2025 Retail Meat Surveillance Laboratory Protocol

National Antimicrobial Resistance Monitoring System (NARMS)
2025 Retail Meat Surveillance Laboratory Protocol

Objective:
Determine the prevalence of antimicrobial resistance in *Salmonella*, *Campylobacter*, *Escherichia coli* and *Enterococcus* isolates from retail meat and poultry samples. Samples of **fresh** (not frozen) chicken without added spices, ground turkey, ground beef, and chicken giblets are purchased from retail grocery stores in the United States.

Sample collection:

PRODUCT	NUMBER TESTED PER MONTH	SAL	CAMPY	E. COLI	ENTERO
Retail Chicken	4	4	4	4	2
Ground Turkey	6	6	NT	6	1
Ground beef	4	4	4	4	1
Chicken Liver	1	1	1	1	1
Chicken Gizzard	1	1	1	1	1
Chicken Heart	1	1	1	1	1
Total	**17**	**17**	**11**	**17**	**7**

Note: Ground pork **will not be collected or tested** for any organism for the 2025 sampling year.

Purchase a total of 17 fresh (not frozen) retail meat samples per month:

- 4 retail chicken (bone-in/skin-on) without added spices
- 6 ground turkey (any fat percentage is acceptable)
- 4 ground beef (80/20 preferred if available)
- 1 each of chicken liver
- 1 each of chicken gizzard
- 1 each of chicken heart

Where to sample:
FDA-CVM will provide a monthly (randomly selected) zip code assignment file, which includes 4 primary zip codes and 5 secondary zip codes. Please visit grocery stores in each of the 4 primary zip codes, which can be on separate trips. Where possible, diversify stores visited within a zip code and equally distribute the number of samples collected among the stores visited. If there are any issues with the primary zip code list, visit stores on the secondary list. **Rural areas are visited the first month starting each quarter (January, April, July, October). For these months, one sample for each meat type will be collected in the rural zip code and the remaining samples will be collected from the city zip codes.**

Chicken parts: For retail chicken samples, purchase fresh bone-in/skin-on breasts. If bone-in/skin-on breasts cannot be purchased, purchase fresh bone-in/skin-on thighs or wings. If fresh whole chickens or mixed parts are purchased, test the chicken breast. For a whole chicken, using sterile scissors (sterile scalpel or a sterile knife), cut off and test a chicken breast. If chicken breasts, thighs, or wings cannot be found, purchase fresh chicken legs. **One or two of the retail chicken samples must be organic or raised without antibiotics.**

Chicken giblets: Purchase fresh chicken giblets separated by type: liver, heart, and gizzard. If fresh chicken giblets separated by type cannot be found, purchase a package of fresh mixed giblets. **Do not test more than one type of giblet from the mixed package.** If a whole chicken is purchased with giblets stuffed inside, these giblets are considered as **one** chicken giblet package. When available, purchase giblets with different "sell by dates". This is preferred, but not required.

Ground turkey and ground beef: If organic ground turkey or ground beef are available, they can be purchased, but this is not a requirement. If ground turkey is not available, collect ground turkey breast or ground turkey patties which have not been previously frozen.

Record demographic information for each purchased meat sample on the monthly log sheets. Demographic information must include store name and address, zip code, brand name, sell-by date, purchase date, lab processing date, raising claim, brand code and establishment number. Record whether the meat or poultry sample was packaged in the store and the packaging type[1]. If the information is provided on the meat package, record the country(ies) of origin. Keep samples on ice during transport from the grocery stores to the laboratory. Refrigerate samples at 4°C (without ice) at your site and begin laboratory processing within 96 hours after purchase.

[1]Examples of the packaging types located in Appendix A (pgs. 9-12).

Processing Day 1:

Fresh samples should be processed within 96 hours of purchase. Media should be brought to room temperature prior to use on each day as needed below. Do not open packages until ready to begin processing. Place intact packages of meat and poultry samples on a clean surface and aseptically open. Ensure external surface and edges of wrappings do not touch meat or poultry samples. Aseptically remove meat or poultry sample with sterile instruments (e.g., tongs, gloves, or spoons). Each site should photocopy or photograph all meat and poultry package labeling and save for 5 years from date of capturing photograph.

Set Up Process:
Aseptically place one retail chicken breast with bone-in/skin-on from each package of chicken into a sterile, filter plastic bag (example: stomacher bag with filter). More than one piece of chicken may be used to achieve a 50 g sample when substituting chicken breast with another bone-in/skin-on chicken part. More than one piece of the chicken giblet may be needed to achieve a 50 g sample. If two pieces are needed, it must be two hearts, two livers or two gizzards. Aseptically place 50 g of ground turkey from each package into a sterile, filter plastic bag. Aseptically place 50 g of ground beef into a sterile, filter plastic bag. Repeat this process for each package of meat and poultry. There should be one sterile, filter bag for each meat and poultry sample. Add 250 ml buffered peptone water to each sterile, filter bag. For bone-in samples, carefully hand massage samples for 3 minutes (being careful not to puncture the bag) or use a mechanical shaker at 200 rpm for 15 minutes. For ground meat products, stomach at low speed (230 rpm) for 90 seconds, hand massage for 3 minutes, or use a mechanical shaker at 200 rpm for 15 minutes until clumps are dispersed.

Enrichment Preparation:
Aseptically transfer 50 ml rinsate from the filtered side (liquid only) of each bag into a separate sterile flask (or other suitable sterile container) for *Campylobacter*. For sites doing *E. coli* and/or *Enterococcus*, an additional 50 ml rinsate from each bag will be transferred into separate sterile containers.

Salmonella
Leave chicken parts, chicken giblets and ground meats in the remaining Buffered Peptone Water (BPW) and incubate at 35°C for 24 hours.

Campylobacter
Add 50 ml double strength (2X) Bolton broth to each container of 50 ml rinsate used for *Campylobacter* isolation. Mix thoroughly, but gently to avoid aeration, incubate containers or bags (caps loosened or closed loosely) in a microaerophilic atmosphere (85% N2, 10% CO2, 5% O2), or in a jar with a CampyPak for 24 hours at 42°C. Do not stack containers. This will prevent samples from achieving the appropriate microaerophilic atmosphere.

Campylobacter Note:
For 2025 testing year, retail chicken, giblets and ground beef samples WILL be tested for *Campylobacter* only.

E. coli

Add 50 ml double strength (2X) MacConkey broth to each container of 50 ml rinsate used for *E. coli* isolation. Mix thoroughly and incubate at 35°C for 24 hours.

Enterococcus

Add 50 ml double strength (2X) Enterococcosel broth to each container of 50 ml rinsate used for *Enterococcus* isolation. Mix thoroughly and incubate at 45°C for 24 hours.

Processing Day 2:

Salmonella

LAMP Screening Assay (see BAM Chapter 5: *Salmonella*, and navigate to C.28 for a detailed protocol):

Gently mix the overnight bag of BPW enrichment. Transfer 1 ml from the filtered side of the bag to a microcentrifuge tube. Vortex briefly. Extract sample DNA using PrepMan ultra sample preparation reagent; briefly, centrifuge first at 900 x *g* for 1 min to remove large particles and transfer supernatant to a clean tube. Centrifuge again at 16,000 x *g* for 2 min and discard supernatant. Suspend the pellet in 100 µl of PrepMan, heat at 100 ± 1°C in a dry heat block for 10 min, cool to room temperature, and centrifuge again at 12,000 x *g* for 2 min. Transfer supernatant to a clean tube as your DNA extract.

Turn on the Genie II instrument and enter sample information. Gather LAMP supplies and thaw reagents and controls at room temperature. Prepare the LAMP reaction mix by adding the appropriate amount (refer to BAM C.28 protocol, Table 3a on page 5 for volume adjustment based on the number of samples) of ISO-004 (15 µl per sample), 10× primer mix (2.5 µl per sample), and water (5.5 µl per sample) to a microcentrifuge tube. Include one positive control (PC) and one no template control (NTC) in every LAMP run. Distribute 23 µl into each well of a Genie strip. In a separate location of the same lab or in another lab (to avoid cross-contamination), add 2 µl of sample DNA extract to each well and cap tightly. Flick the Genie strips with your wrist to ensure all reagents have pooled at the bottom of the tube. Load the Genie strips into Genie II block A and/or B and start the run.

Data interpretation is as follows:

1. Examine the control wells first. The NTC well should be blank for “Peak Ratio” and “Anneal Peak.” The PC well should have a “Peak Ratio” < 10 min and “Anneal Peak” around 89 ± 2°C. Repeat the run if either control is invalid.
2. All samples with “Peak Ratio” ≤ 20 min and the correct “Anneal Peak” (89 ± 2°C) are considered LAMP-positive for Salmonella. Continue with Salmonella Processing Day 2 through Day 5.
3. For wells with the correct “Anneal Peak” (89 ± 2°C) but “Peak Ratio” > 20 min or blank, manually observe amplification curves and either repeat LAMP or record samples as presumptive LAMP-positive and continue with Salmonella Processing Day 2 through Day 5.
4. For samples screening negative by Salmonella LAMP, stop here at Processing Day 2. Record Salmonella negative result on the log sheet.

Transfer 0.1 ml from overnight BPW to a test tube containing 10 ml RVR10 (Rappaport- Vassiliadis) medium. Incubate test tubes of RVR10 medium in a water bath at 42°C for 18-24 hours.

Campylobacter

Carefully mix 2X Bolton broth avoiding aeration. Dip a cotton tip (*Campylobacter* does not do well with synthetic tipped swabs) swab into one container until saturated and swab the first quadrant of a Campy Cefex Agar (CCA) plate. Discard swab. Using a loop, streak the remainder of the plate to obtain isolated colonies. Repeat procedure for each container. Incubate the plates in a microaerophilic atmosphere (85% N2, 10% CO2, 5% O2) or in a jar with a CampyPak at 42°C for 24 hours.

E. coli

Streak one 10 µl loopful of broth from one container of 2X MacConkey broth onto the first quadrant of a MacConkey (MAC) 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.

Enterococcus

If no growth or blackening is observed in a container, sample is negative and can be discarded; complete the log sheet and place a check mark under the no growth column. If growth and blackening is observed in the container, streak one 10 µl loopful of 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 that has growth *and* blackening. Incubate plates at 35°C for 24 hours.

Processing Day 3:

Salmonella

For each RVR10 culture, vortex or mix the enrichment broth and streak one 10 µl loopful of broth onto the first quadrant of one XLT-4 plate, incubate at 35°C for 18-24 hours.

Salmonella Note:

Optional Selective Agars: Chromogenic agar, HE agar, or a selective non-H2S producing agar for *Salmonella* may be used in conjunction with XLT-4 to obtain a positive isolate. These suggested agars do not replace XLT-4 agar.

Campylobacter

Examine each CCA plate for typical *Campylobacter* colonies (round to irregular with smooth edges; thick translucent white growth to spreading, film-like transparent growth). If typical growth is present, select one typical, well-isolated colony and streak for isolation onto a blood agar plate (BAP). If the CCA plate does not have isolated colonies, streak again to another CCA before subculturing to BAP. Incubate the BAP in a microaerophilic atmosphere (85% N2, 10% CO2, 5% O2) or in a jar with a CampyPak at 42°C for 24 hours. Repeat procedure for each CCA plate. If a CCA plate does not have any typical colonies or little growth, re-incubate the plate at 42°C for an additional 24 hours. Chromogenic agar may be used in conjunction with CCA in identifying *Campylobacter*. Indicate on the log sheet when you are using Chromogenic agar and sending an isolate, from an agar other than the CCA.

E. coli

Examine each MAC plate for typical *E. coli* colonies (pink colonies). If no typical growth is observed on MAC agar plate, sample is negative and can be discarded. Record negative result on log sheet for *E. coli*. If typical growth is present, select one typical, well-isolated colony and streak for isolation onto a blood agar plate. If the MAC plate does not have isolated colonies, streak again to another MAC before subculturing to BAP.

Incubate blood agar plates at 35°C for 18-24 hours.

Enterococcus

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.

Processing Day 4:

Salmonella

Examine each XLT-4 plate for typical *Salmonella* colonies (pink colonies with or without black centers; colonies may have large, glossy black centers or may appear as almost completely black colonies). If typical or atypical growth is present, pick two colonies from XLT-4. If the XLT-4 plate does not have isolated colonies, streak again to another XLT-4 before subculturing to BAP. If typical growth is present, confirm that the two colonies are *Salmonella*. Once confirmed, please pick one colony for isolation onto a blood agar plate. Incubate blood agar plate at 35°C for 18-24 hours. Repeat for each sample. If no typical growth is observed on XLT-4, sample is negative and can be discarded. Record negative result on log sheet for *Salmonella*.

Campylobacter

For re-incubated CCA plates: Examine each re-incubated CCA plate for typical colonies (round to irregular with smooth edges; thick translucent white growth to spreading, film-like transparent growth). If no typical growth is observed, sample is negative and can be discarded. Record negative result on log sheet for *Campylobacter*. If typical growth is present on CCA plate, select one typical, well- isolated colony and streak for isolation onto a blood agar plate. If the CCA plate does not have isolated colonies, streak again to another CCA plate before subculturing to BAP. Repeat procedure for each CCA plate. Incubate blood agar plate(s) in a microaerophilic atmosphere (85% N2, 10% CO2, 5% O2) or in a jar with a CampyPak at 42°C for 24 hours.

For blood agar plates: Examine each blood agar plate for purity. If growth is pure and colonies are typical**,** perform a Gram stain, oxidase, and catalase tests to confirm growth as *Campylobacter* (motility and hippurate tests are optional). If there is no typical growth on blood agar plate, or no colonies are positive for *Campylobacter* (Gram-negative rods with corkscrew motility, catalase positive, oxidase positive), sample is negative and can be discarded. Record negative result on log sheet. If hippurate test is performed and is positive, record on log sheet as *C. jejuni*. If positive for *Campylobacter* and hippurate negative, use PCR testing or sequencing to confirm as *C. coli*. (Note: a validated PCR method is optional and must have approval by FDA-CVM prior to use). If *Campylobacter* positive and no speciation is done, record on log sheet as *Campylobacter* species. Repeat procedure for each sample blood agar plate. Swab the growth from the blood agar plate and suspend in Brucella broth with 10-15% glycerol mixture and freeze at -60 to -80°C. Ship all isolates in a cryovial on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

E. coli

Examine each blood agar plate for purity and typical *E. coli* colonies. If no typical growth is observed, sample is negative and can be discarded. Record negative result on the log sheet. If typical growth is observed, perform an indole test (oxidase optional) on each blood agar plate. If the growth is pure, and indole positive (oxidase negative), swab the growth from the blood agar plate and suspend in 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.

Enterococcus

Examine each BHI agar plate for purity and typical Enterococcus colonies. If no typical growth is observed, sample is negative and can be discarded. Record negative result on the log sheet. 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. Record negative result on the log sheet. If Gram-positive cocci are observed, perform a catalase test. If catalase negative, confirm further with a PYR test (positive: pink to red color development; negative: cream, yellow or no color change; follow manufacturer's instructions for use). If catalase positive or PYR negative, plates may be discarded. Record results on the log sheet. If results produce catalase negative and PYR positive results, record the isolate as positive for *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.

Processing Day 5:

Salmonella

Examine each blood agar plate for purity. If pure, confirm as *Salmonella* by standard biochemical methods, API, VITEK or MALDI-TOF and serotype (optional). For sites that confirm and serotype both isolates and get 2 different serotypes from one meat sample, sequence both isolates and forward both isolates to CVM for further testing. Repeat for each blood agar plate. Swab the growth from the blood agar plate and suspend in Brucella broth with 10-15% glycerol mixture and freeze at -60 to -80°C. Ship all isolates on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

Campylobacter

Examine each blood agar plate for purity. For plates that were re-incubated from Processing Day 3 with low growth, recheck for growth and continue processing. If growth is pure and colonies are typical**,** perform a Gram stain and an oxidase, catalase (motility and hippurate tests optional) to confirm growth as *Campylobacter*. If there is no typical growth on blood agar plate or no colonies are positive for *Campylobacter* (Gram-negative rods with corkscrew motility, catalase positive, oxidase positive), sample is negative and can be discarded. Record negative result on the log sheet. If positive for *Campylobacter* and hippurate positive, record on log sheet as *C. jejuni*. If positive for *Campylobacter* and hippurate negative, use PCR testing or sequencing to confirm as *C. coli*. (Note: a validated PCR method is optional and must have approval by FDA-CVM prior to use). Repeat procedure for each blood agar plate. If an isolate is positive for *Campylobacter* and no speciation is done, record on log sheet as *Campylobacter* species*.* Swab the growth from the blood agar plate and suspend in Brucella broth with 10-15% glycerol mixture and freeze at -60 to -80°C in Brucella broth with 10-15% glycerol mixture and ship all isolates on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

Enterococcus

Examine each blood agar plate for purity and typical *Enterococcus* growth. Swab the growth from the blood agar plate and suspend in Brucella broth with 10-15% glycerol mixture and freeze at -60 to -80°C. Ship all isolates on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

Metadata Entry into Log Sheets:

Please refer to the log sheet tutorial. Please reach out to Yesha at yesha.shrestha@fda.hhs.gov with any questions or concerns.

Whole Genome Sequencing*:

*Refer to PulseNet and GenomeTrakr sequencing protocols. Also, refer to WGS Workflow, Template and NCBI submission document for additional instructions.

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. Please use cryogenic labels (freezer labels). 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).

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:

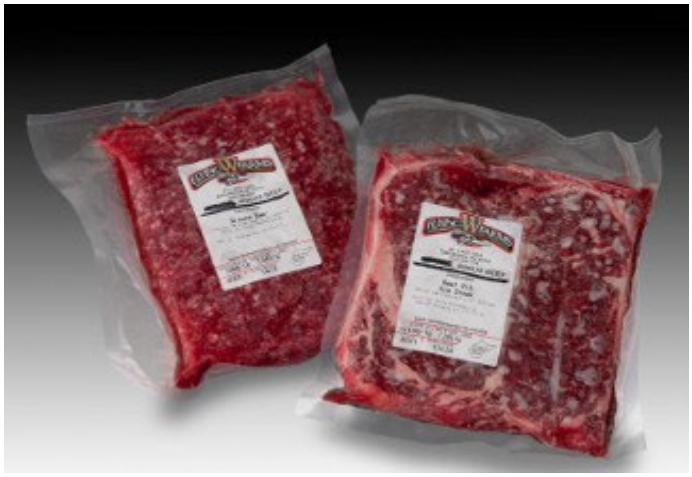
Prior to shipping isolates to FDA-CVM, sites must e-mail FDA-CVM a shipping notification and a copy of the completed electronic log sheets to **NARMSRetail@fda.hhs.gov**. Packages should be sent overnight. Please ship isolates so they will arrive at FDA-CVM by Friday of each week. If the isolates will 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
FDA, Center for Veterinary Medicine
Office of Applied Science
8401 Muirkirk Road
Laurel, MD 20708
Phone: 240-402-5447

Appendices

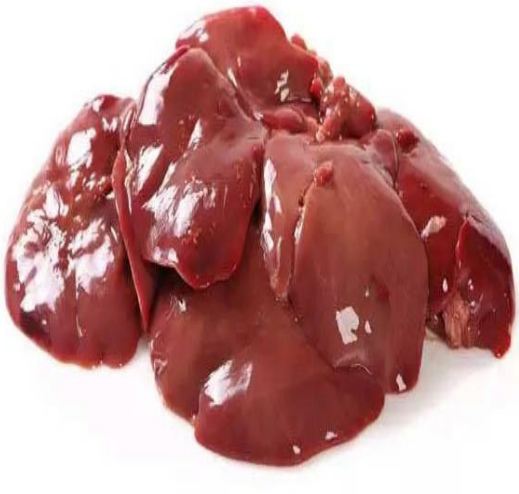
Appendix A: Retail Meat Package Types

MAP packed (modified atmosphere packaging)

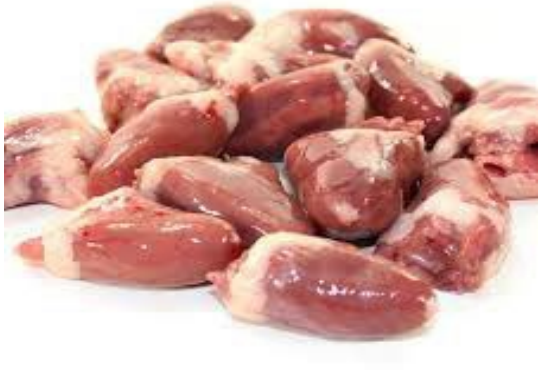


Vacuum packaging

Saran wrapped meat packaging

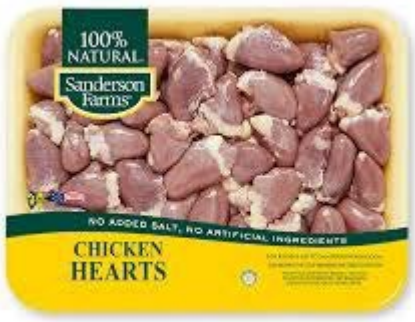


Fresh CL (chicken liver)

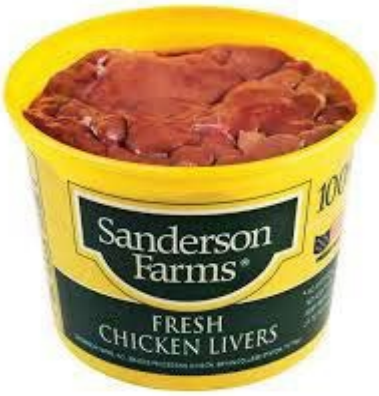


Fresh CH (chicken heart)

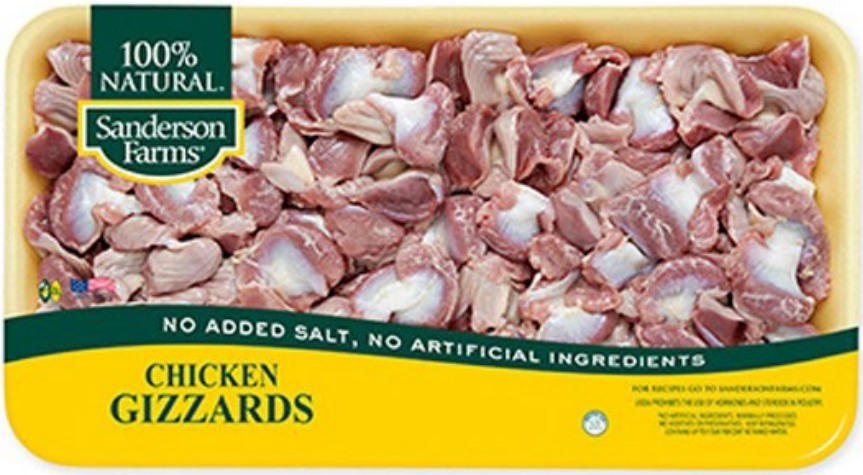
Fresh CG (chicken gizzards)



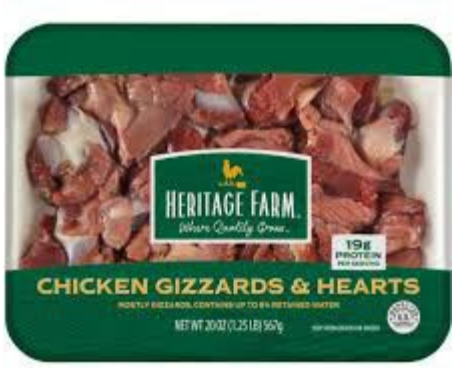
Packaged CH (chicken hearts)



Packaged CL (chicken livers)



Packaged CG (chicken gizzards)



Packaged mix of CG (chicken gizzards) & CH (chicken hearts)