

Review of the VIDAS-*Listeria* (AOAC OMA 999.06) method-matrix extension data from FDA regulatory activities from 2016 through 2019.

R. Derike Smiley, Ph.D.

Arkansas Laboratory, Office of Regulatory Science/Office of Regulatory Affairs, U.S. FDA, 3900 NCTR Rd., Building 26, Jefferson, AR, 72079
Email: Ronald.Smiley@fda.hhs.gov

Abstract

The VIDAS-*Listeria* detection assay (AOAC Official Method 999.06) is routinely used by FDA field laboratories for screening regulatory samples for *Listeria monocytogenes* as well as other species of *Listeria*. Despite having achieved “Official Method of Analysis” status from the AOAC International, the method was only validated for a limited number of food matrices. The regulatory nature of FDA work necessitates that all analytical methods be validated for each matrix for which they are used. To adhere to this obligation, the FDA field laboratories continually assess the VIDAS-*Listeria* assay, for non-validated matrices, using method-matrix extension validation. A review of spike detection results revealed that six matrices can be considered validated, 10 matrices are inappropriate, and an additional five matrices have a single spike failure and require further testing to resolve their validation status. *L. monocytogenes* spikes had a lower detection rate compared to *L. innocua* across all matrices. Continual monitoring and review of the spiked-matrix data is needed to ensure the FDA field laboratories are adhering to good regulatory practices by using appropriate methods for analyses of the products they regulate.

1. Introduction

Listeria monocytogenes is a foodborne pathogen that is frequently the focus of U.S. FDA routine and targeted inspections. The reference method for the detection, isolation, and subsequent confirmation of this organism is detailed in the FDA Bacteriological Analytical Manual (FDA BAM) Chapter 10 (<https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam>). Briefly, the reference method uses a short, four-hour, non-selective enrichment followed by the addition of selective agents and continued incubation for a total of 48 hours. Specific details on times, temperatures, selective agents, concentrations, tolerances and sample set up can be found within the online reference listed above. The Laboratory Information Bulletin (LIB) is a tool for the rapid dissemination of laboratory methods, interesting observations, preliminary study results, or other information which may be of interest to FDA field laboratories. It does not necessarily report completed scientific works or studies. Users of the information presented in FDA LIBs must verify for themselves by appropriate study and procedures that the methods, techniques, or information presented within is reliable and accurate for their intended use. Reference to any commercial materials, equipment, or process does not in any way constitute regulatory approval nor FDA endorsement or recommendation.

Presumptive detection and isolation of *L. monocytogenes* are accomplished in the same step using the streak plate method with both Oxford agar and one chromogenic agar. Isolation of *L. monocytogenes* can be attempted after approximately 24 hours of enrichment and allowing for around 24 hours to incubate the streak plate gives roughly 48 hours for the minimum time to presumptive-positive. The reference method requires a full 48 hours of enrichment incubation plus a full 48 hours of plate incubation to declare a negative result. Because of the regulatory sample burden and since many of the products that are tested for *L. monocytogenes* are perishable, the FDA often uses validated rapid methods that reduce the analysis time.

The VIDAS-*Listeria* assay is an enzyme linked immune-fluorescence assay (ELFA) with AOAC International Official Method of Analysis (OMA) status (AOAC OMA 999.06). This method utilizes the same selective enrichment procedure as the FDA reference *L. monocytogenes* method. The VIDAS-*Listeria* screening method is performed after 48 hours of selective enrichment giving roughly a minimum of 50-52 hours until presumptive positive or time to negative results; the exact time will depend on the number of samples that are processed at a single time. Because of the regulatory nature of the analyses performed and because the FDA field laboratories wish to adhere to ISO 17025 accreditation, all methods used for the detection of foodborne pathogens must be validated for each food matrix. Ideally, the methods would be validated prior to use; however, this is problematic due to the total number of regulatory tests constantly being performed and the wide variety of food matrices encountered. To resolve this dilemma, the FDA field laboratories have adopted an abbreviated validation procedure known as method-matrix extension. When a non-validated matrix is encountered, an analytical test portion (typically 25 g) is spiked (1-30 CFU/25 g) and the spiked sample is tested concurrently with the analytical samples. A “non-validated” matrix refers to a matrix that has not had its validation status established; a matrix that fails validation is referred to here as an “inappropriate” matrix indicating that the method and matrix are not compatible. The results of the spike detection are accumulated until a requisite number has been reached and then the validation status is established.

2. Methods

2.1 Data Retrieval

Data (Sample Number, Accomplishing Laboratory, Sampling District, PAC Code, PAF Code, Completion Date, Laboratory Class, Product Code, Product Description, Method Source Code, Method Code, Sub-sample,

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Rapid Method Results, Conventional Method Results, Spike Results, Genus/Species used for spiking, Selective Agar Results, Selective Agar Used, Kit Compare Remarks, Description Text, and Product Label) were obtained from FACTS using the ORA Reporting, Analysis, and Decision Support System (ORADSS SAP Business Objects Business Intelligence Suite, 4.0 Feature Pack). Data retrieval included spiked-matrix results from all accomplishing laboratories for method code T999.06, between January 01, 2015 and December 31, 2019.

2.2 Data Selection

The spiked-matrix results included in the cumulative data set, which covers the timeframe January 01, 2015 through December 31, 2019, were selected based on the completeness of the product description and a reported spiking level between 1 and 30 CFU/25 g. The product description and the product industry code were used to determine the matrix identity for categorization; the matrix identity had to be established with reasonable certainty to be included in the analysis. If a sample (i.e. FACTS number) had multiple spike-detection entries with the same result, then only one was included in the cumulative dataset. The reason for this was to ensure matrix diversity within the study. For regulatory use, a method should be robust and not be affected by seasonal, geographic, or applied minimal processing. If the same sample had multiple spike-detection entries with differing results, then all entries were omitted from the cumulative dataset. If the sample subsequently test positive for the presence of *Listeria* sp., then the spike-detection results were omitted from the cumulative dataset since an accurate estimate of the initial *Listeria* sp. levels was not possible.

2.3 Spiked-matrix Data Analysis

After being categorized, all food products/matrices were evaluated based on the following criteria derived from the FDA Foods Program Research Science Steering Committee (RSSC) (<https://www.fda.gov/science-research/field-science-and-laboratories/method-validation-guidelines>) (last accessed 03/18/2020); 1) seven to 19 spiked-matrix detection results with no negative detection results or 2) 20 of more spiked-detection results with <5% negative detection results. Food products/matrices meeting either of these were considered validated for the method. If a matrix has between 7 and 20 spiked-matrix

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results with a single detection failure, then the category is considered pending as the matrix could potentially be validated based on criterion 2 once additional results are collected.

3. Results and Discussion

3.1 Data Summary

The FDA field laboratories only recently (approximately early 2016) began collecting spiked-matrix detection results for the VIDAS-*Listeria* assay despite having used the assay for many years for regulatory purposes. There was no spike data captured for 2015 even though it was included in the ORADSS data retrieval criteria. There were 575 spike detection results captured in FACTS. After removing some entries for the reasons discussed in section 2.2, there were 544 remaining useable observations. Sixty product groupings were generated that had more than two spike detection observations; this accounted for 478 of the total useable spike detection results that were available. The remaining 66 spike results reflect orphan product categories with a single spike detection observation.

3.2 Method-matrix extension validation results

Currently, only six matrices can be considered validated based on FDA Foods Program RSSC guidance criteria (Table 1). All six were validated based on Criterion 1 described in section 2.3. Four of the validated products (minimally processed leafy green vegetables, bell chili peppers, plums, and strawberries) are fruit and vegetable products (AOAC category 2). The remaining two validated matrices are seafoods (AOAC category 6). There are an additional five matrices that have the minimum of seven spike detection results but possesses a single failure (Table 2). These products cannot be validated using criteria 1 from section 2.3. Spike detection efforts should continue for these five matrices until 20 observations are obtained, at which time a validation determination can be made based on criteria 2 from section 2.3. There are 10 matrix categories which were shown to be non-compatible with the VIDAS-*Listeria* method (Table 3). One matrix, guacamole, is particularly concerning since it constitutes approximately 30% of the total analyses performed using this method. Method-matrix extension is a validation process and once a product has been determined to be inappropriate, usage of that method should stop, and future analyses should be performed using the FDA BAM reference method. Finally, there were 40 matrices which had between two and six spike detection

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results (Table 4). Continued spiking efforts and periodic monitoring of the results are needed and will ultimately reveal the validation status of these products.

3.3 Spiking with *L. monocytogenes* or *L. innocua*

The VIDAS-*Listeria* assay is only specific to the genus level and will yield a positive signal for all known species of *Listeria* if they are at sufficient levels following enrichment. There were 420 instances where the spike organism was specified to the species level; there were 101 and 319 spike detection observations using *L. monocytogenes* and *L. innocua*, respectively (Table 5). Across all matrices, *L. monocytogenes* spikes were detected at a rate of 77% and *L. innocua* spikes at a rate of 94% (Table 5). Guacamole was the only matrix with enough observations to make a meaningful comparison between the detection of *L. monocytogenes* and *L. innocua* within an individual food (Table 6). The detection rates were 77% and 98% for the same two species, respectively. Given the discrepancy between the detection sensitivities for the two *Listeria* species, it might seem logical to use only *L. innocua* for spiking activities as this would likely increase the number of food products that are ultimately validated. However, *L. monocytogenes* is the pathogenic species and from both a regulatory and human health perspective it is the most important of the *Listeria* species. Therefore, it is more important that the VIDAS-*Listeria* method be validated for the pathogenic rather than the non-pathogenic species.

3.4 Method-matrix extension root matrices

Method-matrix extension validation is a complicated concept in that it is not as rigorous as a full laboratory study, yet the results are often treated with the same merit as the latter. The reconciliation is that a matrix which is validated by the abbreviated process (i.e. method-matrix extension) should be rooted to a similar matrix that received a more in-depth laboratory evaluation. The more similar the two matrices the more resolute the interpretation of the results of the method-matrix extension study. How food products are categorized will affect their perceived similarity and there are no firm rules governing this task, only guidance. If the categories are too specific, then the FDA field laboratories are left with too many orphan categories where the validation status is never established due to infrequent testing. On the other hand, if the categories are too broad then subtle differences between similar products that might affect the performance of the assay could go unnoticed. This results in food products being deemed validated when, in truth, they are not

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compatible for the method. Generally, the more complex the food product the more complicated it is to categorize; additionally, the more complex the food product the greater the variation between different manufacturers of the product. The FDA Foods Program RSSC's guidance (referenced in section 2.3) suggests that for method-matrix extension the test matrix and the root matrix need only be within the same category of foods. While not explicit, the term "category" likely refers to one of the eight AOAC food categories (1) Meat and Poultry, (2) Fruits and Vegetables, (3) Dairy Products, (4) Egg Products, (5) Miscellaneous, (6) Seafoods, (7) Animal Feed, and (8) Spices. This level of similarity seems overly broad especially since it includes a category labeled "miscellaneous". AOAC guidance (<https://www.fda.gov/food/laboratory-methods-food/best-practices-microbiological-methodology>) stipulates that the matrix being validated by method-matrix extension should be in the same class as the original validation study root matrix. While this level of classification is much more precise than that of the FDA Foods Program RSSC, it leads to too many orphan product categories that will require years to obtain enough observations to establish their validation status due to infrequent analyses.

Table 7 includes the AOAC product category, AOAC product class, the root matrix from the original validation study, and the validation study level of the root matrix. The most predominant AOAC class of food analyzed was "Fruits and Vegetables" followed by "Seafoods"; these two classes accounted for 70% of all products tested. The only fruit or vegetable matrix that was included in the original VIDAS-*Listeria* validation study was frozen, minimally processed green beans (Gangar et al., 2000). "Fruits and Vegetables" is a diverse category and includes not only frozen but also fresh, heat processed, dried, and fermented products. This category also contains the tree nuts and vegetable/fruit juices. This category varies greatly in pH with values ranging from <3 for some citrus fruits to >7 for some vegetables. Green beans are a suitable root matrix for frozen minimally processed vegetables and will likely suffice for similar vegetables in the fresh state and those subjected to mild heat treatments (e.g. blanching). However, green beans may not sufficiently mirror lower pH fruits such as berries, melons, and citrus nor do they sufficiently reflect vegetables with an irregular or rough consistency such as cauliflower, broccoli, or lettuce. Additional laboratory-controlled challenge studies are likely needed within the fruits and vegetables category to ensure those products being validated by method-matrix extension are properly anchored to a root matrix. AOAC category "6. Seafood" was represented in the original validation study by raw frozen fish (Gangar, et al., 2000). While this matrix may adequately mirror other seafoods such as raw fresh fish and perhaps even some cooked fish, it likely is not adequate for

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predicting the analytical behavior of smoked fish, molluscan shellfish (e.g. oysters), crustaceans (e.g. shrimp), or molluscan cephalopods (e.g. squid). Additional laboratory validation studies are needed to increase the number of available root matrices within the category “Seafood” in order to adequately encompass all seafood matrices encountered by the FDA field testing laboratories.

4. Concluding Remarks

The FDA field laboratories only recently began collecting and tracking *Listeria* spike detection results to establish the validation status of the VIDAS-*Listeria* assay. The validation status could only be determined for 16 products. For those products that failed method-matrix extension validation, future regulatory testing should be performed using the FDA BAM reference method for *Listeria* detection/recovery. Matrices with too few observations or for which a validation determination cannot be made based on criterion 2 (section 2.3) should continue to be spiked and periodically evaluated until their status is resolved.

There is one limitation of method-matrix extension that can be exploited to allow failed matrices to be re-tested by full laboratory-controlled validation study. In other words, the results of method-matrix extension are not absolute. When evaluating the effectiveness of an alternate method (i.e. VIDAS-*Listeria* assay), it need not be superior to the reference method, only equivalent. When the FDA field laboratories encounter a spike detection failure, they do not confirm the failure using the reference method; it is assumed that the reference method would have recovered the spike organism. This assumption may not be accurate for all FDA regulated matrices encountered by the field laboratories. It is possible that both methods (i.e. BAM reference and VIDAS-*Listeria*) would have failed to detect *Listeria* if they had been performed simultaneously on those spiked samples that failed. It is therefore possible to re-test matrices that failed method-matrix extension with a full laboratory validation study where both methods are tested concurrently. The alternative is to accept the assumption and method-matrix extension results and conduct all future regulatory analyses, of the failed matrix, using the FDA BAM reference method.

References and Additional Readings

AOAC International Presidential Task Force on Best Practices for Microbiological Methodology: Appendix B. available at (<https://www.fda.gov/food/laboratory-methods-food/best-practices-microbiological-methodology>) last accessed 04/23/2020.

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FDA Foods Program Research and Science Steering Committee. "Guidelines for the validation of analytical methods for the detection of microbial pathogens in foods". 3rd ed. available at (<https://www.fda.gov/science-research/field-science-and-laboratories/method-validation-guidelines>) last accessed 04/23/2020.

Gangar V, Curiale MS, D'Onorio A, and Shultz A. (2000). VIDAS enzyme-linked immunofluorescent assay for detection of *Listeria* in foods: collaborative study. J. AOAC Int. 83(4):903-918.

Hitchins AD, Jinneman K, and Chen Y. (2017). Bacteriological Analytical Manual, Chapter 10. "Detection of *Listeria monocytogenes* in foods and environmental samples, and enumeration of *Listeria monocytogenes* in foods. available here (<https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam>) last accessed 04/23/2020.

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Table 1. Product matrices meeting method-matrix extension validation criteria for the VIDAS-*Listeria* method.

| Matrix | Total Samples | VIDAS Positive | VIDAS Negative | Sensitivity | Validation Criterion |
|-----------------------|----------------------|-----------------------|-----------------------|--------------------|-----------------------------|
| Greens* | 19 | 19 | 0 | 1.00 | 1 |
| Imitation Crab | 9 | 9 | 0 | 1.00 | 1 |
| Peppers, Bell | 10 | 10 | 0 | 1.00 | 1 |
| Plums | 8 | 8 | 0 | 1.00 | 1 |
| Shrimp, Cooked | 10 | 10 | 0 | 1.00 | 1 |
| Strawberries | 8 | 8 | 0 | 1.00 | 1 |

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Table 2. Pending product matrices that require additional testing to resolve their validation status.

| Matrix | Total Samples | VIDAS Positive | VIDAS Negative | Sensitivity |
|-------------------------------------|----------------------|-----------------------|-----------------------|--------------------|
| Fish, Smoked | 17 | 16 | 1 | 0.94 |
| Hummus | 8 | 7 | 1 | 0.88 |
| Melons* | 13 | 12 | 1 | 0.92 |
| Poultry, Raw | 7 | 6 | 1 | 0.86 |
| Wheat Grain Bakery Products* | 8 | 7 | 1 | 0.88 |

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Table 3. Product matrices determined to be inappropriate for the VIDAS-*Listeria* method.

| Matrix | Total Samples | VIDAS Positive | VIDAS Negative | Sensitivity |
|-------------------------|---------------|----------------|----------------|-------------|
| Cheese | 15 | 11 | 4 | 0.73 |
| Coconut | 4 | 1 | 3 | 0.25 |
| Crustaceans* | 19 | 17 | 2 | 0.89 |
| Cucumber | 10 | 8 | 2 | 0.80 |
| Eel, Cooked | 6 | 3 | 3 | 0.50 |
| Fish, Fresh/Frozen | 12 | 8 | 4 | 0.67 |
| Fish, Ntrl/Artfcl Dried | 2 | 0 | 2 | 0.00 |
| Guacamole | 170 | 158 | 12 | 0.93 |
| Jellyfish, Instant | 2 | 0 | 2 | 0.00 |
| Pet Food, Raw | 9 | 6 | 3 | 0.67 |

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Table 4. Food matrices having two to six spike detection results for the VIDAS-*Listeria* method.

| Matrix | Total Samples | VIDAS Positive | VIDAS Negative | Sensitivity |
|------------------------------|---------------|----------------|----------------|-------------|
| Anchovies | 4 | 3 | 1 | 0.75 |
| Asparagus | 2 | 2 | 0 | 1.00 |
| Beans, Green | 2 | 2 | 0 | 1.00 |
| Blackberries | 2 | 2 | 0 | 1.00 |
| Brussels Sprouts | 3 | 3 | 0 | 1.00 |
| Celery | 3 | 3 | 0 | 1.00 |
| Chili Pepper (dried, powder) | 3 | 2 | 1 | 0.67 |
| Clams, Cooked | 3 | 2 | 1 | 0.67 |
| Corn Snacks | 5 | 5 | 0 | 1.00 |
| Crab, Cooked | 6 | 5 | 1 | 0.83 |
| Crawfish, Cooked | 4 | 4 | 0 | 1.00 |
| Dietary Supplements* | 4 | 4 | 0 | 1.00 |
| Fish, Cooked | 3 | 3 | 0 | 1.00 |
| Fish, Roe | 3 | 3 | 0 | 1.00 |
| Jellyfish, Instant | 2 | 0 | 2 | 0.00 |
| Lobster, Cooked | 3 | 3 | 0 | 1.00 |
| Milk Curd | 2 | 1 | 1 | 0.50 |
| Mixed Vegetables | 3 | 3 | 0 | 1.00 |
| Mollusks, Cephalopods* | 4 | 4 | 0 | 1.00 |
| Nectarines | 6 | 6 | 0 | 1.00 |
| Nuts* | 2 | 2 | 0 | 1.00 |
| Onion, Scallions | 2 | 1 | 1 | 0.50 |
| Orange Juice | 2 | 2 | 0 | 1.00 |

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| Matrix | Total Samples | VIDAS Positive | VIDAS Negative | Sensitivity |
|-------------------------|---------------|----------------|----------------|-------------|
| Peaches | 2 | 2 | 0 | 1.00 |
| Pet Food, Moist | 4 | 4 | 0 | 1.00 |
| Pet Treats, Jerky | 2 | 2 | 0 | 1.00 |
| Rabbit, Raw | 3 | 3 | 0 | 1.00 |
| Raspberries | 2 | 2 | 0 | 1.00 |
| Salsa | 2 | 1 | 1 | 0.50 |
| Seaweed | 2 | 2 | 0 | 1.00 |
| Shrimp Dumpling | 2 | 2 | 0 | 1.00 |
| Shrimp, Dried | 5 | 4 | 1 | 0.80 |
| Sprout Irrigation Water | 2 | 2 | 0 | 1.00 |
| Squash | 2 | 2 | 0 | 1.00 |
| Squid, Cooked | 2 | 1 | 1 | 0.50 |
| Squid, Dried | 2 | 1 | 1 | 0.50 |
| Tomatoes | 2 | 2 | 0 | 1.00 |
| Vegetable Dumpling | 2 | 2 | 0 | 1.00 |
| Yam | 3 | 3 | 0 | 1.00 |
| Yogurt | 2 | 2 | 0 | 1.00 |

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Table 5. Comparison of *L. monocytogenes* and *L. innocua* spike detections across all food matrices for the VIDAS-*Listeria* method.

| Species | POS Detections | NEG Detections | Total | Detection Rate |
|-------------------------|----------------|----------------|-------|----------------|
| <i>L. monocytogenes</i> | 101 | 31 | 132 | 0.77 |
| <i>L. innocua</i> | 319 | 21 | 340 | 0.94 |

Table 6. Comparison of *L. monocytogenes* and *L. innocua* spike detections in guacamole for the VIDAS-*Listeria* method.

| Species | POS Detections | NEG Detections | Total | Detection Rate |
|-------------------------|----------------|----------------|-------|----------------|
| <i>L. monocytogenes</i> | 30 | 9 | 39 | 0.77 |
| <i>L. innocua</i> | 128 | 3 | 131 | 0.98 |

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Table 7. AOAC matrix categories, root matrices, and validation study level for food product classification when analyzing method-matrix extension spiking data for the VIDAS-*Listeria* method.

| Matrix | AOAC Matrix Category | AOAC Matrix Class | Root Matrix | Validation Study Level |
|------------------------------|--------------------------|---|-------------|------------------------|
| Anchovies | 6. Seafood | A. Finfish | Raw Fish | Single Laboratory |
| Asparagus | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Beans, Green | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Blackberries | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Brussels Sprouts | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Celery | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Cheese | 3. Dairy Products | A. Fermented and Non-fermented | Cheese | Single Laboratory |
| Chili Pepper (dried, powder) | 8. Spices | Class 5 | None | NA |
| Clams, Cooked | 6. Seafood | B. Molluscan Shellfish | None | NA |
| Coconut | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Corn Snacks | 5. Miscellaneous | None | None | NA |
| Crab, Cooked | 6. Seafood | C. Crustaceans | None | NA |
| Crawfish, Cooked | 6. Seafood | C. Crustaceans | None | NA |
| Crustaceans* | 6. Seafood | C. Crustaceans | None | NA |

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| Matrix | AOAC Matrix Category | AOAC Matrix Class | Root Matrix | Validation Study Level |
|-------------------------|--------------------------|---|-------------|------------------------|
| Cucumber | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Dietary Supplements* | NA | NA | None | NA |
| Eel, Cooked | 6. Seafood | None | None | NA |
| Fish, Cooked | 6. Seafood | A. Finfish | Raw Fish | Single Laboratory |
| Fish, Fresh/Frozen | 6. Seafood | A. Finfish | Raw Fish | Single Laboratory |
| Fish, Ntrl/Artfcl Dried | 6. Seafood | A. Finfish | Raw Fish | Single Laboratory |
| Fish, Roe | 6. Seafood | A. Finfish | Raw Fish | Single Laboratory |
| Fish, Smoked | 6. Seafood | A. Finfish | Raw Fish | Single Laboratory |
| Greens* | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Guacamole | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Hummus | 5. Miscellaneous | None | None | NA |
| Imitation Crab | 6. Seafood | A. Finfish | Raw Fish | Single Laboratory |
| Jellyfish, Instant | 6. Seafood | NA | None | NA |
| Lobster, Cooked | 6. Seafood | C. Crustaceans | None | NA |
| Melons* | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Milk Curd | 3. Dairy Products | A. Fermented and Non-fermented | Cheese | Single Laboratory |

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| Matrix | AOAC Matrix Category | AOAC Matrix Class | Root Matrix | Validation Study Level |
|------------------------|--------------------------|---|---------------|------------------------|
| Mixed Vegetables | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Mollusks, Cephalopods* | 2. Seafood | D. Squid/Octopus | None | NA |
| Nectarines | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Nuts* | 2. Fruits and Vegetables | F. Nutmeats | Green Beans | Single Laboratory |
| Onion, Scallions | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Orange Juice | 2. Fruits and Vegetables | C. Juice and Juice Concentrates | None | NA |
| Peaches | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Peppers, Bell | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Pet Food, Moist | 7. Animal Feed | B. <75% Dry Matter | None | NA |
| Pet Food, Raw | 7. Animal Feed | B. <75% Dry Matter | Ground Turkey | Single Laboratory |
| Pet Treats, Jerky | 7. Animal Feed | A. >75% Dry Matter | None | NA |
| Plums | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Poultry, Raw | 7. Animal Feed | B. <75% Dry Matter | Ground Turkey | Single Laboratory |
| Rabbit, Raw | 7. Animal Feed | B. <75% Dry Matter | Ground Turkey | Single Laboratory |
| Raspberries | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Salsa | 5. Miscellaneous | D. Dressings, Condiments, Marinades | None | NA |

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| Matrix | AOAC Matrix Category | AOAC Matrix Class | Root Matrix | Validation Study Level |
|------------------------------|-----------------------------|---|--------------------|-------------------------------|
| Seaweed | 5. Miscellaneous | None | None | NA |
| Shrimp Dumpling | 6. Seafood | C. Crustaceans | None | NA |
| Shrimp, Cooked | 6. Seafood | C. Crustaceans | None | NA |
| Shrimp, Dried | 6. Seafood | C. Crustaceans | None | NA |
| Sprout Irrigation Water | 5. Miscellaneous | None | None | NA |
| Squash | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Squid, Cooked | 6. Seafood | D. Squid/Octopus | None | NA |
| Squid, Dried | 6. Seafood | D. Squid/Octopus | None | NA |
| Strawberries | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Tomatoes | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Vegetable Dumpling | 5. Miscellaneous | None | None | NA |
| Wheat Grain Bakery Products* | 5. Miscellaneous | A. Cereals and Grains | None | NA |
| Yam | 2. Fruits and Vegetables | A. Fresh, B. Frozen and/or Heat Processed | Green Beans | Single Laboratory |
| Yogurt | 3. Dairy Products | A. Fermented and Non-Fermented | Cheese | Single Laboratory |

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