbial sampling, by technical staff wearing new sterile gloves. Samples will be chosen from the bottom, middle and top of the pallet. Chosen containers will be handled by an exterior surface during unstacking and selection. Gloves will be changed as necessary to mitigate cross-contamination.

- b. Forty-eight total containers will be selected for microbial sampling at each site:

- i. Two shipments will be assessed per location.

Note: Where available, the shipments should be from different corrugated manufacturers. If shipments from two different corrugated manufacturers are not available, samples should be taken from two different shipments from the same corrugated manufacturer.

- ii. Twenty-four containers will be sampled per corrugated shipment.

- iii. Four pallets per shipment.
- iv. Six containers per pallet.
- v. Two containers from the top of the pallet, two from the middle and two from the bottom of each pallet.

5. On-site sampling area:

An on-site area to conduct the swabbing of each unfolded corrugated box will be established with effective separation from on-going local operations, de-palletizing and selection activities, and any other potential sources of contamination or sampling interference. The on-site area will be prepared to facilitate proper aseptic technique in sampling/sample handling:

- a. An on-site work bench or table, small folding table, or similar platform will be used for sampling activities.
- b. Prior to sampling, the table surface should be sprayed with a hard-surface sanitizing antimicrobial (bleach and/or 70% alcohol), and/or covered with a new sheet of protective lab paper. This activity will be performed between each pallet being tested.

6. Container Identification:

Each container selected for sampling will be labeled with a unique identifier and include:

- a. Pallet specific prefix to include a corrugated manufacture identification number (to be provided) and a pallet-specific identifier.
- b. Container specific information to include:
 - i. Position on the pallet: T (top), M (middle) or B (bottom).
 - ii. Consecutive number: 1-6.

7. Microbial Sampling

- a. Sampling of the container will be performed using aseptic techniques, and in accordance with Primus Labs SOP 14-20 "Environmental (Sponge) Sampling".
- b. Two microbial sponges will be taken per random containers.
 - i. One sponge will be used to wipe the entire interior bottom surface.
 - ii. One sponge will be used to wipe the interior side and corner surfaces.
- c. Interior dimensions of the container will also be recorded.

8. Sample Transportation

- a. All individual sample bags containing swabs/sponges will be uniquely labeled with permanent ink or bar- code label and placed in a master container per individual corrugated box, pallet location, pallet and delivery.
- b. A "Sample Log Sheet" will be generated for each sampling event, reflecting transit time and receipt at the laboratory. This Sample Log Sheet will be signed by the Sampler and Laboratory Personnel to verify its accuracy.
- c. All samples will be placed in a cooler with blue ice, with the temperature of the cooler and three individual sample bags recorded upon receipt at the laboratory.
- d. If samples are not processed immediately upon receipt at the laboratory, they will be placed in a secure area in a walk-in cooler or refrigerator at 2.0 to 4.0°C. Total time from sampling to processing is not to exceed 24 hours.

9. Microbial Sample Identification

Each microbial sample will be labeled with the container identifier and a notation regarding what part of the container was sampled.

- a. Interior bottom – B.
- b. Interior side/corners – S.

10. Standard Microbial Methods:

- a. All microbial swabs/sponges will be processed in triplicate using standard quantitative microbiological methods for the Enterobacteriaceae and Coliforms. Sponges will be processed in accordance with Primus Labs SOPs 14-05 (Coliforms) and 14-116 (Enterobacteriaceae), respectively.
- b. The number of colony forming units (CFU) for each of the triplicate samples will be recorded.
- c. The average CFU per swab and per surface area swabbed will be generated and recorded.

11. Laboratory data reporting:

Results of standard microbial analyses including individual sample and the sample averages (per sponge and per surface area) will be compiled and submitted as raw tabular data.

APPENDIX B

2017 Annual Corrugated Test Data Summary

2017 Annual Corrugated Test Data - Florida 1, United States

Samples Taken

Number of Containers Sampled	Number of Samples(2/container)
48	96

Total organisms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	41	6	0	1

Total Organisms per Sponge Sample

Number of Sponge Samples (two samples/Container)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	RLU range for Sponge Samples with ≥1000 but <10,000 CFU/Sample
96	88	7	0	1

Coliforms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container
48	42	6

Coliforms per Sponge Sample

Number of Sponge Samples (two samples/Container)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample
96	88	8

Enterobacteriaceae per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	47	0	0	1

Enterobacteriaceae per Sponge Sample

Number of Sponge Samples (two samples/Container)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	Number of Sponge Samples with ≥1000 but <10,000 CFU/Sample
96	95	0	0	1

2017 Annual Corrugated Test Data - California, United States

Samples Taken

Number of Containers Sampled	Number of Samples(2/container)
48	96

Total organisms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	42	5	0	1

Total Organisms per Sponge Sample

Number of Sponge Samples (two samples/Container)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	RLU range for Sponge Samples with ≥1000 but <10,000 CFU/Sample
96	90	5	0	1

Coliforms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	42	5	0	1

Coliforms per Sponge Sample

Number of Sponge Samples (two samples/Container)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
96	90	5	0	1

Enterobacteriaceae per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	48	0	0	0

Enterobacteriaceae per Sponge Sample

Number of Sponge Samples (two samples/Container)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	Number of Sponge Samples with ≥1000 but <10,000 CFU/Sample
96	96	0	0	0

2017 Annual Corrugated Test Data - Florida 2, United States

Samples Taken

Number of Containers Sampled	Number of Samples(2/container)
48	96

Total organisms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	45	3	0	0

Total Organisms per Sponge Sample

Number of Sponge Samples (two samples/Container)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	RLU range for Sponge Samples with ≥1000 but <10,000 CFU/Sample
96	93	3	0	0

Coliforms per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container
48	45	3

Coliforms per Sponge Sample

Number of Sponge Samples (two samples/Container)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample
96	93	3

Enterobacteriaceae per Container

Number of Corrugated Containers Sampled	Number of Corrugated Containers with <10 CFU/Container	Number of Corrugated Containers with ≥10 but <100 CFU/Container	Number of Corrugated Containers with ≥100 but <1000 CFU/Container	Number of Corrugated Containers with ≥1000 but <10,000 CFU/Container
48	48	0	0	0

Enterobacteriaceae per Sponge Sample

Number of Sponge Samples (two samples/Container)	Number of Sponge Samples with <10 CFU/Sample	Number of Sponge Samples with ≥10 but <100 CFU/Sample	Number of Sponge Samples with ≥100 but <1000 CFU/Sample	Number of Sponge Samples with ≥1000 but <10,000 CFU/Sample
96	96	0	0	0