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ADMINISTRATION

FY 2014 – 2016 Microbiological Sampling Assignment
Summary Report: Whole Fresh Avocados

Office of Compliance
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EXECUTIVE SUMMARY

The U.S. Food and Drug Administration (FDA) set out to collect and test whole fresh avocados in 2014 as part of the agency's new proactive and preventive approach to deploying its sampling resources with the ultimate goal of keeping contaminated food from reaching consumers.

The new approach, detailed in the Background section of this report (page 5), centers on the testing of a large number of samples of targeted foods over a relatively short period, about 18 months, to ensure that enough data are available to inform decisions. This approach may help the agency determine if there are common factors – such as origin, variety or season – associated with pathogen findings.

The FDA began the avocado assignment in May 2014 under its new sampling model. The agency collected 1,615 samples of avocados to test to determine the prevalence of *Salmonella* and *Listeria monocytogenes* in the commodity (i.e., the number of samples that tested positive for the pathogen out of the total number of samples tested). The agency collected about 70 percent of its samples from imported avocados and the rest from domestically grown avocados, approximately proportionate to their respective U.S. market shares at the outset of the assignment. The FDA designed its sampling plan such that if contamination of one percent or greater was present in the commodity, the agency would be likely to detect it. The FDA monitored the assignment closely to gather lessons learned and to make changes to its sampling procedures if needed to address trends or food safety issues.

Three months into the assignment, the FDA updated its approach to its *Listeria monocytogenes* testing to focus on the avocado pulp (i.e., the fruit's edible portion), as opposed to its exterior, to better evaluate public health concerns associated with the pathogen, namely the extent to which it may be present in the part of the fruit that people eat. The agency made the change to its test method as part of its assignment monitoring and upon considering that, at the time, no outbreaks or individual illnesses had been linked to *Listeria monocytogenes* on the fruit's exterior (i.e., its skin).

Based on the test results, the FDA found the overall prevalence of *Salmonella* on the samples collected to be 0.74 percent. Breakdowns by avocado origin, variety and season are provided in the Pathogen Findings section of this report (page 10).

Based on the test results, the FDA found the overall prevalence of *Listeria monocytogenes* in the avocado pulp samples to be 0.24 percent and in the avocado skin samples to be 17.73 percent. The report addresses these findings in its various sections and aggregates the discussion of them in a dedicated appendix, in addition to providing breakdowns as described above.

The FDA's assignment was not designed to determine the concentrations of the pathogens in the samples. At low levels of exposure, *Listeria monocytogenes* does not cause severe illness in healthy adults. However, pregnant women, older adults and persons with weakened immune systems (such as organ transplant recipients, or those with diabetes or cancer) are susceptible to small amounts of the pathogen.

As described in the Public Health Impact section of this report (page 17), the FDA conducted [whole genome sequencing](#) (WGS) of all the avocado samples that tested positive for a pathogen under this assignment and subsequently analyzed them to determine whether any of the detected pathogens may be linked to human illness. The FDA found some of the *Listeria monocytogenes* strains isolated from both skin and pulp samples to be highly related to *Listeria monocytogenes* strains found in ill persons. However, the available epidemiological information did not indicate whether the consumption of avocados was implicated in the illnesses.

All three of the *Listeria monocytogenes*-positive samples of avocado pulp were detected by the FDA in imported avocados. In response to those detections, the FDA refused entries of avocados from lots associated with the positive samples and put the responsible firms and their product on [Import Alert 99-23](#), “Detention without Physical Examination of Produce Due to Contamination with Human Pathogens.”

Listeria monocytogenes was detected by the FDA in avocado skin samples from both domestic and imported product. Of the 64 positive avocado skin samples, 33 were from domestic avocados and 31 were from imported avocados. The FDA notified the responsible firms of the test results as soon as the results were available.

Salmonella was detected by the FDA in 12 avocado skin samples from domestically grown product. No samples from imported avocados tested positive for *Salmonella*. In response to the detections, the FDA worked with the firms that owned or released the product to conduct voluntary recalls and followed up with facility inspections.

The findings of this assignment affirm that *Salmonella* may be present on avocados and that *Listeria monocytogenes* may be present on or in the fruit. Moreover, the findings underscore the need for avocado growers to comply with the FDA’s Produce Safety Rule¹ as applicable, and for importers of the fruit to comply with the FDA’s Foreign Supplier Verification Programs Rule² as applicable. Avocados require appropriate protection from environmental pathogens during growing, harvesting, packing and holding, as this study indicates.

Consumers can take steps to reduce possible microbial risks related to avocados. [Foodsafety.gov](#) recommends that consumers “wash all produce thoroughly under running water before eating, cutting or cooking.” The site also advises, “Even if you plan to cut the rind or peel off the produce before eating, it is still important to wash it first so dirt and bacteria aren’t transferred from the knife onto the fruit.” Foodsafety.gov also recommends that consumers scrub firm produce (which includes avocados) with a clean produce brush, and then dry it with a clean cloth towel or paper towel to further reduce bacteria that may be present.

¹ [The Produce Safety Rule](#) establishes, for the first time, science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption.

² [The Foreign Supplier Verification Programs Rule](#) requires that importers perform certain risk-based activities to verify that food imported into the United States has been produced in a manner that meets applicable U.S. safety standards.

Other practices associated with avocado consumption may reduce the risk to consumers as well. Consumers commonly slice avocados and extract the fruit's pulp prior to eating it, discarding the fruit's peel as they would a banana peel or an orange rind. Consumers also typically eat avocados shortly after slicing the fruit as its pulp tends to brown quickly once exposed to oxygen. These practices generally limit the amount of the pathogen, if present, that consumers may be exposed to.

The FDA will continue to evaluate methods to reduce microbial contamination of avocados and avocado products. Consistent with that work, the agency has published [draft guidance](#) to help industry comply with the Produce Safety Rule. The agency likewise will work with industry and other food safety experts on best practices that may be used to reduce contamination of avocado skin with *Listeria monocytogenes*. In addition, the agency began a related large-scale assignment in 2017 to sample processed avocados for *Salmonella* and *Listeria monocytogenes* to determine the extent to which those pathogens may be present in the processed product, the main ingredient in guacamole. The processed avocado assignment is slated to conclude in 2019.

The FDA also will continue to sample whole fresh avocados using its longstanding approach to food sampling, which centers on (but is not limited to) the following criteria:

- A firm has a previous history of unmitigated microbial contamination in the environment (e.g., human illness, recalled or seized product, previous inspectional history, or environmental pathogens without proper corrective actions by the facility), or
- Inspectional observations that warrant collection of samples for microbiological analyses.

Employing the approaches described above, the FDA will sample avocados as warranted and take other steps consistent with its mission to protect consumers.

BACKGROUND

The FDA Food Safety Modernization Act (FSMA) amended the Federal Food, Drug, and Cosmetic Act (FD&C Act) to provide the FDA with additional authority to better prevent problems before they occur. To develop better prevention-based systems, the FDA needs data and other information to help identify hazards that must be addressed and minimized. That is why sampling is an important part of the agency's preventive approach to food safety and why the FDA has developed a new sampling model designed to identify patterns that may help predict and prevent contamination by disease-causing bacteria.

The new model complements the FDA's longstanding approach to sampling, which has employed for-cause and targeted strategies to monitor known hazards. The FDA will continue its longstanding approach to sampling while also undertaking larger, in-depth surveys of products and commodities to help evaluate risks. These large sample collections enable the FDA to determine the prevalence of contamination (i.e., the number of samples that tested positive for a pathogen in proportion to the total number of samples tested for the given commodity) in instances where it does not otherwise have enough data to do so. Such studies also may shed

light on areas of needed focus or issues of food safety that must be addressed or help identify effective industry practices to control or minimize food safety hazards.

As a starting point for the new model – and because it is not feasible to sample every product and/or commodity extensively – an FDA work group developed a system to score commodities based on microbial risk. The group reviewed sampling data collected over a five-year period, systematically considering criteria such as linkage to foodborne illness, consumption of product without a mitigating kill step, and available research studies. Products that scored comparatively high were evaluated by subject matter experts to determine their feasibility as candidates for a large-scale survey and the remaining data needs for the commodity. Following the work group review, the FDA chose to sample whole fresh avocados, raw milk cheese (aged 60 days), and sprouts (seeds, finished product and spent irrigation water) in FY2014-2016, as the first commodities under the new model. This report details the rationale and findings for the sampling and testing of whole fresh avocados.

Why Avocados?

Prior to this assignment, the FDA obtained data pertaining to avocados that indicated a need to better understand the prevalence(s) of *Salmonella* and *Listeria monocytogenes* in this commodity and to identify common factors among contaminated samples, if possible, in an effort to help protect consumers. Specifically, from 2001 to 2013, the FDA collected and tested 429 avocado samples for microbial hazards. Of those 429 samples (most of which were imported, processed product), 77 of them, or 18 percent, were violative, warranting regulatory follow-up, including the addition of firms to import alerts and/or recalls. Also, avocado products were associated with four recalls from 2003 to 2011 due to the presence of foodborne pathogens: *Salmonella* in fresh avocado, in one instance, and *Listeria monocytogenes* in frozen/processed products, in three instances. The fruit can be a favorable growth medium for pathogens given its high lipid and moisture content, low carbohydrates, and non-acidic pH level.

Additionally, a 2013 study³ by the Centers for Disease Control and Prevention (CDC) found that *Salmonella* contamination of salsa or guacamole had resulted in 26 outbreaks and 1,872 illnesses during the 35-year period examined. The study did not directly implicate whole fresh avocados in an outbreak of foodborne illness, but avocado is the main ingredient in guacamole and may be used in salsa.

Given that whole fresh avocados represent the beginning of the supply chain for guacamole and other products made from avocados – and because the FDA did not have reliable estimates of the prevalence(s) of *Salmonella* and *Listeria monocytogenes* associated with whole fresh avocados – the agency decided the commodity would be a good starting point in its surveillance sampling of avocados and avocado products.

Avocado Production

³ Kendall, M., Mody, R., Mahon, B., Doyle, M., Herman, K. & Tauxe, R. (2013). [Emergence of salsa and guacamole as frequent vehicles of foodborne disease outbreaks in the U.S., 1973–2008](#). *Foodborne Pathogens and Disease*.

Avocados are cultivated in tropical and subtropical climates. Major commercial producers include Colombia, the Dominican Republic, Mexico, Peru and the United States.

According to 2012 data from the USDA Agricultural Marketing Service, domestically grown avocados made up about 32 percent of the U.S. market and imported avocados made up about 68 percent of the U.S. market leading up to the start of the FDA's sampling assignment.⁴ Most of the avocados produced in the United States are grown in California and Florida; these two states' avocado production accounted for about 27 percent and 4 percent of the total U.S. market share, respectively, in 2012. Mexico is the biggest exporter of avocados to the United States. Avocados harvested in Mexico accounted for nearly 60 percent of the U.S. market in 2012.

Most of the avocados consumed in the United States are the Hass variety. Hass avocados have a distinctive, pebbled surface that turns from a green color to a purplish-black color when ripe. California produces the vast majority of the Hass avocados grown in the United States, with peak production in the spring and summer. The Hass variety also is the primary avocado variety imported by the United States from Latin America, which produces Hass avocados year-round.

The other category of avocado eaten in the United States is commonly referred to as 'green skin,' a broad grouping that includes many varieties. Green-skin avocados can be larger than the Haas variety and have a smoother skin that is comparatively easy to peel. Most of the avocados grown in Florida are green skin. The leading varieties cultivated in Florida vary from year to year, but include Choquette, Hall, Lula and Pollock.⁵ Florida avocado production occurs primarily in the spring and summer.

OBJECTIVES

The objectives of the FDA's FY2014-2016 avocado sampling assignment were:

- To determine the prevalence(s) of *Salmonella* and *Listeria monocytogenes* associated with whole fresh avocados.
- To determine if there are common factors associated with positive findings (such as avocado origin, variety, or season).
- To take appropriate regulatory action in response to violations.

SAMPLE COLLECTION

The FDA collected 1,615 avocado samples from May 2014 through November 2015 for purposes of this assignment. The samples were collected in approximate proportion to their U.S. market share based on origin (i.e., domestic vs. import).

⁴ The FDA designed its sampling assignment in 2013 using [USDA data on "Fresh Fruit and Vegetable Shipments" from 2012](#), the most current at the time. Since then, the share of imported avocados on the U.S. market has grown to about 80 percent, according to [2016 USDA data](#).

⁵ Morton, J. (1987). Lauraceae: Avocado and Related Species. *Fruits of Warm Climates*. (p. 91–102). Miami, FL.

Agency field staff collected samples one at a time from individual lots. In cases where the collection site(s) featured multiple lots, the field staff collected one sample from each lot. This approach, which carefully avoided commingling samples from different lots, was designed to help the agency identify the source of the contamination, if present, and to facilitate targeted removal of adulterated product from the food supply.

As directed by the assignment, the field staff collected samples to ensure that they were representative of the lot and to allow the agency to obtain cross sections of operation types (such as packinghouses, distribution centers or retail stockrooms) for the domestic samples and countries of origin for the import samples. Additionally, the FDA collected the samples over 18 months, enabling the capture of seasonal data as well.

Domestic Samples

The FDA collected 478 samples of domestically produced avocados (29.6% of the total under this assignment). Agency field staff collected these samples from growers, distribution centers/warehouses, packinghouses, and retail stockrooms, which are settings where the fruit was not yet available to consumers (Table 1).

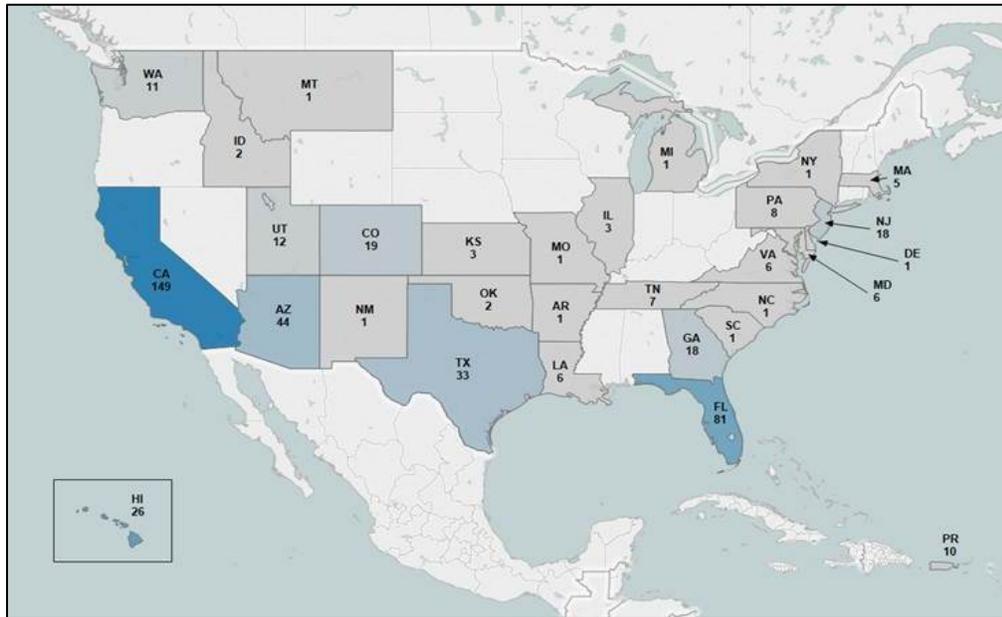
Table 1: Domestic Sample Collection Sites

Collection Site	Number of Samples Collected	Percentage of Domestic Samples	Percentage of All Samples
Retail Stockroom	219	45.8%	13.6%
Distribution Center/Warehouse	201	42%	12.4%
Grower	30	6.3%	1.9%
Packinghouse	28	5.9%	1.7%
Total	478	100%	29.6%

Sample Collection by U.S. States

The FDA collected samples from 29 U.S. states and Puerto Rico. The agency took into account production statistics and the availability of product on the market to inform sample allocation. The agency’s preference was to collect samples as early in the supply chain as feasible, and thus it allocated the greatest numbers of samples to states with the highest avocado production, namely California and Florida (Figure 1).

Figure 1: Sample Collection by U.S. States



This map shows the number(s) of domestic samples collected by state. The darker the color, the greater the number of samples collected.

Import Samples

The FDA collected 1,137 samples of imported avocados (70.4% of the total under this assignment). Most of these avocados were grown in Mexico (86%), followed by Peru (9%), the Dominican Republic (3%), Chile (2%), and Grenada (less than 1%).

FDA field staff used two approaches in collecting import samples: port-of-entry and domestic import (DI) collection. Of the 1,137 import samples, 360 samples (31.7%) were collected at ports of entry or other locations where the product was being held prior to its release into domestic commerce.

Additionally, 777 samples (68.3%) were collected as DI samples and counted toward the import sample total. DI samples are collected after the imported product is released into domestic commerce. They often are collected near their port of entry, usually at a warehouse, but may also be collected from retail stock rooms. DI sampling allows for imported products to be released and sold domestically or to undergo processing. For purposes of this report, DI samples are included in the import sample data (Table 2).

Table 2: Import Sample Collection Sites

Collection Site	Number of Samples Collected	Percentage of Import Samples	Percentage of All Samples
Ports of Entry	360	31.7%	22.3%
Domestic Import (DI)	777	68.3%	48.1%
Total	1,137	100%	70.4%

Sample Collection: Hass vs. Green Skin

Given that most of the avocados consumed in the United States are the Haas variety, the FDA collected more Hass avocados than green-skin avocados, with the Hass variety accounting for 69.3 percent of all samples collected, versus 30.7 percent for green skin (Table 3).

Table 3: Hass vs. Green-Skin Samples

Variety/Type	Number of Samples Collected	Percentage of Samples Collected
Hass	1,120	69.3%
Green Skin	495	30.7%
Total	1,615	100%

Hass avocados likewise accounted for 80 percent of the import samples collected, versus 20 percent for green-skin import samples. With respect to the domestic collection, Hass avocados accounted for 44 percent of the samples collected, versus 56 percent for green-skin samples.

Sample Composition

Each sample consisted of 20 subsamples. Each subsample consisted of an individual avocado, with 10 subsamples tested for *Salmonella* and 10 subsamples tested for *Listeria monocytogenes*. Per the assignment testing scheme, if one subsample tested positive for the target pathogen, the sample was deemed to be positive for the organism.

This approach – the collection and testing of samples composed of multiple subsamples – is more reflective of actual conditions, and it increases the odds of finding pathogens if present, given that microbial hazards may not be uniformly present.

The field staff collected all samples aseptically to prevent contamination during the collection process. The FDA’s aseptic sampling methods, which entail the use of sterile implements and containers, and prescribed collection procedures, are published in the agency’s [Investigations Operations Manual](#) (Section 4.3.6).

PATHOGEN FINDINGS

This section provides the prevalence of *Salmonella* on avocados as well as the prevalence of *Listeria monocytogenes* on the fruit and in its pulp, based on the FDA’s test results. The test methods the FDA used are described in [Appendix A](#).

Pathogen Findings: *Salmonella*⁶

⁶ The FDA tested the fruit’s exterior for *Salmonella*. The agency did not update its approach to its *Salmonella* testing as it did with respect to its *Listeria monocytogenes* testing because the low infectious dose for *Salmonella* even in populations not considered especially vulnerable is documented and widely known, and thus the method was adequate for its intended purpose.

The FDA detected *Salmonella* in 12 of the 1,615 samples, a *Salmonella* prevalence of 0.74 percent. The pathogen was present in 11 samples of green-skin avocados and one sample of the Hass variety. None of the import samples tested positive for *Salmonella* (Table 4). A list of the *Salmonella*-positive samples by serotype is provided in [Appendix B](#).

Table 4: *Salmonella* Findings

Variety/Type	Origin	Number of Samples	Number of Samples Positive for <i>Salmonella</i>
Hass	Domestic	212	1
	Import	908	0
Green Skin	Domestic	266	11
	Import	229	0
Total	NA	1,615	12

Pathogen Findings: *Listeria monocytogenes* (Pulp Samples)

The FDA tested 1,254 avocado pulp samples for the presence of *Listeria monocytogenes* and detected the pathogen in three of them, a *Listeria monocytogenes* prevalence of 0.24 percent. The agency detected the pathogen in two samples of Hass pulp and one sample of green-skin pulp. None of the pulp samples of U.S.-produced avocados tested positive for the pathogen (Table 5).

Table 5: *Listeria monocytogenes* (Pulp Samples)

Variety/Type	Origin	Number of Samples	Number of Pulp Samples Positive for <i>Lm</i> *
Hass	Domestic	142	0
	Import	739	2
Green Skin	Domestic	222	0
	Import	151	1
Total	NA	1,254	3

* *Listeria monocytogenes*

Pathogen Findings: *Listeria monocytogenes* (Skin Samples)

Prior to changing its test method to focus on the fruit’s edible portion, the FDA tested 361 avocado skin samples for the presence of *Listeria monocytogenes* and detected the pathogen in 64 of them, a *Listeria monocytogenes* prevalence of 17.73 percent (Table 6). The FDA discusses this finding in the Statistical Evaluation section of this report (page 14.)

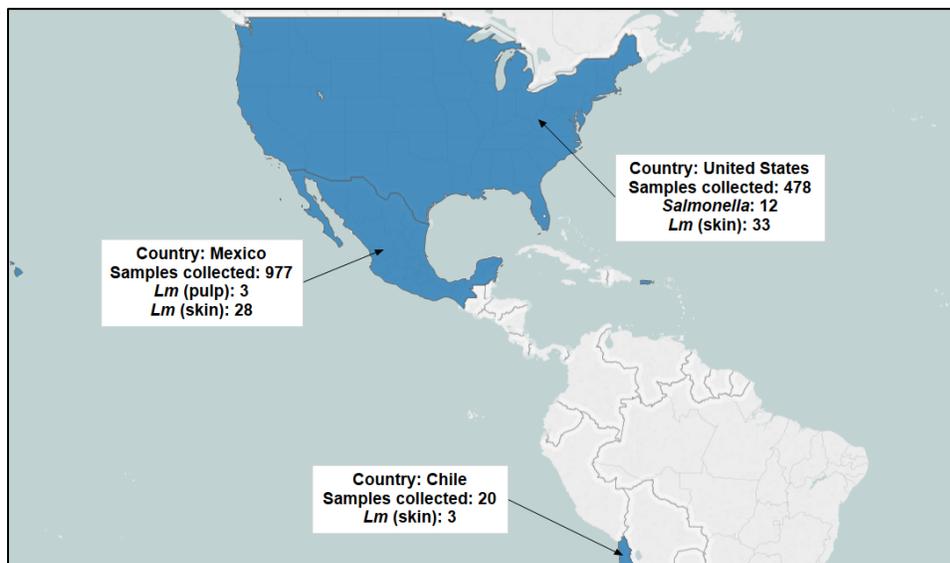
Table 6: *Listeria monocytogenes* (Skin Samples)

Variety/Type	Origin	Number of Samples	Number of Skin Samples Positive for <i>Lm</i>
Hass	Domestic	70	26
	Import	169	21
Green Skin	Domestic	44	7
	Import	78	10
Total	NA	361	64

Pathogen Findings: By Country of Origin

The FDA obtained country-of-origin information for all samples collected (Figure 2). The 12 samples that tested positive for *Salmonella* were collected by the FDA from domestically grown avocados. The three import samples of avocado pulp that tested positive for *Listeria monocytogenes* were collected by the agency from avocados grown in Mexico. The 64 avocado skin samples that tested positive for *Listeria monocytogenes* were collected by the agency from avocados grown in the United States (33), Mexico (28), and Chile (3).

Figure 2: Pathogen Findings by Country of Origin

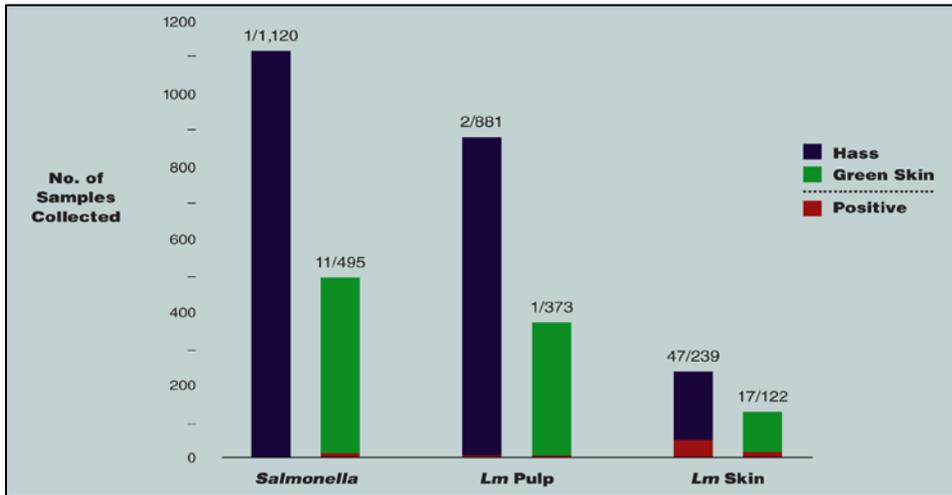


This map displays pathogen findings by country or territory of origin with the sources rendered in blue. The Caribbean island rendered in blue is Puerto Rico, a U.S. territory. For each collection total, the FDA tested some samples for *Lm* using the skin method and others using the pulp method, as noted earlier in this section of the report.

Pathogen Findings: By Hass vs. Green Skin

The FDA detected *Salmonella* in one sample of a Hass avocado versus 11 samples of green-skin avocados. The FDA detected *Listeria monocytogenes* in two pulp samples of Hass avocados versus one pulp sample of a green-skin avocado. The agency detected *Listeria monocytogenes* in 47 skin samples of Hass avocados versus 17 skin samples of green-skin avocados. Once again, the FDA discusses the prevalence of *Listeria monocytogenes* in the skin samples in the Statistical Evaluation section of this report.

Figure 3: Pathogen Findings by Hass vs. Green Skin

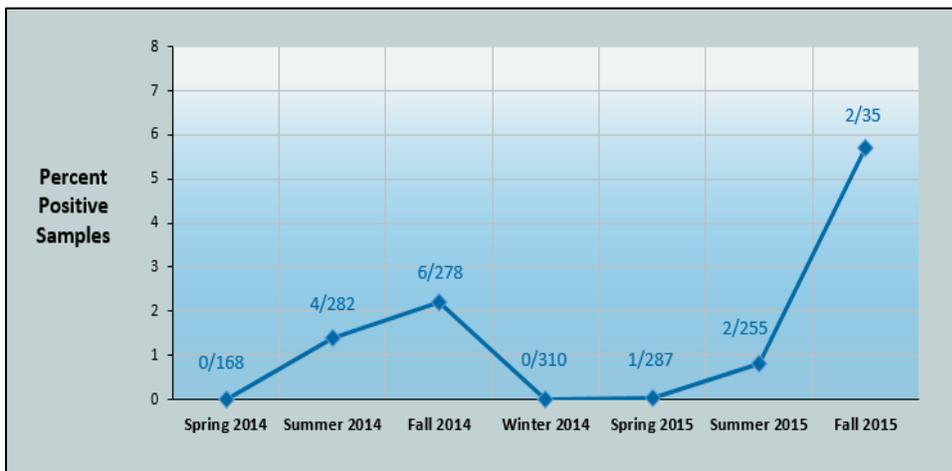


This chart shows the pathogen findings by Hass vs. green-skin avocados for each target pathogen. The fraction at the top of each bar reports the number of samples that tested positive out of the number of samples collected (of the type indicated).

Pathogen Findings: By Season

All the samples that tested positive for *Salmonella* and all the pulp samples that tested positive for *Listeria monocytogenes* were collected by the FDA in the summer or fall of 2014 and 2015, except for a single sample collected in the spring of 2015 (Figure 4). The agency has no seasonal data to report with respect to the avocado skin samples tested for *Listeria monocytogenes* because that testing occurred only during the first three months of the assignment.

Figure 4: Pathogen Findings by Season



The fractions in the graph's plot area report the number(s) of samples that tested positive for a pathogen out of the total number of samples collected for the indicated season. The date ranges defining the seasons may be obtained at the [U.S. Naval Observatory](http://www.navy.mil/navalobservatory) site.

Pathogen Findings: By 'Repeat Violation' Firms (De-Identified), and Related Actions

For purposes of this subsection, 'repeat violation' firms are defined as firms responsible for one or more positive samples during each of two or more sample collections. Of the eight domestic firms found to be responsible for *Salmonella* positives, the FDA detected multiple samples positive for the pathogen at two firms in the Southeast (Table 7).

Table 7: Pathogen Findings by ‘Repeat Violation’ Firms (De-Identified), and Related Actions

Firm ID	Firm Type	Firm Location	Collection Date	Pathogen	Action
A	Grower/ Repacker	Southeast	7/2014	<i>Salmonella</i>	Class 1 Recall
			9/2014	<i>Salmonella</i>	Follow up Inspection Conducted *
B	Grower	Southeast	10/2014	<i>Salmonella</i>	Class 1 Recall
			10/2014	<i>Salmonella</i>	Class 1 Recall
			10/2015	<i>Salmonella</i>	Follow up Inspection Conducted *

* The FDA classified the outcomes of the follow-up inspections as “NAI” (No Action Indicated), which suggests that the firms took effective corrective measures after their initial inspection.

STATISTICAL EVALUATION

The FDA estimated the overall prevalence of *Salmonella* on avocados as well as the overall prevalence of *Listeria monocytogenes* in the fruit’s pulp and on its skin, based on the data collected under this assignment. Where possible, the agency also estimated the prevalence of these pathogens in the commodity by origin, variety and season.

Overall Prevalence

The FDA found the prevalence of *Salmonella* on avocados to be 0.74 percent with a 95 percent confidence interval of 0.38 percent to 1.29 percent (Table 8).

Table 8: *Salmonella* Findings

No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1,615	12	0.74%	0.38%	1.29%

The FDA found the prevalence of *Listeria monocytogenes* in the avocado pulp to be 0.24 percent with a 95 percent confidence interval of 0.05 percent to 0.70 percent. The agency found the prevalence of *Listeria monocytogenes* on the avocado skin to be 17.73 percent with a 95 percent confidence interval of 13.93 percent to 22.07 percent (Table 9).

Table 9: *Listeria monocytogenes* Findings

Test Method	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Pulp Method	1,254	3	0.24%	0.05%	0.70%
Skin Method	361	64	17.73%	13.93%	22.07%

The FDA cautions against making inferences about the estimated 17.73 percent prevalence of *Listeria monocytogenes* on the avocado skin based solely on the analytical results of this study. Of note, it is not clear to what extent this prevalence may increase the risk to consumers. The FDA’s assignment was not designed to determine the concentrations of the pathogens in the samples. At low levels of exposure, *Listeria monocytogenes* does not cause severe illness in

healthy adults. However, pregnant women, older adults and persons with weakened immune systems (such as organ transplant recipients, or those with diabetes or cancer) are susceptible to small amounts of the pathogen.

As a related matter, common consumer practices associated with avocado consumption may reduce the risk of illness. Consumers commonly slice avocados and extract the fruit’s pulp prior to eating it, discarding the fruit’s peel as they would a banana peel or an orange rind. Consumers also typically eat avocados shortly after slicing the fruit as its pulp tends to brown quickly once exposed to oxygen (a process called enzymatic browning). These practices generally limit the amount of the pathogen, if present, that consumers may be exposed to.

Origin: Domestic vs. Import

The FDA did not design its sample collection to compare bacterial prevalence by country of origin (i.e., domestic versus import) and therefore cautions against making inferences based solely on the analytical results that follow, which are provided for informational purposes. The prevalence of *Salmonella* in domestically grown avocados was higher than in imported avocados, based on the data collected (Table 10). A Fisher’s Exact Test shows that the difference is statistically significant ($p < 0.0001$). The FDA is not aware of other bacterial surveillance sampling studies of avocados that may be considered for comparison. The difference in the *Salmonella* prevalence between domestically produced and imported avocados may be attributable to one or more risk factors that are associated with country of origin, or may simply reflect chance given that these are subgroup findings. Further study would be required to both replicate the finding and determine what factor(s) may have contributed to the observed difference.

Table 10: Pathogen (*Salmonella*) Findings: Domestic vs. Import

Origin	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	478	12	2.51%	1.30%	4.34%
Import	1,137	0	0.00%	0.00%	0.32%

The prevalence of *Listeria monocytogenes* in the skin samples of domestically produced avocados was higher than in the skin samples of imported avocados, based on the data collected (Table 11). A Fisher’s Exact Test shows that the difference is statistically significant ($p < 0.001$). Further study would be required to replicate this subgroup finding, and to determine what factor(s) may have contributed to the observed difference.

Table 11: Pathogen (*Listeria monocytogenes*) Findings for Skin Samples: Domestic vs. Import

Origin	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	114	33	28.95%	20.84%	38.19%
Import	247	31	12.55%	8.69%	17.34%

There was no significant difference in the prevalence of *Listeria monocytogenes* in the avocado pulp samples based on origin (Table 12).

Table 12: Pathogen (*Listeria monocytogenes*) Findings for Pulp Samples: Domestic vs. Import

Origin	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Domestic	364	0	0.00%	0.00%	1.01%
Import	890	3	0.34%	0.07%	0.98%

Hass vs. Green Skin

The FDA did not design its sampling to compare bacterial prevalence based on Hass vs. green skin, and so cautions against making inferences based solely on the analytical results that follow, which are provided for informational purposes. A comparison of the *Salmonella* prevalence (Table 13) in the samples of green-skin versus Hass avocados using a Fisher’s Exact Test shows that the *Salmonella* prevalence in green skin avocados was significantly higher than in Hass avocados ($p < 0.0001$). Further study would be required to replicate this subgroup finding, and to determine what factor(s) may have contributed to the observed difference.

Table 13: Pathogen (*Salmonella*) Findings: Hass vs. Green Skin

Variety/Type	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Hass	1,120	1	0.09%	0.00%	0.50%
Green Skin	495	11	2.22%	1.11%	3.94%

There was no significant difference in the prevalence of *Listeria monocytogenes* by Hass vs. green skin, for either the pulp or skin samples (Table 14).

Table 14: Pathogen (*Listeria monocytogenes*) Findings: Hass vs. Green Skin

Test Method	Variety/Type	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Skin Method	Hass	239	47	19.67%	14.82%	25.28%
	Green Skin	122	17	13.93%	8.33%	21.37%
Pulp Method	Hass	881	2	0.23%	0.03%	0.82%
	Green Skin	373	1	0.27%	0.01%	1.48%

Season

The FDA did not design its sampling to compare bacterial prevalence based on season and therefore cautions against making inferences based solely on the analytical results that follow, which are provided for informational purposes. The *Salmonella* prevalence in the avocado samples (Table 15) was significantly higher in the summer and fall than in the winter and spring ($p < 0.01$). The higher prevalence in the summer and fall coincide with the seasons of greatest production volume in the United States. With respect to *Listeria monocytogenes*, there was no significant difference in the prevalence of the pathogen in the pulp samples by season. The agency has no seasonal findings to report with respect to the skin samples tested for *Listeria*

monocytogenes, again because that testing occurred only during the first three months of the assignment.

Table 15: Pathogen (*Salmonella*) Findings by Season

Season	No. of Samples Collected	No. of Samples Positive	Estimated Prevalence	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Spring	455	0	0.00%	0.00%	0.81%
Summer	537	5	0.93%	0.30%	2.16%
Fall	313	7	2.24%	0.90%	4.55%
Winter	310	0	0.00%	0.00%	1.18%

REGULATORY APPROACH

The Federal Food, Drug, and Cosmetic Act (FD&C Act) authorizes the FDA to take regulatory action regarding adulterated food. Regulatory tools at the agency’s disposal include warning letters, import alerts, import refusals, administrative detentions, seizures, injunctions, suspension of registration, and mandatory recalls (if a firm does not conduct an adequate voluntary recall).

Avocados that test positive for *Salmonella* are adulterated under Section 402(a)(1) of the FD&C Act in that they bear or contain a poisonous or deleterious substance which may render them injurious to health. Such foods may be subject to regulatory action, as detailed below.

Avocados that test positive for *Listeria monocytogenes*, based on testing of the fruit’s pulp, are adulterated under Section 402(a)(1) of the FD&C Act in that they bear or contain a poisonous or deleterious substance which may render them injurious to health. Such foods may be subject to regulatory action, as detailed below.

PUBLIC HEALTH IMPACT

Consistent with its mission to protect consumers, the FDA analyzed the pathogens that it detected in the avocado samples to identify their genetic patterns and determine whether those pathogens may be linked to human illness.

The FDA and state laboratories on contract employed two technologies to conduct the genetic analysis, [pulsed-field gel electrophoresis](#) (PFGE) and [whole genome sequencing](#) (WGS), both commonly used to identify disease-causing microorganisms. Explanations of these technologies and the analytical results for each pathogen are provided in [Appendix C](#).

The FDA’s genetic analysis showed that two of the avocado pulp samples had produced *Listeria monocytogenes* isolates that were highly related to three isolates from ill persons. However, the available epidemiological information did not indicate whether the consumption of avocados was implicated in the illnesses.

The analysis also showed that 31 of the avocado skin samples had produced *Listeria monocytogenes* isolates that were highly related to 142 isolates from ill persons. Again, however, the available epidemiological information did not indicate whether the consumption of avocados

was implicated in the illnesses.⁷ Furthermore, most of the clinical isolates were highly related to isolates from other food or environmental sources, precluding attribution.

With respect to the *Salmonella*-positive samples, the CDC and state public health laboratories had not yet begun to routinely sequence *Salmonella* isolates from ill persons during the period when this assignment was conducted, and so the FDA could not compare the isolates from the avocado samples to isolates from clinical samples.

Of note in this analysis is the increasing importance of WGS, which enables the FDA to differentiate between organisms with a precision that other technologies do not allow. For that reason, the FDA will continue to expand its WGS efforts, gradually moving away from lower resolution approaches.

It is important to note that not all consumers exposed to contaminated foods become ill. Additionally, not all persons who become ill seek care in the public health system, and among those who obtain care, not all receive microbial testing. Regardless of whether or not a link to reported human illness can be demonstrated, removal of contaminated foods from the marketplace serves to prevent potential human illnesses.

In conducting this assignment, when the FDA detected *Salmonella* in a domestic sample, the agency worked with the firm that owned the affected avocados to conduct a voluntary recall as indicated, or carried out a follow-up inspection, and in some cases did both.⁸ When the FDA detected a *Listeria-monocytogenes* contaminated sample at a port of entry, the agency refused entries associated with the positive sample and placed the responsible firm and product on Import Alert 99-23, thereby requiring additional controls for future entries.

Avocados are a raw agricultural commodity, and as such the samples collected and tested by the FDA were not packaged. That being the case, when the agency detected a pathogen in a sample, the available information did not definitively identify the point of origin of the contamination, as it could have occurred at any of several junctures in the distribution chain.

Finally, this sampling assignment prompted the FDA to undertake a separate but related laboratory study with the ultimate goal of helping to protect consumers from microbial hazards associated with avocados. The related study, begun in 2014, sought to determine whether *Listeria monocytogenes* can penetrate the skin of an avocado or pass through its stem scar such that it may contaminate the fruit's pulp. The researchers artificially inoculated avocados using *Listeria monocytogenes* from the culture collection at the FDA's Center for Food Safety and Applied Nutrition. The researchers found that *Listeria monocytogenes* was not likely to penetrate the intact avocado skin, but internalization can occur through the avocado stem scar, via the

⁷ To improve investigations of foodborne illness, the FDA asked the CDC to include avocados on its National Hypothesis Generating Questionnaire, administered to patients who are part of illness clusters, to help them recall foods they may have eaten. The CDC added avocados (in any form) to its questionnaire in 2017.

⁸ Recalls were conducted in all instances as indicated. In one case, for example, the operators of a packinghouse that had not distributed the affected product voluntarily destroyed the lot of avocados associated with the sample that tested positive, and thus there was no product to recall.

fruit's vascular system. The *Journal of Food Protection* published the study⁹ in 2016, and the agency microbiologists who conducted the research presented their findings at a produce industry conference.

CONCLUSION AND NEXT STEPS

The FDA accomplished the objectives that it set at the outset of this assignment, the most fundamental being to determine the prevalence(s) of *Salmonella* and *Listeria monocytogenes* associated with whole fresh avocados.

The FDA found the prevalence of *Salmonella* on avocados to be 0.74 percent with a 95 percent confidence interval of 0.38 percent to 1.29 percent. The FDA found the prevalence of *Listeria monocytogenes* in the avocado pulp to be 0.24 percent with a 95 percent confidence interval of 0.05 percent to 0.70 percent. The agency found the prevalence of *Listeria monocytogenes* on the fruit's skin to be 17.73 percent with a 95 percent confidence interval of 13.93 percent to 22.07 percent.

As explained in the Statistical Evaluation section of this report (page 13), the FDA cautions against making inferences about the *Listeria monocytogenes* prevalence associated with the exterior of the fruit based solely on the analytical results from this study. Of note, it is not clear to what extent this prevalence may increase the risk to consumers. The FDA's study was not designed to determine the concentrations of the pathogens in the samples. At low levels of exposure, *Listeria monocytogenes* does not cause severe illness in healthy adults, but pregnant women, older adults and persons with weakened immune systems (such as organ transplant recipients, or those with diabetes or cancer) are susceptible to small amounts of the pathogen. At the same time, common consumer practices associated with avocado consumption may reduce the risk of illness by limiting the amount of the pathogen, if present, that consumers may be exposed to.

Due to resource constraints and anticipated challenges with sample availability, the FDA limited the primary objective of its study to determining the overall prevalence(s) of the target pathogens associated with avocados. Despite the limitations, the FDA also evaluated the data preliminarily and throughout the assignment for signals (variations in prevalence by origin, variety and season) to determine whether more targeted sampling or further study may be warranted. The agency concluded that its next step should be to sample processed avocados for *Salmonella* and *Listeria monocytogenes* to determine the extent to which those pathogens may be present in the processed product, and thus began an assignment to do so in 2017. That assignment is slated for completion in 2019.

The FDA performed statistical tests where possible to determine whether the *Salmonella* prevalence and the *Listeria monocytogenes* prevalence(s) for the skin and pulp samples differed statistically by origin, variety or season. Again, the agency did not design its sample collection to compare bacterial prevalence by observed subgroups findings, which may have occurred by

⁹ Chen, Y., Evans, P., Hammack, T.S., Brown, E.W. & Macarisin, D. [Internalization of *Listeria monocytogenes* in Whole Avocado](#). *Journal of Food Protection*, Vol. 79, No. 8, 2016, Pages 1440–1445.

chance, and so cautions against making inferences based solely on the following findings, which are provided for informational purposes.

Origin (i.e., Domestic vs. Import): The prevalence of *Salmonella* in the samples of domestically produced avocados was significantly higher than in the samples of imported avocados ($p < 0.0001$). The prevalence of *Listeria monocytogenes* in the skin samples of domestically produced avocados was significantly higher than in the skin samples of imported avocados ($p < 0.001$).

Variety (i.e., Hass vs. Green Skin): The prevalence of *Salmonella* in the samples of green-skin avocados was significantly higher than in the samples of Hass avocados ($p < 0.0001$).

Season: The prevalence of *Salmonella* in the avocado samples was significantly higher in the summer and fall than in the winter and spring ($p < 0.01$).

The FDA took regulatory action as warranted in response to each violative sample. To address the violative domestic samples, the agency worked with the responsible firms to conduct recalls and followed up with inspections of growers and packinghouses to ascertain their adherence to recommended good agricultural and manufacturing practices. The FDA also notified the relevant states of each violative domestic sample as products grown and sold in the same state fall under that state's jurisdiction. To address the violative samples of imported avocados, the FDA refused entries in lots associated with each positive sample and placed the responsible firms and their product on Import Alert 99-23.

The findings of this assignment affirm that *Salmonella* may be present on avocados and that *Listeria monocytogenes* may be present on or in the fruit. Moreover, the findings underscore the need for avocado growers to comply with the FDA's Produce Safety Rule as applicable, and for importers of the fruit to comply with the FDA's Foreign Supplier Verification Programs (FSVP) Rule as applicable.

The FDA has published [draft guidance](#) to help industry comply with the Produce Safety Rule. The rule's compliance dates vary depending on farm size, type, and whether the farm may be eligible for a qualified exemption. The agency has posted updated information on the rule's [compliance dates](#).

The FDA has published [draft guidance](#) to help industry comply with the FSVP Rule. Compliance dates vary based on the size of the foreign supplier, the nature of the importer, and whether the foreign supplier must comply with the requirements of other FSMA rules. Updated information on the FSVP rule's [compliance dates](#) is posted at FDA.Gov.

The FDA will continue to evaluate methods to reduce microbial contamination of avocados and avocado products. The presence of harmful bacteria in the commodity remains a concern to the FDA in view of this assignment's findings and the history of recalls associated with the fruit. This includes working with industry and other food safety experts on best practices that may be used to reduce contamination of avocado skin with *Listeria monocytogenes*. The agency has

initiated a surveillance assignment to sample processed avocados for *Salmonella* and *Listeria monocytogenes*. The agency updates the assignment preliminary results quarterly on [FDA.Gov](https://www.fda.gov).

In addition to the efforts described above, the FDA will continue to sample avocados using its for-cause and targeted strategies to monitor known hazards, and as further warranted, consistent with its mission to protect consumers.

Analysts tested the samples using aseptic methods specific to each pathogen, as follows:

Salmonella

A soak method was used to detect *Salmonella* contamination on the surface of the avocado. Analysts soaked whole avocados in a pre-enrichment broth of buffered peptone water (without blending) and incubated them for 24 hours at 35 degrees Celsius. The analysts then used VIDAS Easy (2001.03) or VIDAS SLM (OMA 996.08 or 2004.03) immunoassay instrument(s) to detect *Salmonella*. The FDA's [Bacteriological Analytical Manual](#) culture method for *Salmonella* was then used to confirm the VIDAS results.

Listeria monocytogenes

The FDA developed an initial method (i.e., a soak method) to test the avocados for *Listeria monocytogenes* and a second method once the FDA decided to focus on the edible portion of the fruit. It was not possible to test the same avocado using both methods because the test preparations were not compatible.

Skin Sample Method. A soak method was used to detect *Listeria monocytogenes* contamination on the surface of the avocado. Analysts soaked the fruit in a *Listeria monocytogenes* enrichment broth and subsequently analyzed the skin according to VIDAS *Listeria* (999.06 or 2004.06) immunoassays or the method described in the chapter on *Listeria monocytogenes* in the FDA's [Bacteriological Analytical Manual](#) to detect *Listeria monocytogenes*. Each method employed its own enrichment scheme. The FDA's [Bacteriological Analytical Manual](#) culture method for *Listeria monocytogenes* was then used to confirm the VIDAS results.

Pulp Sample Method. Analysts disinfected the fruit's exterior then aseptically cut it to collect the edible portion, specifically underneath the stem scar. They then incubated the pulp in a *Listeria* enrichment broth and analyzed the pulp using VIDAS *Listeria* (999.06 or 2004.06) immunoassays or the method described in the chapter on *Listeria monocytogenes* in the FDA's [Bacteriological Analytical Manual](#) to detect *Listeria monocytogenes*. Each method employed its own enrichment scheme. The FDA's [Bacteriological Analytical Manual](#) culture method for *Listeria monocytogenes* was then used to confirm the VIDAS results.

APPENDIX B: SALMONELLA-POSITIVE SAMPLES BY SEROTYPE

Organized by sample ID, the table below provides the serotype of each strain of *Salmonella* detected. Some samples produced multiple isolates. Antigenic formulas are provided in cases where the FDA observed unnamed serotypes.

Sample ID	Isolate No. 1	Isolate No. 2	Isolate No. 3	Isolate No. 4	Isolate No. 5
844352	O-1,3,19z4z23-	IV 50z4z23-	IV 50z4z23-	IV 50z4z23-	
819244	IV 48:z4,z32:-	IV 48:z4,z32:-	IV 48:z4,z32:-		
865619	Saintpaul				
864954	Rissen	Rubislaw	Rubislaw	Rissen	
838211	Aberdeen	Aberdeen			
838213	Aberdeen	Aberdeen	Aberdeen	Aberdeen	Aberdeen
874038	V 48:z4,z32-	V 48:z4,z32-	V 48:z4,z32-		
883888	IV 44:z4,z23-				
867060	IV 50:z4,z23-				
854240	IV 50:z4,z23-	IV 50:z4,z23-			
917530	Muenchen				
923713	Rubislaw	Rubislaw	Rubislaw	Rubislaw	

APPENDIX C: GENETIC EVALUATION

This section describes the FDA's further analysis of the samples that tested positive for pathogens – and their comparison to clinical isolates – to determine whether those pathogens, or pathogens of the same species, may have caused foodborne illness.

In carrying out its further analysis, the FDA employed two technologies, pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS), which are commonly used to identify microorganisms. Information on each technology is provided below.

Pulsed-Field Gel Electrophoresis Evaluation

PFGE is a laboratory technique used to separate DNA fragments for purposes of bacterial subtyping. Analysts take bacterial cells from an agar plate, treat them with specific enzymes to cut them based on their DNA sequence, mix them with an agarose gel and subject them to an electric field that separates the DNA fragments by size. The analysts then stain the gel so they can view the DNA under ultraviolet light, photograph it with a digital camera and analyze its pattern.

Following the PFGE analysis, the FDA queried the [PulseNet USA](#) database, the nation's established repository of PFGE test results, to see whether any of the PFGE patterns associated with the samples that tested positive for a pathogen under this assignment matched any of the PFGE patterns reported previously in association with ill individuals.

Importantly, while the FDA uses indistinguishable PFGE patterns to cluster genetically similar bacterial strains and investigate potential foodborne illness outbreaks, other information, usually food histories from ill persons and isolates from the site where the food was grown or processed, are needed to determine that an adulterated food caused an illness, or multiple illnesses in the case of an outbreak.

Salmonella: The FDA's evaluation found that most of the *Salmonella* strains from the avocado samples were indistinguishable from clinical isolates in the PulseNet USA database. The 12 avocado samples that tested positive for *Salmonella* produced 32 isolates. The FDA queried the PulseNet USA database to compare those isolates to reported human biological (i.e., clinical) isolates from December 15, 2013 to December 15, 2015, covering the period of this assignment's sample collection and testing. The FDA's analysis found 20 of the avocado isolates across nine samples to be indistinguishable by PFGE from clinical isolates. The bulleted information below provides details on those 20 isolates and the follow-up activities that the FDA undertook.

- Two isolates recovered from one avocado sample were indistinguishable from two clinical isolates, from Oregon and Idaho, respectively. Upon detecting the pathogen in the avocado sample, the FDA worked with the responsible firm to carry out a Class 1 recall and subsequently conducted an inspection of its packinghouse. Environmental sampling carried out as part of the inspection yielded no pathogens.
- Three isolates from one avocado sample were found to be indistinguishable by PFGE from 83 clinical isolates, with 77 of them collected from people in Florida. Upon

detecting the pathogen in the avocado sample, the FDA worked with the responsible firm to carry out a Class 1 recall. The agency conducted an initial follow-up inspection in January 2015 and documented objectionable conditions and practices. The agency conducted an additional inspection in October 2016, documented further violations, and collected environmental samples that were found to be positive for *Salmonella*. The firm has taken corrective action and the FDA continues to monitor it closely.

- One avocado isolate from a discrete sample was indistinguishable by PFGE from 18 clinical isolates, 15 of which were collected in Florida, and one each in Indiana, Oklahoma, and Tennessee. Upon detecting the pathogen in the avocado sample, the FDA worked with the responsible firm to carry out a Class 1 recall.
- Two avocado isolates from one sample were indistinguishable by PFGE from 16 clinical isolates all emanating from persons in Florida. Upon detecting the pathogen in the avocado sample, the FDA worked with the responsible firm to carry out a Class 1 recall and subsequently conducted an inspection of its packinghouse. Environmental sampling carried out as part of the inspection did not detect any pathogens.
- Two avocado isolates from one sample were indistinguishable from six clinical isolates (three from Florida, two from Georgia, and one from Massachusetts). Upon detecting the pathogen in the avocado sample, the FDA worked with the responsible firm to carry out a Class 1 recall.
- Seven avocado isolates across two samples were indistinguishable by PFGE from eight clinical isolates, with a geographical concentration of seven from Puerto Rico and one from New Jersey. After detecting the pathogen in the avocado samples, the FDA conducted an inspection of the responsible facility. In carrying out the inspection, the FDA found *Salmonella* in the facility; however, the strains detected in the environmental samples did not match the strain detected on the avocado samples, based on the WGS results (done on product and environmental samples but not clinical isolates; see “WGS Evaluation” subsection below). Further, the strains detected in the environmental samples were isolated from non-food contact surfaces, and thus the FDA concluded that no additional action was warranted apart from future monitoring.
- One avocado isolate from a discrete sample was indistinguishable by PFGE from one clinical isolate collected in Florida. Upon detecting the pathogen in the avocado sample, the FDA notified the retailer (a farmer’s market produce stand, where the sample had been collected), which destroyed the remaining product.
- Two avocado isolates from one sample were indistinguishable by PFGE from 27 clinical isolates, 25 of which were collected in Florida, along with one each in California and Washington. Upon detecting the pathogen in the avocado sample, the FDA worked with the responsible firm to carry out a Class 1 recall. The FDA also subsequently conducted an inspection of the responsible facility, including collecting environmental samples and additional avocado samples. The additional avocado samples tested negative for pathogens. Of the environmental samples, six subsamples collected from areas adjacent to food contact surfaces within the facility tested positive for *Salmonella*. Those six subsamples were not found to be indistinguishable by PFGE from the initial avocado sample that tested positive for *Salmonella*. Given the inspectional findings, this firm will be subject to heightened inspectional scrutiny and possible enforcement action if deviations persist.

Listeria monocytogenes: The FDA and CDC have transitioned their genetic evaluation of *Listeria* from PFGE to whole genome sequencing, which provides greater resolution of the data (i.e., sharper detail and thus more reliable DNA comparison between isolates). The next subsection provides the results of the bioinformatics analysis of the *Listeria monocytogenes* strains isolated from the avocado pulp and skin samples.

Whole Genome Sequencing (WGS) Evaluation

[Whole genome sequencing](#) reveals the complete DNA make-up of an organism at a single time, enabling the FDA to better understand variations both within and between species. This in turn helps the FDA to differentiate between organisms with a precision that other technologies do not allow.

Salmonella: WGS of clinical *Salmonella* isolates was not routinely performed by the CDC and state public health laboratories during the period when this assignment was conducted, and so the FDA was not able to compare the WGS profiles of isolates from its avocado samples to isolates from clinical samples.

***Listeria monocytogenes* (Pulp Samples)**: Though not widespread at the time, beginning in the fall of 2013, WGS technology has been used for analysis of clinical *Listeria monocytogenes* isolates in the United States. The FDA compared whole genome sequences from the three *Listeria monocytogenes* strains isolated from avocado pulp samples to clinical case sequences housed in the National Center for Biotechnology Information database. Two of the three *Listeria monocytogenes* isolates from the avocado pulp samples were highly related to isolates from clinical sources, but the available epidemiological data obtained by public health officials in the states and at the CDC did not indicate whether the clinical cases (i.e., ill persons) had eaten avocados. The remaining isolate taken from avocado pulp did not match any clinical isolates in the database.

***Listeria monocytogenes* (Skin Samples)**: The FDA's analysis showed that 31 of the avocado skin samples had produced *Listeria monocytogenes* isolates highly related to 142 isolates from ill persons. Of those 142 clinical isolates, 44 were unique to avocados, with the remainder featuring additional isolation sources (i.e., other foods, or environmental sources). However, the available epidemiological information did not indicate whether the consumption of avocados was implicated in the illnesses.

As described in the body of this report, the FDA began collecting and testing avocados for *Listeria monocytogenes* in May 2014 as part of the agency's new approach to deploying its sampling resources.

Test Methods

The FDA initially used a soak method to detect *Listeria monocytogenes* on the fruit's exterior. From May 13 to August 26, 2014, analysts soaked whole avocados in a *Listeria monocytogenes* enrichment broth and then analyzed the samples as described in the Test Methods appendix of this report.

On September 2, 2014, the FDA updated its approach to its *Listeria monocytogenes* testing to focus on the avocado pulp, as opposed to the fruit's exterior, to better evaluate public health concerns associated with the pathogen, namely the extent to which it may be present in the part of the fruit that people eat.

Rationale for Changing the *Listeria monocytogenes* Test Method

Prior to changing its test method, the FDA considered several factors and continues to evaluate their significance, including:

No history of outbreaks. The *Listeria monocytogenes* prevalence that the FDA found in the skin samples using the soak method was not consistent with outbreak data for the hazard-commodity pair. No outbreaks or individual illnesses had been linked to *Listeria monocytogenes* on the fruit's skin at the time (August 2014) that the decision to change the test method was made.

WGS analysis conducted on samples collected as part of this assignment found that some of the avocado skin samples had produced isolates that were highly related to clinical isolates; however, the available epidemiological information did not indicate whether the consumption of avocados was implicated in the illnesses.

Consumer practices. Common consumer practices can have the effect of limiting the growth and reproduction of *Listeria monocytogenes*, if present, prior to consumption of the fruit.

- Consumers typically slice avocados then extract and eat the pulp. The peel of most commercial varieties of avocados has a leathery texture not conducive to consumption; consumers discard it as they would a banana peel or an orange rind.
- Consumers slice avocados and typically eat the pulp soon afterward. This is largely because avocado pulp tends to brown quickly once exposed to oxygen, a process known as "enzymatic browning."

Safe preparation reduces possible risks. If *Listeria monocytogenes* is present on the skin of an avocado, the act of slicing and/or related handling of the fruit could transfer the pathogen to the pulp. However, consumers can reduce any possible risks by taking simple steps. [Foodsafety.gov](http://foodsafety.gov)

advises consumers to “wash all produce thoroughly under running water before eating, cutting or cooking.” The site further notes: “Even if you plan to cut the rind or peel off the produce before eating, it is still important to wash it first so dirt and bacteria aren’t transferred from the knife onto the fruit.” Foodsafety.gov also recommends that consumers scrub firm produce (including avocados) with a clean produce brush, and then dry it with a clean cloth towel or paper towel to further reduce bacteria that may be present.

Pathogen Findings

Based on the data collected under this assignment, the FDA found the prevalence of *Listeria monocytogenes* in the avocado pulp to be 0.24 percent and on the fruit’s skin to be 17.73 percent. The FDA cautions against making inferences about the *Listeria monocytogenes* prevalence associated with the exterior of the fruit based solely on the analytical results from this study. The agency discusses this finding in the Statistical Evaluation section of this report (page 14).

Compliance

FDA and state laboratory supervisors assign samples to classes based on whether they are “regulatory (classes 1, 2, 3 and 5)” or “non-regulatory (class 4)” in nature, and on the laboratory test results. The agency designated the samples of avocado pulp that tested positive for *Listeria monocytogenes* to be Lab Class 3 (“Adverse Findings”). The agency designated the samples of avocado that tested positive for *Listeria monocytogenes* on the fruit’s exterior to be Lab Class 4 (“No Classification Required”), based on factors cited earlier in this appendix and because the FDA did not consider them to have met its threshold for regulatory action. For each Lab Class 4 sample, the agency notified the responsible firm of its findings.

Further Study

The FDA conducted a study to better understand whether *Listeria monocytogenes* can penetrate the skin of an avocado or pass through its stem scar such that it may contaminate the fruit’s pulp. The researchers artificially inoculated avocados using *Listeria monocytogenes* from the culture collection at the FDA’s Center for Food Safety and Applied Nutrition. The researchers found that *Listeria monocytogenes* was not likely to penetrate the intact avocado skin, but internalization can occur through the avocado stem scar, via the fruit’s vascular system. The FDA did not design the study to determine the frequency of *Listeria monocytogenes* contamination. The *Journal of Food Protection* published the study¹⁰ in 2016, and the agency microbiologists who conducted the research presented their findings at a produce industry conference.

Strengthening Information Collection

At the beginning of this assignment, the CDC’s National Hypothesis Generating Questionnaire did not include mention of avocados. Outbreak investigators use the questionnaire, administered

¹⁰ Chen, Y., Evans, P., Hammack, T.S., Brown, E.W. & Macarisin, D. [Internalization of *Listeria monocytogenes* in Whole Avocado](#). *Journal of Food Protection*, Vol. 79, No. 8, 2016, Pages 1440–1445.

to patients who are part of recognized illness clusters, in part to help the patients recall foods they may have eaten, on the chance that one or more of those foods may have been the vehicle of the contamination. Following outreach by the FDA, the CDC included avocados (in any form) in the 2017 version of the questionnaire and otherwise going forward.