NARMS

National Antimicrobial Resistance Monitoring System

2025 RETAIL SEAFOOD LABORATORY PROTOCOL

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Objective:
Determine the prevalence of antimicrobial resistance in *Aeromonas*, *Enterococcus* and *Vibrio* isolates from retail seafood samples. Samples of fresh or previously frozen seafood are purchased from retail grocery stores in the United States.

Sample collection:

Product	Number Tested Per Month	Vibrio	Enterococcus	Aeromonas
Shrimp	2	2	2	2
Tilapia	2	2	2	2
Salmon	2	2	2	2
Total	**6**	**6**	**6**	**6**

- Look for raw tilapia, shrimp, and salmon. The preference is to collect fresh or previously frozen seafood, if available. If fresh or previously frozen shrimp, tilapia, or salmon are not available in a grocery store, frozen seafood may be collected. Salmon should be SKIN-ON, if possible. All seafood can be domestically - reared or imported.

- More than one seafood sample may be collected from the same store if samples have different brands, or distributors, or sell by dates. Seafood can be purchased in bulk or by weight.

- Record the demographic information for each sample including store name and location, brand name, sell-by date, purchase date, lab processing date, and country of origin for each meat sample on the electronic log sheet. The selection of country of origin is at the lab's discretion. Please try to select as wide a variety as possible. Record if the product was frozen, previously frozen, or fresh. If the package indicates whether the seafood was farm-raised or aqua-cultured, please indicate that as well.

- **IMPORTANT NOTE:** The monthly sampling and testing schedule is decided at the lab's discretion. Please keep in mind that fresh or previously frozen seafood expire more quickly than retail meats, and therefore will shorten the time to process. If frozen seafoods are collected, they can be processed at later dates.

Sample Set-up:
Media should be brought to room temperature prior to inoculation for use on each day as needed below. Do not open packages until ready to begin processing. **Fresh seafood samples should be processed within 96 hours after purchase**. Place intact packages of shrimp and salmon samples on a clean surface and aseptically open. **Preferably, do not thaw frozen samples before analysis.** If frozen sample must be tempered to obtain analytical portion, thaw below 45°C for <15 minutes with continuous agitation in thermostatically controlled water bath or thaw within 18 hours at 2-5°C. Aseptically remove shrimp or salmon samples with sterile instruments (e.g., tongs, gloves, or

spoons).
***Vibrio Species*:**

Day 1:
Aseptically weigh 25g of each commodity of (**shrimp, tilapia, and salmon)** separately into a sterile stomacher bag. Add 225ml of Alkaline Peptone Water (APW) and stomach for 2 minutes at 230 RPM or blend sample for 2 minutes. Incubate enrichment at 35°C for 24 ± 2 hours.

Day 2:
Streak overnight enrichment onto TCBS agar incubate at 35°C for 18-24 hours. Repeat for each sample.

Day 3:
Observe TCBS agar plates. If typical growth, pick green and yellow colonies. Pick one isolated colony of each color to a blood agar plate (BAP). For each sample pick 1 to 2 colonies from TCBS agar. Incubate each BAP at 35°C for 24 ± 2 hours. If no typical growth is observed, the sample is negative and can be discarded; complete the log sheet and select no for *Vibrio* spp.

Day 4:
Examine each blood agar plate for purity and typical *Vibrio* colonies. Perform oxidase test on each blood agar plate. If oxidase positive, keep. If oxidase negative, discard. FDA will identify all isolates sent to us. If the growth is pure, swab the growth into Brucella broth with 10-15% glycerol mixture and freeze at -60 to -80°C. Repeat procedure for each BAP. Ship all isolates on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

***Enterococcus and Aeromonas* species:**

Day 1:
Aseptically weigh 25g of shrimp, salmon, and tilapia separately into a sterile stomacher bag. Add 225 ml of Buffered Peptone Water (BPW) broth and stomach for 2 minutes at 230 RPM or blend sample for 2 minutes.

Day 2:
***Enterococcus* species**: Streak one 10 µl loopful of BPW broth onto the first quadrant of an Enterococcosel agar plate. Streak the remainder of the plate to obtain isolated colonies. Repeat procedure for each container. Incubate plates at 35°C for 24 hours.

***Aeromonas* species:** Streak one 10 µl loopful of BPW broth onto the first quadrant of a Glutamate Starch Phenol Red Agar plate. Streak the remainder of the plate to obtain isolated colonies. Repeat procedure for each container. Incubate at 29°C for 24-48 hours.

Day 3:
Enterococcus species: Examine each Enterococcosel agar plate for typical Enterococcus colonies (brownish black to black zones around colonies). If no typical growth is observed on the Enterococcosel agar plate, sample is negative and can be discarded. Record negative result on log sheet for Enterococcus. If typical growth is present, select one typical, well-isolated colony and

streak for isolation onto a BHI (or other non-blood containing) agar plate. If the Enterococcosel plate does not have isolated colonies, streak again to another Enterococcosel plate before subculturing to BHI. Repeat procedure for each Enterococcosel agar plate. Incubate BHI plate(s) at 35°C for 24 hours.

Aeromonas species: Examine each Starch agar for typical Aeromonas colonies. Typical colonies are yellow to honey-colored. If typical growth is present, select one isolated colony. Streak one colony to BAP and incubate at 35°C for 24 ± 2 hours. If no typical growth is observed on Starch agar plates, the sample is negative and can be discarded; indicate results on log sheet for Aeromonas.

Day 4:

***Enterococcus* species**: Examine each BHI agar plate for purity and typical enterococci colonies. If no typical growth is observed, sample is negative and can be discarded; complete the log sheet and select no for *Enterococcus*. If typical growth is observed, Gram stain the suspected colonies. If the Gram stain is atypical, sample is negative for enterococci and can be discarded; complete the log sheet and select no for *Enterococcus*. If Gram-positive cocci are observed, perform a catalase test. If catalase negative, confirm further with a PYR test. If catalase positive or PYR negative, plates may be discarded; complete the log sheet and select no for *Enterococcus*. If results produce catalase negative and PYR positive, record the isolate as *Enterococcus*. Repeat procedure for each BHI agar plate. Subculture one well isolated colony from BHI to blood agar plate. Incubate at 35°C for 24 hours.

***Aeromonas* species:** Examine each blood agar plate for purity and typical *Aeromonas* colonies. Pick one colony for oxidase test. If it is oxidase positive, please keep for freezing. If it is oxidase negative, please discard. If the growth is pure swab the growth into Brucella broth with 10-15% glycerol mixture and freeze at -60 to -80°C. Repeat procedure for each blood agar plate. Ship all isolates on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

Day 5:

Enterococcus* species:* Examine each blood agar plate for purity and typical *Enterococcus* colonies. If the growth is pure, swab the growth into Brucella broth with 10-15% glycerol mixture and freeze at -60 to - 80°C. Repeat procedure for each BAP. Ship all isolates on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

Preparing Isolates for Shipment:
Label each vial with NARMS isolate ID (MANDATORY). The NARMS isolate ID on the vial should match the NARMS isolate ID on the log sheet. Labels **should not** be handwritten or taped to the tube. Handwritten or taped on labels come off during the freezing process. Laboratories should keep duplicates of strains within their culture collections until notified by FDA-CVM that the duplicates may be discarded (isolates can be discarded once the NARMS report for the testing year has been published).

Isolate IDs should follow the following format:
Vibrio: Year + State + Month + Source + Sample Number-V+colony number (Example: 23SC08SH01-V1 or 23SC08SH01-V2 if green and yellow colonies are present)

Enterococcus: Year + State + Month + Source + Sample Number-E (Example: 23SC08SH01-E)
Aeromonas: Year + State + Month + Source + Sample Number-A (Example: 23SC08SH01-A)

Source Key:
Shrimp: SH **Salmon:** SA **Tilapia:** TI

Packaging the Isolates:
Ship all isolates in cryogenic vials with parafilm wrapped tops to keep tops from becoming unscrewed (**DO NOT use excessive parafilm on the tubes**). Cryogenic vials should be properly wrapped with absorbent material to prevent leakage during shipment. Place cryogenic vials in a shipping container with plenty of dry ice placed in a box for shipping (EXTRA dry ice during warmer months). Cryogenic vials should be shipped to FDA-CVM in accordance with current shipping of hazardous material guidelines. A physical copy of the electronic log sheets must be included with each isolate shipment to FDA-CVM. Each site should retain copies of log sheets for their records.

Shipping the Isolates and Notification:
Prior to shipping isolates to FDA-CVM, sites **must** e-mail FDA-CVM a shipping notification and a copy of the completed electronic log sheets to **NARMSRetail@fda.hhs.gov**. Packages should be sent overnight. Please ship isolates so they will arrive at FDA-CVM by Friday. If the isolates will not arrive by Friday, please store them in your freezer and ship the following Tuesday. Please hold isolates so they do not arrive at FDA-CVM on a holiday. Shipments must occur each month. Send all shipments to **Shawn McDermott** at the following address:

Shawn McDermott
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