National Food Safety Guidelines for Cantaloupe and Netted Melons

Appendix B: Microbiological Testing

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ABBREVIATIONS AND DEFINITIONS

Terminology	Definitions
AFNOR	AFNOR International; a Paris-based association that develops and provides standards, certification and training in France and internationally
AOAC	AOAC International; a global organization that develops and promotes standard methods of analysis for food and environmental safety.
cGMPs	current Good Manufacturing Practices
EHEC	Enterohemorrhagic *E. coli*
FDA BAM	U.S. Food and Drug Administration's Bacteriological Analytical Manual
GAPs	Good Agricultural Practices
GHPs	Good Handling Practices
ICMSF	The International Commission on Microbiological Specifications for Foods
ISO	International Organization for Standardization
STEC	Shiga toxin-producing *E. coli*
USDA MLG	U.S. Department of Agriculture's Microbiological Laboratory Guidebook

PURPOSE

Microbiological testing and profiling are important tools offering valuable insight into the static or dynamic microbial content of food, a process, and/or facility. Often, microbial testing is used, in conjunction with visual and physicochemical testing, to monitor whether preventative measures such as good agricultural practices (GAPs), good handling practices (GHPs), good manufacturing practices (GMPs), and/or cleaning and sanitation are working and effective. The primary purpose is to meet product specifications and established standards to minimize opportunities for microbially adulterated food to enter the marketplace. When testing programs are used in food safety, it is important to ensure that they are accurate, reliable, and appropriate for their intended purpose. To achieve this, a testing program should be designed to carefully consider the purpose of the testing, the sampling plan needed, and the testing methodology, and should include means to monitor and evaluate the overall program as performed to ensure the testing output is fit-for-purpose.

This appendix summarizes how to best manage your testing program, what testing can and cannot do for cantaloupe operations, and outlines practical approaches to sampling whole netted melons, and water. Note: Cantaloupe and netted melons are used interchangeably in this document.

PURPOSE AND ROLE OF MICROBIOLOGICAL TESTING FOR NETTED MELONS

For netted melons, microbiological testing is generally established to verify the performance of processes or food safety practices implemented to ensure the scientifically validated controls and performance criteria are being met. Testing can support the industry:

1. To determine adherence to accepted safety parameters
2. To determine adherence to GHPs, GMPs, GAPs
3. To determine the utility of food or ingredients for a particular purpose
4. To predict product stability

This document focuses on the first two categories: (i) To determine adherence to accepted safety parameters of product, water, or surfaces based on a microbial criterion (e.g., testing for pathogens or fecal indicators), and (ii) determining how well a standard operating procedure was performed (i.e., verifying the satisfactory performance of cleaning and sanitizing harvesting equipment).

MICROBIOLOGICAL TESTING PERFORMANCE

Microbiological testing programs have two essential components: sampling and analysis (testing). The value of any test result depends on how well these two components work together. Strong laboratory methods cannot compensate for weak or unrepresentative sampling, and excellent sampling cannot overcome a poorly performing test method. For netted melons, where contamination, if present, is typically low-level and clustered, understanding both components of the testing program is critical for interpreting results accurately and making informed food safety decisions.

Analytical Testing Performance

The performance of a testing method is crucial because it directly impacts the accuracy, reliability, and sensitivity of detecting contaminants or pathogens in food products. Robust testing methodology increases the likelihood that the results are reflective of reality, minimizing the risk of false positives or false negatives.

When selecting and using any testing method, walk through the checklist below and confer with laboratory personnel to confirm the method is fit-for-purpose and properly documented.

1. Ensure the laboratory is accredited, and in good standing, by either US or international accreditation bodies (such as ISO 17025 or equivalent). Many ISO 17025 accreditation bodies have online directories where certificates can be found. For example, A2LA, a common accreditation body for laboratories, has an online lab portal that can be found here: https://customer.a2la.org/index.cfm?event=directory.index
2. Confirm that test methods used by the laboratory are appropriate for the intended use and are based on recognized or validated methodologies (e.g., AOAC, AFNOR, FDA BAM, or equivalent), as applicable. Documentation supporting the method's scientific basis, modifications, or validation status should be maintained by the laboratory and made available upon request. Information on the validation and where to access these validations can be found in Table 1 below.
3. Review and document official validation documentation/summary (e.g., AOAC report).
    - Note if the method is not officially validated for the matrix or sample size/type, the responsibility to conduct a matrix extension verification study lies with the laboratory and should follow the AOAC Appendix J and/or FDA Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds.
4. Document method conditions (see Template A at the end of this appendix) to be used during analysis. For microbiological testing, include:
    - Sample size (e.g., grams, milliliters, sponge, swab, sampling cloth)
    - Sampling process is used to obtain samples (e.g., 'n' grabs, random sampling, stratified random, continuous sampling device (CSD), etc.)
    - Incubation time to be used (e.g., 18 hours, 24 hours, 48 hours)
    - Sample-to-enrichment ratio (e.g., 1:10, 1:5, 1:3)
5. Document any modifications to the official validation method (e.g., wet-pooling, reduced time, reduced enrichment, media substitutions, etc.).
6. Determine and document corrections and verifiable corrective actions to be taken when out-of-tolerance results are obtained within the testing program.
7. Manage test reporting and reviews using a means to analyze data and trend/track results.
8. Collect and store metadata around the samples – product preharvest, harvest, and pre-shipping management, time, location, what was sampled, sampling method (random, targeted, etc.)
9. Maintain method information such as sample type, reporting unit (e.g. per x g, per swab, per melon), enrichment time, sample-to-enrichment ratio, parameter tested, etc. See Template A at the end of this appendix for what to document.
10. Establish a process for selecting and monitoring your testing laboratory(s). Make sure your laboratory meets your operation's needs and keep documentation that they perform this reliably over time.
11. Maintain a simple, consistent supplier-approval program. This should outline how new vendors (in this case, laboratory services) are evaluated, how records are kept, and how ongoing performance is reviewed.
12. Ask for and review results from proficiency tests the laboratory has conducted and any "check samples" you send to verify that your laboratory or testing provider is producing accurate and consistent results. This helps confirm the reliability of your testing program.

Table 1: Validated Method Sources

Validation Body / Authority	What They Validate	Where to Find Their Validated Methods
AOAC INTERNATIONAL: Official Methods of Analysis (OMA) & Performance Tested Methods (PTM)	Microbiological & chemical methods for foods, produce, environmental samples	**AOAC OMA Database:** https://www.aoac.org/official-methods-of-analysis/ **AOAC PTM Directory:** https://members.aoac.org/AOAC/RI/PTM_Validated_Methods.aspx
AFNOR Certification	Microbiology alternative methods validated by ISO 16140	**AFNOR NF Validation List:** https://nf-validation.afnor.org/en/food-industry/
FDA – Bacteriological Analytical Manual (BAM)	Pathogen & indicator testing for food, produce, environmental swabs	**FDA BAM Chapters:** https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam
FDA – Foods Program Compendium of Analytical Laboratory Methods	Includes both microbiological (BAM) and chemical methods	**FDA Compendium:** https://www.fda.gov/food/laboratory-methods-food/foods-program-compendium-analytical-laboratory-methods
EPA – Clean Water Act Methods (40 CFR Part 136)	Microbiological & chemical water methods (surface water, irrigation water, ag water)	**EPA CWA Methods:** https://www.epa.gov/cwa-methods **EPA Approved Microbiological Methods:** https://www.epa.gov/cwa-methods/approved-cwa-microbiological-test-methods
USDA AMS Microbiology Laboratory Guidebook (MLG)	Methods used for USDA-regulated commodities (mostly meat/poultry)	**USDA MLG:** https://www.fsis.usda.gov/news-events/publications/microbiology-laboratory-guidebook
FDA Equivalent Testing Methodology for Agricultural Water - Produce Safety Rule (21 CFR 112)	FDA table listing ag water methods equivalent to EPA method 1603 for *E. coli* is water	https://www.fda.gov/food/laboratory-methods-food/equivalent-testing-methodology-agricultural-water-produce-safety-rule-21-cfr-112

SAMPLING PERFORMANCE

Sampling is the first and most influential part of a microbiological testing program. The goal is to collect a sample that reasonably reflects the true condition of a field, block, water source, or product lot. Because growers cannot test every cantaloupe harvested, every liter of water used, or every surface that touches the fruit, sampling design must be a balance between practicality and statistical power. The following considerations highlight how sampling choices directly influence what your testing program can realistically detect:

- **Larger sample mass increases the chance of detecting low-level contamination.** Whether using whole netted melons, tissue composites, or cloth/sponges, collecting more material increases the likelihood of capturing organisms if they are present.
- **More sampling units (n) improve detection when contamination is sparse.** When only a small portion of the lot, or certain times/sections of water may be contaminated (e.g., a few cantaloupes out of the whole field or water is contaminated during late hours or at the edges of the pond) taking a greater number of sampling units significantly strengthens detection power.
- **Contamination is rarely uniform.** Microorganisms cluster in certain areas of a field or on certain netted melons. A sampling plan must account for this uneven distribution to avoid missing contamination by chance.
- **Document what, where, and how you sampled.** Good documentation provides context for interpreting results and supports stronger decision-making.
  - **What:** water, surface, cantaloupe
  - **Where:** location, water source/point, equipment type
  - **How:** mass, netted melons, sample volume, number of grabs
- **Increase sampling and information gathering during higher-risk periods.** Events such as heavy rain, wildlife activity, water-quality changes, or sanitization deviations may require larger sample sizes or more frequent sampling. Collecting additional data during these times helps compare conditions to normal operations and supports stronger, risk-based decisions.
- **Recognize the limits of sampling.** No sampling plan can guarantee the absence of contamination. The goal is to design the most representative, practical, and risk-based sampling approach for cantaloupe operations.

SAMPLING APPROACHES

Numerous options exist when deciding to test a particular sample or matrix, and choosing the most representative sample for the intended purpose of testing is critical. In the case of testing produce, it is common to see product testing per a certain sample mass of 25 g, 125 g, 375 g, 1500 g, or for environmental samples such as water, soil, equipment/facility, and biological soil amendments, sample amounts of 100 mL, 10 g, sponge/swab, or 25-375 g.

For whole netted melons, sample masses make less sense as they are a whole fruit-commodity (meaning that you cannot sample just a part of it). For bulky items like whole netted melons, see Table 2 to review options for whole fruit testing. Similarly, you may encounter other sampling methods for water, you can review some of the sampling methodologies below (for more detailed information on water sampling, review Appendix C.).

Table 2: Sampling approaches for food safety testing

Sampling Approach	Pros/Cons	Ideal for Matrix
Solid matrix Testing (food, soil)		
Tissue	Destructive testing but easier to take multiple "n" as grabs are usually small.	Fresh-cut netted melons
Whole unit	Hard to take many 'n' as every 'n' represents one unit Needs a large volume of media for larger units, quickly challenging practicality for sample submission and processing.	Whole netted melons, individually packaged products. Best for detecting surface contamination, lower performance has been shown for internalized contamination.
Cloths, swabs, sponges	Nondestructive sampling approaches that can capture larger aggregative areas. These are increasingly used for whole, intact fruit sampling.[1,2] Depending on the sampling approach and time in the processing, the aggregate sample can target the surface and/or internal part of the netted melon if cut/processed.	Whole fruit, surfaces, and equipment
Water Testing		
Specific volume samples (100 mL, 1 L, 10 L)*	Commonly used in routine testing and lower cost. May not capture low-level contaminants at smaller sample volumes.	Suitable for water sources, and ideal for routine microbial testing of coliforms and generic *E. coli*. **Statistical power: smaller sample volumes will have lower statistical power for detecting contamination. Similarly, smaller sample masses will not capture the heterogeneity of the water matrix).*
Moore swabs (including modified Moore swabs)[3]	Passive sampling devices deployed in flowing water for an extended period (hours to days) to capture microorganisms over time. Effective for capturing intermittent or low-concentration contaminants over time in flowing water systems. They can be labor intensive as they require retrieval after a period of time.	Best for detecting pathogens in flowing water. Often used by researchers to characterize water sources/areas during a specified time period.
Filter-based samples	Concentrates large volumes of water, allowing for the detection of low-level contaminants. Requires specialized equipment and advance lab capabilities.[4,5]	Suitable as part of investigative sampling.

TESTING DATA COLLECTION AND REVIEW

Once the testing program has been established, data collection and review need to be conducted as part of a "process control" program. The frequency of data collection will depend on the type of testing being performed. For instance, for a netted melon field, pathogen testing could be performed to accept a lot or as a requirement from a customer. Meanwhile, for agricultural water, water quality from a single source may be assessed by weekly or monthly testing of indicators. The frequency of testing must be defined to assess how the system may be changing over time and to provide confidence that the food or matrix (e.g., cantaloupe or water) has not exceeded established microbiological limits. Once the frequency of data collection has been established, it is important to define a way to analyze the test results. For example, in statistical process control, trends over time (e.g. positivity rate of my fields per week or levels of the same water source over time and especially after an observed or known shift in risk, (i.e., rain event > 2 inches or placement of masses of soil amendment next to an irrigation canal) are frequently analyzed.

Data Management

To properly implement these systems, effective data management needs to be followed. Some simple concepts can be found in *Best Practices for Sharing Tissue and Water Data* for GreenLink®. Below is a summarized version of best data management practices. Use machine-readable software (e.g., Microsoft Excel) to maintain your data. You can analyze your data using Excel or statistical software such as R, Python, or SAS.

- Follow these data format best practices when storing your results (Table 2)
  - Columns: Each column represents a unique piece of data or metadata (e.g., sample ID, date collected, EHEC/STEC result).
  - Rows: Each row represents an individual observation or sample.
- Data types:
  - Numeric: For numbers, avoid adding empty spaces before or after a number. Avoid putting units within the cell. The units should be identified in a separate cell or as part of a dictionary (e.g. 100, 126, etc.).
  - Text: Ensure text entries are consistent for the following areas
    - Font case: all-lower case, all-upper case, sentence case
    - Be consistent when referring to the same item. For example, if you are recording the variety "Cantaloupe," always use the same term in the same written form and avoid multiple ways to refer to this item (e.g., "Cantaloupe, Melon"; "Cantaloupes"; etc.)
      - Include commodity and variety as below
        - [Melon][Cantaloupe]
        - [Melon] [Galia]
    - If a template is specified, try to match the format established in the template. An example could be that all text entries should be in lowercase letters (e.g., salinas, cantaloupe, etc.).
  - Categorical: For categorical data fields, use drop-down menus with the specified categories (e.g., type a, type b, type c). Using drop-down menus helps avoid adding additional unwanted categories.
- Missing values: Leave blank or put NA. Avoid putting in values that could be misinterpreted, such as "0" or "-".

Table 3: Illustration of table, columns, and rows for data management

		Data or Metadata for the sample			
		Column 1 Data field 1	Column 2 Data field 2	Column ... Data field ...	Column x Data field x
Individual samples	Row 1 Sample 1				
	Row 2 Sample 2				
	Row 3 Sample 3				
	Row y Sample y				

WHY TEST? SHOULD I TEST PRODUCT?

Microbiological testing is a tool. Before sampling any product/field/site, clarify what decision the result will drive (e.g., insight into a situation, release of product to commerce, "for cause" investigation, etc.). In agricultural fields, contamination has often been found to be rare, of low pathogen concentration, and unevenly distributed. These characteristics of contamination limits the applicability and power of routine pathogen product testing to "clear" lots. Product testing with these types of conditions is generally impractical to implement due to the large number of samples that would need to be taken to be statistically capable of detecting a pathogen if it were present. As such testing program design is critical for when/if testing should be used.

Strategically planned and well-designed testing programs can offer insight into whether controls are working, if conditions have drastically changed, and/or help target corrective actions when needed. Indiscriminately applied testing without careful planning often leads only to added cost and little to no functional impact on improved safety.

Primary reasons for the test

- Decision to release or divert: Due to the reasons mentioned above (low levels and pattern of contamination), lot acceptance testing is often not encouraged due to practical limitations in designing enough statistical power into the sampling program. However, if lot acceptance testing is to be employed, verify that a defined lot meets a criterion (e.g., absence of Salmonella) under a documented testing program.
- Best practices require pre-defined decision rules, including how to respond if an adjacent lot tests positive, if multiple lots test positive within a short time window, or if patterns emerge that suggest a broader contamination event. Factors to consider include (but are not limited to):
  - o If an adjacent lot is positive, design a means to consider adjacent lots. Example: consider if it is warranted to treat the associated lot as potentially linked due to shared conditions or handling steps. Consider input, machinery, harvest crews, etc. in the assessment.

  - Do additional investigatory sampling in adjacent lot/lot that tested positive to learn and identify potential root causes. Evaluate inputs used, irrigation information, activities that may have been occurring on adjacent land, etc.
  - Multiple positive test results should trigger further exploration. Nearby and adjacent lots with shared similar growing and environmental characteristics may also be impacted and should be thoroughly assessed.
- Verification of controls: Testing (often using indicators), when designed appropriately, can help confirm over an extended timeline (i.e. quarter or season) that sanitation, water quality, or hygienic design programs/changes are effective.
- Investigation / corrective action: Testing can support root-cause analysis after a deviation, complaint, crop-destruct event, or outbreak signal. Ensure testing design is optimized for the situation to ensure greatest value from testing.
- Regulatory or customer requirement: Satisfy specific specifications in contracts or regulations. Due to testing limitations, it is strongly encouraged that conversations about the intent, value, and purpose of this type of testing be held to ensure it delivers on need/expected value (see points below).

When routine product pathogen testing adds little value

- Expected prevalence is very low and contamination is patchy (i.e., not uniformly spread in a field/lot). In these scenarios, individual samples (low number of samples) will have low detection power/likelihood and can often create a false confidence in the safety of a lot.
- Testing is pursued as a substitute for risk reduction controls (e.g., hygienic design, PEC, water/source controls, exclusion/harvest decisions). Testing alone is not a mitigation or management practice. Testing is a tool to inform, monitor, or identify potential issues (when well designed).

1 MicroTally Mitt_Sampler vs Rinse Sampling on Cantaloupe

2 Aggregated Sampling of Cantaloupe_Using MicroTally Mitt Samplers

3 Sbodio A, Maeda S, Lopez-Velasco G, Suslow T. 2013. Modified Moore swab optimization and validation in capturing *E. coli* O157:H7 and Salmonella enterica in large volume field samples of irrigation water. Food Res Int. 51:654-62. Modified Moore swab optimization and validation in capturing E. coli O157:H7 and Salmonella enterica in large volume field samples of irrigation water - ScienceDirect

4 Leskinen SD, Kearns EA, Jones WL, Miller RS, Bevitas CR, Kingsley MT, Brigmon RL, Lim DV. 2012. Automated dead-end ultrafiltration of large volume water samples to enable detection of low-level targets and reduce sample variability. J Appl Microbiol. 113(2):351-60.

5 McEgan R, Rodrigues CA, Sbodio A, Suslow TV, Goodridge LD, Danyluk MD. 2013. Detection of *Salmonella* spp. from large volumes of water by modified Moore swabs and tangential flow filtration. Lett Appl Microbiol. 56(2):88-94.

TEMPLATE A DOCUMENTATION OF TESTING METHOD CONDITIONS

Goal: Describe conditions used for testing product for human pathogens; details will allow additional interpretations of an data analysis for results originating from a testing plan.

Instructions: Provide this form to the laboratory performing your product testing. File the completed form with you food safety plan. Lab, complete the form for client records. Deviations from the lab are not authorized without client notification and acceptance as testing methodology is defined in food safety records/plans.

Food matrix to be tested (every item with unique conditions must be listed – e.g., cantaloupe, water)	**Analyte to be tested for.** (Salmonella, STEC, etc.)	**Method certification** (AOAC, AFNOR, internal, etc.)	**Initial detections confirmed by what method?** (BAM, MLG, ddPCR, IMS, etc.)	**Amount Tested* (x Mass, x Cantaloupes, x Swabs)** **amount physically tested*	**Sample to media enrichment ratio (1:2, 1:3, 1:4, 1:5, 1:10, etc.)**	**Incubation time ex: 10-12 hours. 2-hour range acceptable.**	**Is there a regrow/ secondary enrichment or DNAse step?**	**Are detection samples wet-pooled post-enrichment?**

Are all steps performed above included in the official method certification/validation (e.g. enrichment ratios, wet-pooling, confirmation methods, etc.)

Lab company:

Title of person completing this form:

Name of person completing this form:

Date:

REFERENCES AND RESOURCES

- CAC/GL 50-2004. General guidelines on sampling. Microsoft Word - CXG_050_2004e.doc
- FDA. Bacteriological Analytical Manual. Bacteriological Analytical Manual (BAM) | FDA
- FDA. Guidelines for the validation of analytical methods for the detection of microbial pathogens in foods and feeds. FDA Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds
- ICMSF. 2011. Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance. Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance | SpringerLink
- ISO 874. 1980. Fresh fruits and vegetables – Sampling. Reviewed in 2023. ISO 874:1980 - Fresh fruits and vegetables — Sampling
- Leskinen SD, Kearns EA, Jones WL, Miller RS, Bevitas CR, Kingsley MT, Brigmon RL, Lim DV. 2012. Automated dead-end ultrafiltration of large volume water samples to enable detection of low-level targets and reduce sample variability. J Appl Microbiol. 113(2):351-60.
- McEgan R, Rodrigues CA, Sbodio A, Suslow TV, Goodridge LD, Danyluk MD. 2013. Detection of *Salmonella* spp. from large volumes of water by modified Moore swabs and tangential flow filtration. Lett Appl Microbiol. 56(2):88-94.
- Sbodio A, Maeda S, Lopez-Velasco G, Suslow T. 2013. Modified Moore swab optimization and validation in capturing *E. coli* O157:H7 and *Salmonella enterica* in large volume field samples of irrigation water. Food Res Int. 51:654-62.
- USDA FSIS. Microbiological Laboratory Guidebook. Microbiology Laboratory Guidebook | Food Safety and Inspection Service