
Response to Questions Posed by the Department of Defense Regarding Microbiological Criteria as Indicators of Process Control or Insanitary Conditions

Adopted 10JUN2015, Washington, DC

NATIONAL ADVISORY COMMITTEE ON MICROBIOLOGICAL CRITERIA FOR FOODS

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EXECUTIVE SUMMARY

The Department of Defense (DOD) purchases a grocery-store array of foods (hereafter to include bottled water and packaged ice) throughout the world. DOD primarily uses the assessment of a supplier's food safety plan, including its HACCP system, to determine whether a supplier is an acceptable supplier to meet its mission requirements. For these suppliers, DOD can rely less on microbiological testing and more on process-oriented, risk-based preventive controls that ensure the supplier's manufacturing process is controlled and sanitary conditions are maintained. However, some mission requirements include the need to purchase foods where suppliers may not have fully developed food safety plans, including HACCP systems. In these instances, DOD has a need for standardized sampling and testing programs that reflect process control and assess sanitary manufacturing conditions. Such programs, defined herein, would enable DOD to monitor suppliers from centralized locations, prioritize supplier audits, and conduct cost-effective and meaningful verification testing.

To assist DOD with its ability to assess suppliers that do not have well-established food safety plans, the NACMCF Committee (hereafter the Committee) has provided microbiological limits for food categories that reflect process control and sanitary manufacturing conditions. These limits are not microbiological criteria for finished products typically found in a product specification, but are provided to help DOD assess process control and sanitary conditions in those suppliers without evidence of a documented and functioning food safety plan. Combined with process flow diagrams of manufacturing processes, the microbiological limits also provide guidance to DOD auditors when assisting suppliers with corrective and preventive actions taken when there is evidence of insanitary conditions and lack of process control. The processes for statistical analyses of microbiological data for DOD and suppliers are provided to optimize the use of the data in making decisions affecting process control and sanitation. These limits are based on expert opinion, industry recommendations, and published finished-product microbiological criteria from global sources.

RECOMMENDATIONS

- DOD should develop and implement a supplier expectations policy and program to address supplier programs such as crisis management, environmental monitoring, sanitation effectiveness monitoring, pest control, Good Manufacturing Practices (GMPs), Hazard Analysis and Critical Control Point (HACCP) systems, preventive maintenance, the use of statistical process control (SPC), and verification testing, as appropriate to the individual operation.
- DOD should share the information contained herein with suppliers who do not have documented and functioning food safety plans to begin the process of having them develop SPC charts to demonstrate process control and sanitary conditions. These charts should be based on microbiological limits provided in Appendix J. Suppliers also should examine trends in the data from the supplier's Environmental Monitoring Program (EMP) and

sanitation effectiveness monitoring program. A timeline for development and use of these charts should be set.

- DOD should provide a list of expert consultants that can assist suppliers with development and implementation of the SPC charts, and EMP.
- DOD should develop purchasing specifications that include microbiological criteria for foods purchased through the Worldwide Directory as well as for those foods purchased outside of the Directory. These specifications should be set initially based on consultation with industry experts, shared as draft specifications with the supplier community, and fully adopted after feedback and data confirm that the specifications, and the microbiological criteria imbedded therein, ensure safe and wholesome products, and are realistic and practical.
- DOD should communicate microbiological standards, specifications and guidelines to all suppliers and brokers.
- DOD should request that suppliers document their acceptance of the standards, specifications and guidelines in manufacturing food for DOD.
- DOD should require that their suppliers, even if instructed through brokers, use the sampling plan, specified limits, and analytical methods specified in the microbiological criteria (when formally developed and implemented), and maintain documentation for audit purposes.
- DOD should require Certificates of Analysis and consider the use of Certificates of Compliance with each shipment of product received to verify compliance with the specified microbiological criteria (when formally developed and implemented).
- If there is a third-party intermediary that is involved in the food supply chain, the intermediary should be required to receive, maintain and transfer the Certificate of Analysis or Certificate of Compliance with the products.
- Whenever and wherever possible, meat, poultry and processed egg products should be purchased from countries with United States Department of Agriculture (USDA)-equivalent inspection programs, and from manufacturing establishments that meet the requirements of the inspection system. When this is not possible, the manufacturing facility should meet the requirements specified by USDA for production of meat, poultry and egg products. The product specification for fresh (unfrozen) raw meat and poultry should include a maximum time between slaughter and receipt by DOD.
- DOD should leverage the implementation of the Food Safety Modernization Act (FSMA) legislation and regulations, requiring all suppliers that would be regulated by the Food and Drug Administration (FDA) to meet statutory and regulatory requirements as mandated by FSMA and corresponding regulatory rules.
- DOD should use an information technology solution that requires all suppliers to input key data such as location, contacts, product identification, code dating and traceability program, significant hazards, audit scores, regulatory actions (e.g., equivalent to recalls, market withdrawals, non-compliance records), SPC data, and microbiological test data. DOD should capture appropriate data in a standardized electronic spreadsheet or database.
- The risk of potential foodborne pathogens should be considered not only for fresh-cut and frozen fruits and vegetables but also for whole or unprocessed fruits and vegetables.
- The risk of potential foodborne pathogens should be considered not only for processed nuts, spices and herbs but also for unprocessed nuts, spices and herbs.
- DOD should develop procedures to collect appropriate meta-data associated with assay results. Meta-data are data about the data, such as, methods, sample size, analytical unit, point of sampling, and the reason the sample was collected.
- DOD should incorporate evaluation of sampling schemes and SPC into audit procedures for those suppliers using the microbiological limits to assess process control and sanitary conditions.

- DOD should consider enhancing diagnosis and reporting of foodborne illness, and integrating this information amongst the Services, to help identify potential problems within the supply chain.

INTRODUCTION: STATEMENT OF CHARGE TO NACMCF AND THE RATIONALE FOR THE APPROACH TO THE CHARGE

DOD has specific action levels for various microbiological pathogens (e.g., *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Clostridium perfringens*) and microbiological toxins in certain raw and processed meat, poultry, egg products and other products, such as fresh fruits and vegetables, procured globally for U.S. military personnel (U.S. Army Public Health Command (USAPHC), Circular 40-1: Worldwide Directory of Sanitarily Approved Food Establishments for Armed Forces Procurement, 2012; Appendix O, 2013 (27). Hereafter, USAPHC Circular 40-1 is referred to as the Worldwide Directory. In addition, there are bacteria that, when present in higher numbers, may indicate that processing conditions did not adequately prevent bacterial growth or reduce bacterial contamination of the product. DOD has encountered circumstances where the presence of potential pathogens or the numbers of non-pathogenic indicator bacteria have generated concerns about the safety and/or wholesomeness of products. DOD seeks updated microbiological limits to better evaluate process control and insanitary¹ conditions at the point of production.

The Committee agreed with the need to establish microbiological limits to help assess process control and sanitary conditions at DOD suppliers that do not have documented and functioning food safety plans, including HACCP systems. In time, the testing by these suppliers, and to a lesser extent by DOD, should assist these suppliers to develop functioning food safety plans and enable the suppliers to meet the microbiological specifications established by DOD. DOD also expressed interest in the use of criteria such as *Staphylococcus aureus* and *Bacillus cereus* levels in ready-to-eat (RTE) products, mesophilic aerobic plate count (APC) in raw and RTE products, and other possible indicators (e.g., generic *E. coli*, coliforms, *Enterobacteriaceae*, enterococci and gas-forming anaerobes) for establishing that food was manufactured with process controls and under sanitary conditions.

SPECIFIC CHARGE TO THE COMMITTEE

Because of the many questions regarding microbiological limits that might indicate poor process control or insanitary conditions, the Committee was asked for its guidance to clarify the following issues.

Describe processes and important considerations that could be used to develop a microbiological criterion for a particular product (e.g., bagged leafy greens, dairy products, grain-based products, raw ground beef, and RTE sliced luncheon meat) at various points in the process that might indicate poor process control and/or insanitary

¹ The terms insanitary and unsanitary are considered as one and the same in this document. Insanitary is a word that has been used in regulatory language. In this document insanitary is used as this term was provided in the charge to the NACMCF Committee.

conditions. Describe how the processes and considerations could differ in other regions of the world where processing conditions may make certain indicators or levels of indicators more or less appropriate.

At the point of production, how many *Staphylococcus aureus*, *Bacillus cereus*, generic *Escherichia coli*, coliforms, *Enterobacteriaceae*, enterococci and/or gas-forming anaerobes in RTE finished products might indicate: a) a possible process control problem or insanitary conditions, or b) potentially hazardous product unfit for distribution? How might the levels and the applicability of these criteria vary between different RTE products (e.g., processed meat, poultry, egg products, refrigerated meat/poultry salads, and bagged leafy green salads)?

At the point of production, what level of mesophilic aerobic plate count in RTE finished products and in non-intact raw meat and poultry products might indicate a possible process control problem or insanitary conditions? How might these criteria vary between different RTE products (e.g., processed meat, poultry and egg products, and refrigerated meat/poultry salads)? How might these criteria vary between different non-intact raw products (e.g., beef trimmings versus ground product)? How might these levels be expected to change during the expected shelf-life of the product?

Are there other potential indicators (e.g., microbiological, biochemical or molecular parameters) of process control that should be considered? If so, how might these apply at various points in the process to major product categories (e.g., processed meat, poultry and egg products, bagged leafy green salads and refrigerated meat/poultry salads)?

Discuss various sampling plans (e.g., International Commission on Microbiological Specifications for Food, ICMSF, 2- or 3-class plans) that may be applicable for the various analytes and products identified in the questions above.

The Committee notes that the microbiological limits reflecting process control and sanitary conditions requested by DOD should not be misinterpreted as microbiological criteria (specifications and guidelines) for finished food products. It is important that persons reading and using this document do not immediately transfer the limits provided herein to microbiological criteria for foods. Over time, as suppliers without documented and functioning food safety plans, including HACCP systems, use the microbiological limits to establish that their processes are in control and that sanitary conditions exist during manufacturing, they can complement this testing with their development of food safety plans that will demonstrate and ensure that the products purchased by DOD meet the microbiological criteria for finished food products. Once such documented and functioning food safety plans are audited by DOD and found to be effective, testing using the microbiological limits provided herein will be secondary and useful when there is evidence that there is a lack of process control or sanitary conditions and investigative actions are undertaken to determine root causes.

PUBLIC HEALTH FOCUS

With the large number of personnel served by DOD, the wide variety of raw, RTE and fresh foods procured, and the high number of countries, brokers and suppliers, the implications for failures in the food safety systems are considerable. While insanitary conditions and process failures can lead to higher numbers of indicator organisms (or classes of microorganisms such as coliforms or aerobic bacteria detected by APC; hereafter “indicator organisms”), the greater risks are failures leading to increased prevalence of pathogens in foods.

Verification testing by DOD, while limited in scope and absolute numbers of tests, should provide feedback to suppliers to improve controls where necessary. DOD inspection and auditing staff need to be equipped with tools to assist them in their evaluation of suppliers of a wide array of products. One tool will be process flow diagrams that illustrate points in the manufacturing process where loss of control or insanitary conditions can lead to introduction or growth of microbial contamination.

COMMITTEE’S APPROACH TO ANSWERING THE CHARGE

The Committee leveraged the expertise of the Committee members, additional experts and published literature and finished-product microbiological criteria to assist in developing microbiological limits indicative of process control and sanitary conditions for food manufacturing. The Committee prepared process flow diagrams to reflect the major food categories purchased by DOD and used these diagrams to predict unit operations that would lead to an increased prevalence of pathogens and levels of indicator organisms, or growth of contaminants, based on loss of control or insanitary conditions. The diagrams also indicate where in the process there are lethality steps.

SCOPE OF COMMITTEE’S WORK

The Committee focused on major food product categories to address the questions posed by DOD. DOD purchases food products that include what one would find in a retail supermarket. It was not in the scope of the Committee to recommend finished-product microbiological criteria (*i.e.*, product specifications and guidelines with levels of microorganisms describing acceptable, marginally-acceptable and unacceptable products) for the vast array of products. In addition, some food items purchased by DOD will no doubt fall outside of the major food categories included by the Committee. DOD will need to work with food safety experts to address any foods not covered in the major food categories.

The Committee recognized that a food safety program for DOD requires a farm to table approach; but the charge did not ask for the Committee to address producer food safety programs, supplier GMPs, broker responsibilities, management of the microbiological data, information technology to optimize use of supplier testing and DOD verification testing, or food service operations managed by DOD or their contractors. All of these components affect food safety and quality of the food purchased and used by DOD and should be included in its comprehensive food safety plan.

The Committee did not address the variability in food manufacturing around the world. The Committee chose to recommend microbiological limits that reflect traditional processes that are in control and running under sanitary conditions. The Committee did not address the consequences for suppliers whose processes are deemed out-of-control or operating with insanitary conditions. DOD will determine what steps it will take in the event a supplier is unable to substantiate their process is in control or that sanitary conditions exist for manufacturing. This report is intended to assist DOD in meeting mission requirements, particularly when purchasing from suppliers without documented and functioning food safety plans, including HACCP systems.

In addressing the charge, the Committee did not focus on establishing microbiological criteria as part of purchasing specifications, which DOD does not currently use. The Committee does discuss the use of microbiological limits for both assessment of process control and sanitary conditions, and the use of the limits, when and where appropriate, as the initial step toward developing microbiological criteria for lot acceptance.

The Committee did not address the programs and systems for delivering microbiological limits to suppliers, ensuring suppliers implement testing against the limits, reviewing microbiological data from suppliers, targeting of suppliers that do not test or do not meet the limits, collecting and managing data on microbiological quality of the products produced for DOD, and for selecting new suppliers or terminating existing suppliers.

GENERAL

While sampling and testing of food products are tools to verify compliance with preventive and pre-requisite programs, process control and sanitary conditions, HACCP systems and microbiological criteria, the results do not guarantee food safety. For all refrigerated and frozen products, temperature monitoring should be done throughout storage and distribution channels, as well as at receipt by DOD. Appropriate organoleptic and visual evaluation of the product and the means of conveyance in which it was delivered should occur. Where possible, continuous temperature recording documentation associated with the container delivering these products should be reviewed before accepting the products.

For food products classified under the jurisdiction of FDA inspection, the facilities supplying DOD should meet all applicable regulatory requirements, including those promulgated under the authority of the FSMA with regard to preventive controls and product safety. Meat, poultry and egg products that would be classified under the jurisdiction of the USDA Food Safety and Inspection Service (FSIS) should meet the regulatory requirements defined by FSIS for the U.S. and as equivalent for foreign suppliers.

BACKGROUND: DOD PROCUREMENT

DOD procures food products from all 50 states, U.S. territories, and over 60 countries. These food products are made available to active duty and reserve service members and to retirees

and eligible family members who choose to purchase from on-post facilities. Clearly the ability to safeguard these food products and ensure high quality is of paramount importance.

The DOD selection and approval process for new suppliers can take three months. In some situations where foods are required more rapidly, expedited processes are used to approve suppliers. All purchases of food for the military whether on bases, remote locations, ships, or through commissaries or other commercial establishments, should occur using the Worldwide Directory. Most of the purchases occur through the Defense Logistics Agency, but the Defense Commissary Agency also purchases food products for grocery-type operations. Ship supply officers will purchase food products for their ship. There are instances where procurement occurs outside the Worldwide Directory, especially where fresh foods, including meat and poultry, are purchased. In many instances, these non-standard situations are corrected when detected; however, ship supply officers are granted more freedom in buying from unapproved sources. It is noteworthy, and potentially problematic, that fresh fruits and vegetables are currently exempt from requirements to purchase from approved suppliers.

Based on the food product and a DOD informal risk ranking, approved suppliers are scheduled for DOD food protection audits on a quarterly, semi-annual, or annual basis. Food protection audits encompass an establishment's total food safety and food protection systems and programs. Those facilities receiving a passing score are then listed in the Worldwide Directory. The audit scores are based on observations, with major and critical defects noted, and different ramifications on the approval status for each type of finding. Audit documentation is reviewed first at one of the 20 districts, then at one of the five regions, and finally at the Army Public Health Command where new or continued approval is granted. If major or critical failures occur, a corrective action request with a timeframe for completion is made of the supplier. Follow-up is scheduled at a time reflective of the seriousness of the failure.

DOD evaluates the supplier's food safety plan, including HACCP system, to help determine whether the supplier can provide safe and wholesome food products. This evaluation also includes a review of verification testing data that supports the efficacy of the supplier's food safety plan. In instances where a supplier is needed to meet mission requirements, but does not have a documented and functioning food safety plan, DOD requires an alternative means to assess the supplier's processes and sanitary condition of the production environment. Microbiological testing is one of the tools that help with this assessment. The microbiological limits provided herein were requested by DOD to provide guidance on what tests are appropriate for various foods and production processes, and what test results may be indicative of process control and sanitary conditions.

Many food manufacturing facilities reference microbiological criteria from various entities or have established their own criteria to monitor the safety and quality of raw or RTE components used to manufacture finished products. The Codex Alimentarius defines a microbiological criterion as consisting of the following components (30, 31):

- The purpose of the microbiological criterion (e.g., lot acceptance or process control);

- The food, process or food safety control system to which the microbiological criterion applies;
- The specified point in the food chain where the microbiological criterion applies;
- The microorganism(s) and the reason for its selection;
- The microbiological limits (*e.g.*, m, M, or other action levels);
- A sampling plan defining the number of sample units to be taken (n), the size of the analytical unit, and where appropriate, the acceptance number (c);
- Depending on its purpose, an indication of the statistical performance of the sampling plan; and
- Analytical methods and their performance parameters.

DOD has established their own action levels (not-to-exceed limits) for finished products to assist auditors in their evaluation of various processing systems and finished products. DOD procurement requires that food products adhere to U.S. regulatory requirements; however, as mentioned above, exceptions to this requirement may be granted under limited circumstances.

Laboratory analysis forms an integral part of the overall mission of protecting military personnel and DOD beneficiary populations from foodborne and waterborne (hereafter foodborne will include waterborne) illness. The DOD program allows for testing of food products and the environments in which they are produced. Laboratory testing includes qualitative and quantitative analyses for pathogenic and nonpathogenic bacteria, respectively, as well as verifying other wholesomeness and quality parameters. Food testing equipment is located within each DOD deployable veterinary detachment to provide presumptive (considered Level 1 testing by DOD) microbiological testing results, with the staff of each detachment responsible for animal care, food protection, and review of area facilities that supply food. Testing by a food manufacturing facility using an accredited laboratory (*e.g.*, ISO 17025) is required for DOD procurement. Currently, DOD uses microbiological test results in combination with audit findings to determine the status of an establishment regarding initial and on-going approval, or whether product that has been procured is safe and wholesome for military personnel.

Appropriate organoleptic evaluation of food products may be useful to assess quality. While organoleptic examination has its value, it is inherently subjective and dependent upon sensory capabilities that vary from analyst to analyst. Numbers of indicator bacteria such as APC might be more effective for determining quality of products that may have been stored for a significant period of time. However, fresh produce may have appropriate quality for use while also containing substantial comparatively high concentrations of aerobic bacteria.

Food processors, including those who supply DOD with RTE multi-component products (*e.g.*, meals, sandwiches), should be responsible for evaluating individual components (*e.g.*, processed meats, cheese, poultry, egg products and spices) received at their establishments. In many cases, these components may be included as ingredients in the final product without further processing to inactivate biological hazards. The supplier establishments should perform microbiological testing on these raw materials, require microbiological test results from the secondary suppliers on a Certificate of Analysis, or require the listing of microbiological criteria as elements of a Certificate of Conformance that accompanies the raw materials.

A variety of analytes (e.g., aerobic bacteria, *E. coli*, *Enterobacteriaceae*, coliforms, enterococci) currently are monitored on a limited basis by DOD to suggest potential insanitary conditions or poor process control. This report recommends that this testing should be done by suppliers without documented and functioning food safety plans, including HACCP systems, using the microbiological limits provided herein to demonstrate process control and sanitary conditions. Currently, there is no consensus in the U.S. on acceptable microbiological limits for indicator bacteria to indicate a process is in control. Such limits may vary by facility, process and food, and may be best determined through the use of SPC as described herein.

FOOD CATEGORIES

Because of the vast array of food products purchased by DOD, categorization is complex. It is beyond the scope of this document to list or cover all foods purchased by DOD. The major food categories and the subcategories covered herein include:

Beverages

- Bottled water
- Ice, packaged
- Juices and drinks, pasteurized, refrigerated
- Shelf stable

Dairy

- Butter, margarine
- Cheese, hard
- Cheese, soft, semi-soft, surface ripened
- Cultured, pH<4.8
- Cultured, pH>4.8 and < 5.4
- Dried products (does not include dairy ingredients used to make infant formula)
- Frozen desserts
- Milk and milk products (fluid)
- Processed cheese

Egg Products

- Pasteurized, processed
- Shell eggs, raw

Grain-based Products

- RTE, baked items, refrigerated or temperature/time controlled for safety (TCS)
- RTE, baked items, shelf stable or non-TCS
- RTE, cereals
- RTE, cold pressed bars
- Non-RTE, Dry flour-based mixes
- Non-RTE, Pasta, dried or refrigerated

Meals and Entrees

- Non-RTE, ready-to-cook (RTC) meals, includes raw ingredients
- RTE, deli salads, sandwiches, heat-eat meals, sushi
- RTE, sous vide, cook and chill

Meat, Pork, Poultry Products

Non-RTE, beef and pork, raw, intact and non-intact

Non-RTE, poultry, raw

RTE, cooked, perishable

RTE, fermented, dried

Nuts and Nut Butters

RTE, not processed for lethality

RTE, processed for lethality

Produce

Fruits and vegetables, cut, frozen or refrigerated, minimally processed

Fruits and vegetables, whole

Mushrooms

Packaged salads and leafy greens

Vegetable sprouts

Seafood

Non-RTE, raw

RTE, fish, cold smoked

RTE, cooked or hot smoked

RTE, raw molluscan shellfish

Spices and Herbs, Coffee and Tea

PROCESS FLOW DIAGRAMS

The generic process flow diagrams for these food categories are included (Appendix A) to identify for DOD auditors the steps in the manufacturing process where microbiological counts could potentially increase with loss of process control or development of insanitary conditions. In addition, the flow charts illustrate where there are lethality steps that reduce numbers of indicator organisms and pathogens.

Principles Used in Making the Process Flow Diagrams

Steps for receiving and storing packaging materials were omitted to simplify the creation and use of the process flow diagrams. It is expected that a DOD-approved food processing plant would have appropriate control and documentation of these functions, either as part of product-specific preventive controls or HACCP system, or as preventive and pre-requisite programs such as Standard Operating Procedures (SOPs) for receiving and storage. It was recognized that a finished food product could move through many storage and distribution facilities as part of the supply chain. Moreover, it is possible that a finished product of one production system could be an input for another production system. The final two steps were denoted “store finished product” and “distribute finished product” to simplify the creation and use of the process flow diagrams.

For several types of food, there are many different possible combinations of manufacturing steps. Rather than try to show all multiple combinations and step sequences, the steps that

could be used in the relevant portion of the manufacturing process were listed collectively. For example, in the process flow diagram for yogurt, the “add culture” step also includes the information “(may be preceded by concentration)” and the “process” step also includes “filter, heat, separate, concentrate, stir (optional)”. In the coffee process flow diagram the “process raw coffee cherries” step lists the component steps of a wet method and a dry method to process the coffee cherries. The Committee assumes that DOD personnel will be able to recognize the specific steps observed at a food processing plant from among the general manufacturing steps shown on the process flow diagrams.

Interpreting the Process Flow Diagrams

The name of a processing step may be followed by any of the following designations: C, a step at which significant contamination may occur when adequate process controls are not in place, G, a step in the process where growth of microorganisms can occur, K, a step where there is a pathogen kill step, and S, a point where sampling and testing by the supplier are recommended for verification or investigation.

The effectiveness of the expected process controls at preventing contamination may differ considerably from step-to-step and product-to-product. For example, there would be a greater likelihood of contamination during the harvesting of coffee cherries than during the packaging of ground roasted coffee beans. Similarly, less contamination might be expected during yogurt packaging than during the packaging of raw, non-RTE seafood.

Programs for minimizing contamination at the identified steps include Good Agricultural Practices (GAPs), Sanitation Standard Operating Procedures (SSOPs), GMPs, SOPs for specific steps, and purchasing specifications. Steps denoted as potential contamination points may occur before or after a step causing significant reductions in the numbers of microorganisms present in the food. For example, there may be a high level of concern about *L. monocytogenes* contamination of RTE foods during the “package” step and this step will be labeled with a “C.”

Intended Use of the Process Flow Diagrams

DOD personnel should use the process flow diagrams to review the general steps to manufacture the food product under evaluation. From the process flow diagram, DOD personnel should determine the step(s) at which sampling should be done by the supplier without a documented and functioning food safety plan to demonstrate process control and sanitary conditions. When microbiological or organoleptic analyses indicate that any supplier may have shortcomings in process or sanitation controls, DOD personnel should use the process flow diagram to determine steps at which contamination could occur or steps at which a failure to achieve the expected destruction of bacteria may be occurring. It shall be important that DOD consider that test results or organoleptic assessments for finished products at the point of use (e.g., commissaries) may not reflect loss of process control or insanitary conditions at the supplier since factors such as temperature control during storage and distribution can

affect microbiological results and organoleptic properties, and should be taken into account when deriving conclusions about a supplier's manufacturing processes.

MANUFACTURING PROCESSES AND OPPORTUNITIES FOR LOSS OF PROCESS CONTROL

The designation of food categories and subcategories is based on criteria such as the food description itself, type and extent of processing, RTE status, and chemical characteristics of the food. For each subcategory a general process flow diagram depicts the manufacturing process for the foods in that subcategory. If DOD investigates a process following the review of verification test data or as part of an on-site audit, the process flow diagrams provide insights into where in the manufacturing process the investigator or auditor could focus their attention.

Measuring Insanitary Conditions

The Committee believes that the best assessment of insanitary conditions is not necessarily finished product testing, but is typically best achieved through strategic evaluation of the production inputs, cleaning and sanitation practices and their efficacy, and the environmental monitoring and sanitation effectiveness monitoring data generated by the supplier facility as part of their preventive controls program.

SAMPLING AND TESTING

There are various reasons for sampling and testing by DOD itself. While relying primarily on supplier testing, DOD may sample food products at locations such as distribution centers, field locations or commissaries to determine the microbiological quality of the food product at a particular point in the supply chain. The test results from analysis of these samples can provide insights into supplier compliance with specified microbiological limits; although, as pointed out above, the results would be affected by the warehousing, distribution and handling processes and conditions in the supply chain from the time of manufacturing to the point of sampling. For example, the results can provide indirect information regarding temperature control during warehousing and its impact on the shelf life of the food product.

DOD also may take samples during supplier audits. If finished products are sampled, these samples represent verification samples; the test results provide some indication of the ability of the supplier to manufacture safe and wholesome food products and provide an incentive to establish and maintain process control and sanitary conditions. The allocation of verification testing resources should include consideration of the potential presence of biological, chemical and physical hazards, type of food, supplier characteristics and where the supplier is located, audit results, shelf life, the distribution system and likelihood of temperature abuse, as well as the cost of sampling and testing. DOD has an informal risk ranking process that has been used to define audit frequencies. A more systematic and analytical approach to risk ranking of foods and suppliers by DOD considering the factors specified above would enhance controls over food safety and quality, as well as resource allocation.

The DOD process of evaluating suppliers with documented and functioning food safety plans should rely more on the documented evidence supporting effective food safety plans, including verification testing results (EMP, sanitation effectiveness monitoring, and finished product testing where appropriate) generated by the suppliers, with the DOD sampling and testing used only for periodic verification. For those suppliers without documented and functioning food safety plans, DOD should ensure the suppliers are conducting sufficient sampling and testing to demonstrate process control and to establish that their manufacturing is occurring under sanitary conditions, using the guidance provided in this report. When deemed necessary, more finished product verification testing by the supplier and DOD may be appropriate for these suppliers until they develop functioning food safety plans.

Use of Statistical Sampling Plans in the Supply Chain

Currently, DOD, through the USAPHC, maintains the Worldwide Directory but does not stipulate purchase specifications, such as microbiological criteria including sampling plans, microbiological limits, and reference methods for specific microorganism-commodity combinations. This section addressing sampling plans is not intended to provide guidance to DOD (or any other entity) for elaborating microbiological specifications for foods. Instead, the aim is to provide some contextual and statistical background for DOD to consider when evaluating food suppliers, their microbiological data, and the extent to which their manufacturing process is in control.

Strategic microbiological testing of foods, as in-process samples or finished products, provides useful information about microbiological quality, safety, sanitation, and the effectiveness and extent of process control. While it is rarely possible to use microbiological testing of foods to ensure safety and wholesomeness, it is possible to design strategic sampling schemes and select appropriate analytes and assays that can aid in the management and control of suppliers. Testing data can be used to help assess manufacturing and monitoring systems such as HACCP and preventive control programs.

In some instances (e.g., immediate need by DOD for a supplier without a documented and functioning food safety system, rapid development and implementation of HACCP systems and preventive control programs by a supplier may not be possible in the short term. In such instances, use of the microbiological limits provided in this report may be useful for suppliers and DOD to evaluate the food safety and quality performance of the manufacturing process. Furthermore, analysis of the data may help identify improvement opportunities. The Committee recommends that a long-term goal be that all approved suppliers develop and implement effective food safety plans, including HACCP systems, preventive control and prerequisite programs. In doing so, suppliers and DOD can rely less on the use of the microbiological limits described herein and finished-product testing, and more on data associated with the food safety plan that demonstrate the manufacturing process is stable and capable, and sanitary conditions are maintained continuously.

SPC methods are a powerful tool to evaluate process capability and monitor the extent of control within a manufacturing process. In particular, SPC can be used to identify an out-of-control process and consequently flag events warranting investigation for an assignable cause, corrective action and potential preventive action. In this document, we focus on sampling schemes that allow the use of SPC to assess process control and sanitary conditions, particularly, but not exclusively, for suppliers without a documented and functioning food safety plan. Some approaches described herein also may be suitable for a variety of other qualitatively or quantitatively measurable observations such as those identifying chemical hazards or physicochemical measurements; but control of these food process characteristics is beyond the scope of this report.

Finished-Product Testing to Aid in the Management and Control of Suppliers

As mentioned previously, the microbiological limits provided in this report are not microbiological criteria for finished products; although as data generated for SPC accumulate over time, they may help define realistic finished-product criteria that reflect wholesomeness, safety, process control and sanitary conditions. Finished-product testing does have a role for verification that food is manufactured under sanitary conditions with processes that are under control.

As used herein, finished-products refer broadly to food products or ingredients that have completed a manufacturing process by a supplier. It does not necessarily imply a RTE product. For example, beef trim may be considered a finished product from the perspective of a slaughter plant supplying trim to a customer (e.g., a producer of ground beef). Consequently, a finished product of one process may be an input of another.

In order to ensure the integrity of its food supply, DOD should assess a supplier's product as the output of a process that should be under control and delivers wholesome and safe product. This assessment is achieved through reviewing data supporting the supplier's food safety plan, supplier microbiological test data, surveillance of food products at receiving or in distribution, monitoring of process control at the supplier, and supplier audits, among other activities. In what follows, the elements of process control are reviewed, and guidelines are given for statistically-based activities of surveillance and process control monitoring that help ensure process control, sanitary conditions and high-quality finished products. It is important to understand that assessing process control can take many forms including measurement and documentation of critical processing parameters such as time, temperature and pressure, documentation of employee compliance to personnel requirements, verification and monitoring programs for SOPs and SSOPs, and evaluation of microbiological, chemical and physical characteristics of food before, during and after processing.

Process Control

In simple terms as it relates to food manufacturing, storage and distribution systems, process control can be defined as maintaining the output of a specific process within a desired range. Control of a process (or management of a process in general) requires accomplishment of six basic steps:

1. The output of the process must be sampled and quantified on key attributes. Even limited information (e.g., above or below target) can be used to establish control, if the sampling rate is high enough. The higher the information content of the measurement (e.g., enumeration vs. presence/absence), generally the lower the minimum required sampling rate for control.
 2. There must be predefined relevant process control performance limits and targets traceable to the basic requirements for acceptable outputs (e.g., specifications) and the history of the process.
 3. The actual sample output results must be compared to the relevant process control limits.
 4. There must be a predetermined plan of action (POA, such as a corrective action plan) based on the size and frequency of deviation from relevant limits. This POA should include the conditions under which 'take no action' is the proper response to a deviation from control limits. For example, a typical set of POA choices might be: take no action, move to tightened inspection with increased sampling frequency or sample size, conduct a pre-determined internal or external audit of the process that is typical for out-of-control variability, or identify an assignable cause through root-cause analysis and take corrective and preventive actions. The corrective actions specified must be validated to ensure they do help to prevent future deviations.
 5. The proper action must be decided upon based on the observed deviation.
 6. The proper action must be promptly taken to adjust the process. Failure to be prompt is equivalent to lowering sampling frequency and reduces the ability to control the process.
- Failure to execute any of these steps will obstruct control of the process.

Statistical Process Control Limits

A process is considered under statistical control when its output varies as expected within a standard operating range (SOR) of variation (Appendix B). This refers to common cause variation and represents the random variation inherent in a process. When a process becomes out-of-control, its average shifts, variation increases beyond the SOR, or both. This loss of control is typically due to the introduction of a disturbance generated by an assignable cause.

SPC limits bracket the SOR, and indicate the boundary between controlled and out-of-control operations. The SPC limits may be supplemented by additional statistical rules, such as run tests (*i.e.*, a rule defining loss of control based on a run of sequential observations, such as seven measurements over the center line).

SPC limits typically are determined in one of three ways:

1. Theoretically, from careful scientific analysis of the underlying process;
2. Nonparametrically, from quantiles of the empirical distribution function (EDF), derived from historical data; or
3. Parametrically, from quantiles of an assumed model distribution (e.g., lognormal) whose parameters (e.g., mean and standard deviation) are estimated from historical data.

The first way is difficult to carry out successfully, particularly for microbiological data. The third method is typical for non-microbiological applications. However, all three may be useful options for establishing SPC limits in various settings.

There is a trade-off involved in the choice of the quantiles used to establish the SOR. If the upper control limit (UCL) is too low (or the lower control limit, LCL, is too high), the corresponding false alarm rate (FAR) will be too high, and will monopolize resources in performing corrective actions and searching for assignable causes when actually the process is under statistical control. For example, if the UCL is chosen at the 90th percentile, then 10% of testing can be expected to result in false alarms. If the chosen percentile is too high, the FAR will be too low, and the process may drift out of control too far before it is discovered, or the sampling rate would need to be increased to counteract this effect. Similar arguments apply to the LCL used, if any.

Typical quantiles used for the upper control limit in SPC are 95%, 99%, 99.7% or 99.9%. Choice of the quantile is related to FAR, production lots defined in part by time (e.g., hours, days, or months), and the amount of resources budgeted for dealing with exceptions. Absent other information, a reasonable rule of thumb might be to use 95% or 99% limits if the sampling rate is low (e.g., weekly), so there is no more than one or two expected false alarms per year of production; otherwise it is conventional to use 99.7% or 99.9% limits.

It is important to note that there is a difference between a process being in statistical control and meeting specifications. A process is considered under statistical control if it is stable over time and the observed variation is due to common, chance causes inherent to the process (e.g., background noise due to normal variation in ambient temperature and humidity) and there is no between-lot variation. A food manufacturing process being under statistical process control does not imply its capability with respect to meeting microbiological specifications. The ideal situation is when a process is both under statistical control and is capable of manufacturing products that meet specifications. However, a process can be in statistical control and not capable of satisfying specifications. For example, the process consistently generates substandard product. Alternatively, a process can be out of statistical control but capable of satisfying specifications. For example, the process is designed to be robust in regard to deviations from the norm, such that it meets specifications despite high variability. Given seasonal and other sources of variability beyond a supplier's control, the latter situation may be particularly relevant to food production processes.

Process Capability

Observations that fall within the SPC limits indicate the SOR of production at a facility that is under control. They indicate the typical range of results on product (in-process or finished product samples) produced when the process is under control. Specification limits are different in that they indicate the range of results that indicate company or customer requirements.

The degree by which the SPC limits fall within the specification limits reflects the process capability to meet specifications when the process is in control. If the process UCL exceeds the

upper specification limit (USL) or the LCL is less than the lower specification limit (LSL), a fraction of the product produced under normal conditions will not meet the specification, even though the process is in control.

Process capability is traditionally quantified by a Process Capability Index (C_p , Appendix B). Typically a recommendation for a new process is $C_p = 1.45$, or for an established process $C_p = 1.25$. Equivalent nonparametric rules would be that the USL corresponds to the 99.999 percentile for a new process or the 99.99 percentile for an established process. In both instances, the USL is higher than the UCL.

SPC Monitoring via Microbiological Testing

SPC monitoring is meant to verify that a supplier's process of production is operating in statistical control (or in terms of previous discussions, there is control of the production process), and therefore is expected to meet microbiological limits where they have relevance in relation to the process control limits. SPC monitoring requires testing at a frequency that makes the data valuable for assessment of stability and capability.

Microbiological testing presents some unique features not present in other applications where SPC is used. Unless a chemical or physical surrogate variable is used, microbiological testing typically results in a discrete count, not a continuous result. The count may be 0 or 1 (*i.e.*, presence/absence testing) or a plate count, or the result of a sequence of serial dilutions. A zero count represents a concentration below the limit of quantification or detection (*e.g.*, <10/ml or negative in 325 g) for the particular method and test portion size involved.

Because of the discrete count nature of microbiological testing, test results are governed typically by one or more of three distributions:

1. Low prevalence (presence/absence) modeled by the binomial or Poisson distribution;
2. Single dilution plate counts, modeled by the Poisson distribution; and
3. Multiple dilution or large plate counts, governed by the lognormal distribution.

Examples of control charts (that illustrate statistical analysis of microbiological test results) based on DOD data are provided in Appendices C, D, E, F, G and H. In addition, other distributions that characterize microbiological populations include the Poisson lognormal distribution. This distribution is a generalization of the Poisson that assumes that the mean concentration varies log-normally rather than remaining constant throughout the product. Furthermore, the combination of low prevalence and a range of concentrations when the analyte is detected results in a zero-inflated distribution that complicates analysis. Zero-inflated refers to a higher frequency of zero counts than expected under a parametric distribution. For example, if the microbiological counts in a product follow a simple Poisson distribution with a mean concentration of 0.04 CFU/g, zero counts in 25 g portions are expected with a frequency of 37%. If a higher frequency of zero counts is observed, the distribution may be a heterogeneous mixture in which the microorganism is completely absent from some proportion

of the product and present and Poisson-distributed in the remainder. The result would be a zero-inflated Poisson distribution.

Considerations for Finished-Product Testing

The microbiological limits provided in this report for DOD are useful to establish process control and sanitary conditions. If suppliers or DOD test finished products, the results may be useful in assessing the microbiological quality of the product. However, to determine finished-product acceptability, additional samples may be required ($n > 1$), a three-class plan may be more appropriate, and microbiological criteria for a food category (and not provided in this report) shall be required. Considerations for finished-product testing are discussed herein to provide insights and guidance as the suppliers without a documented and functioning food safety plan move from establishing process control and sanitary conditions using microbiological limits to collaborating with DOD to implement microbiological criteria for product acceptance.

Determining the beginning and endpoint of a clearly defined product lot, and delineating it microbiologically from other lots is critical. A product lot may be defined using a number of criteria, such as:

- The food manufactured between defined activities (e.g., clean-up to clean-up);
- The food manufactured within a period of time (e.g., day, week, or month); or
- A defined quantity of manufactured food.

The process of defining lots involves thoughtful balancing of various (and sometimes competing) factors such as sampling costs, the likelihood that a lot is rejected by a customer, and the cost of lot rejection. The International Organization for Standardization (ISO) observes that from the point of view of the cost of sampling inspection, there is an advantage in large lots, provided the same frequency distribution is maintained as lot size increases (12). However, there are a number of reasons for limiting the lot size including: large lots might result in inclusion of widely varying quality (i.e., heterogeneity of assignable causes), storage and handling might preclude the formation of large lots, and the economic consequences of rejecting or recalling large lots might be unacceptably large. In process control, therefore, there are tradeoffs between the increased resolution of frequent testing (e.g., every shift or daily) and the costs of sampling and laboratory analysis. While general rules are available for lot size, frequency of lot sampling, and number of samples per lot, a sampling scheme can be devised to optimize control subject to cost constraints (14).

Lot definition also has implications for SPC when used for assessing the acceptability of a lot. For purposes of SPC, an important consideration is that a lot is produced under reasonably constant conditions so that a lot is a homogeneous volume of contemporaneous production. Statistically, a volume of production is considered homogenous relative to a given characteristic (e.g., concentration of the microorganism) if the characteristic follows the same probability distribution throughout the volume (e.g., lognormal with fixed mean μ and fixed standard deviation σ). It does not mean that the characteristic is the same throughout the volume (2).

That is, the conditions result in a homogenous frequency distribution that may or may not produce a spatially uniform distribution within a lot.

A homogenous distribution is often interpreted in food microbiology to indicate a homogenized product with the same mean concentration throughout (*i.e.*, a Poisson spatial distribution); however, statistically a consistent or homogeneous frequency distribution can result in spatial heterogeneity within a lot (ILSI-Europe, 2010). For example, if two days of production have the same mean concentration ($\mu_1 = \mu_2$) but substantially different variability ($\sigma_1 \neq \sigma_2$), then the two production lots are not characterized by a homogenous (the same) frequency distribution. This concept is important because assignable causes that might occur between lots ought to be different from those that occur within lots. As such, an important aim of SPC methods is to evaluate between-lot variance compared to within-lot variance.

Selection of the appropriate microorganisms when deploying SPC is critical. Typically the best organisms are either a) those that are predictably present within the sample matrix at some quantifiable concentration; or b) those that are neither exceptionally rare (*i.e.*, approaching 0% prevalence) nor ubiquitous (*i.e.*, approaching 100% prevalence) when detected with qualitative assays. In some instances, microorganisms present at low prevalence may be useful for SPC (Appendices D and E).

Sampling Frequency

Product samples may be taken systematically based on units of production or by duration of production, *e.g.*, by shift, day, week, month or quarter. Indicators of process control are best obtained by more frequent sampling. As a general rule, sampling frequency should be high enough to detect the presence of expected assignable causes within the first 10% of their persistence time. SPC cannot function for process control if the sampling frequency is less than twice during the assignable cause persistence time. Cost is associated with sampling and testing, so considerable economic force is exerted to drive the frequency to the minimum possible rate. However, disruptions that cause a loss of process control often persist for only a finite time, and not much is learned if they are either not detected when happening, or are detected too late for corrective action.

Although DOD currently conducts some sampling and testing during screening, auditing, and surveillance, to develop fully the use of SPC, suppliers would need to do sampling and testing at a frequency described above. As such, the supplier needs to have access to a competent laboratory, have the technical ability to collect the appropriate samples, have the financial resources to pay for the program, and have the knowledge of SPC necessary to interpret and use the data.

Even under ideal conditions, a large quantity of data may be required before stable, precise estimates are obtained for process parameters (*e.g.*, mean, variance, prevalence). Shewhart (16) cautioned that assignable causes of variation are almost always present in the early stages of process control and that a long data sequence (*e.g.*, a total sample size not less than 1000)

may be required to demonstrate that a process is in statistical control . However, acquiring additional data is subject to diminishing returns, and requiring a very long sequence of data may not be economically or technically feasible under operational conditions (Appendix I). For example, the only suppliers of perishable foodstuffs required to support DOD operations in austere areas may be small facilities without long production histories. Also, attainment of process control is often a gradual, stepwise process. Therefore, in practice, a pragmatic compromise is often warranted. As a general rule, Shewhart suggested a data sequence of not less than twenty five samples of size four (e.g., sampling 25 lots at 4 samples per lot for a total of 100 samples) is the minimum requirement for concluding that a process is in a state of statistical control (16) . Similarly, the ICMSF (ICMSF, 2011) recommends that a minimum of 30 lots should be examined; but cautions that it may be necessary to conduct an initial process control study for longer periods or in phases.

Sampling Plans for Screening and Auditing Suppliers

Screening of New Suppliers

The first step in screening a new supplier is to have the supplier conduct a self-audit against DOD supplier expectations (currently a pre-audit checklist). With the self-audit, or upon an initial visit, DOD should request that the supplier provide microbiological data that demonstrates that their production process is under control and occurs under sanitary conditions. The supplier could be asked for verification data supporting its food safety plan, or for those suppliers without a documented and functioning food safety plan, SPC charts that help to demonstrate their level of control (although it is unlikely such suppliers will have these charts and will need to be provided direction, such as that given in this report). If either type of supplier does not have the information, DOD should consider whether the supplier is willing to begin the process of demonstrating that their process is under control and is operating under sanitary conditions by collecting verification data to support their food safety plan or by using the microbiological limits provided herein to support that their process is in control and production is occurring under sanitary conditions. Suppliers might be accepted under a probationary status. During the probationary period, finished product testing may be required to assess the acceptability of the supplier's product.

For-cause Auditing (Directed Audits)

When a potential problem has been identified (e.g., failure to achieve a microbiological criterion, prematurely spoiled product, or an outbreak of illnesses associated with consumption of a product), sampling is frequently required to determine the extent and source of the problem. The ICMSF (ICMSF, 2002) refers to investigational sampling, which includes sampling for this objective. While the sampling conducted in the course of for-cause auditing would typically require more extensive sampling than normal sampling, it differs from tightened inspection in that there are no conventional sampling plans specifically designed for determining the extent of a problem and identifying the underlying cause. The success of such sampling depends greatly on knowledge of the process, product, and microorganism. The process flow diagrams

presented in Appendix A should be a useful resource for guiding the sampling conducted during for-cause auditing.

Surveillance at Point of Sale

DOD performs intermittent point of sale surveillance of finished products at locations such as at commissaries. The accumulated data are valuable for various purposes such as assessing not only the suppliers' products and processes, but also the potential for contamination or abuse during transportation, and storage and handling practices throughout the supply chain and at the commissaries themselves. Various sampling plans are appropriate for surveillance purposes including that sampling and testing being performed currently by DOD. However, improvements in standardization of sampling plans and associated meta-data (characterization of the data and the methods used) are warranted.

MICROBIOLOGICAL LIMITS AND CRITERIA

Development of Limits and Criteria

The ICMSF describes the establishment and application of microbiological criteria in considerable depth in two publications, *Microorganisms in Foods 7* (ICMSF, 2002) and *Microorganisms in Foods 8, Use of Data for Assessing Process Control and Product Acceptance* (ICMSF, 2011). The details described in these references will not be repeated here; however, the following discussion relates to how the development of criteria relates to the specific charges posed by DOD.

ICMSF defines three types of microbiological criteria: standards, specifications, and guidelines. Standards are mandatory criteria incorporated into a law or ordinance (normally pathogen oriented). Specifications are part of a purchasing agreement between a buyer and a supplier of a food and may be advisory or mandatory according to use. Guidelines are advisory criteria used to inform food operators and others of the microbiological content that can be expected in a food when best practices are applied (ICMSF, 2002).

Regardless of where food products are manufactured in the world, the finished-product microbiological criteria indicating safe, wholesome products for DOD would be the same. This presents challenges for DOD because manufacturers around the world do not have the same facility design requirements and standards, processing equipment and technology, sampling and testing programs, regulatory requirements, preventive and pre-requisite programs, oversight and auditing, customer expectations and food safety culture. Further complicating the development of microbiological criteria for finished products purchased by DOD is the large number and variety of products and suppliers.

In contrast to establishing appropriate microbiological criteria, if there was interest or a need to truly reflect how microorganisms are related to process capability for each manufactured product, data would need to be captured over many lots of production at each manufacturing site to determine what levels of organisms measured at various points of production reflect

sanitary and insanitary conditions or lack of process control. This requires a site-specific assessment for each product individually to gain an accurate assessment of these data; this resource-intensive effort is not commonly done at manufacturing locations. Setting uniform microbiological limits for process control, while purposeful, may not accurately reflect individual processes and products within that general category. Thus, the suggested microbiological limits (Appendix J) described herein should be considered guidance to DOD representing a provisional starting point for developing empirically based microbiological data and a basis for discussion of DOD expectations with suppliers that do not have documented and functional food safety plans.

Microbiological analyses and comparison of the test results to microbiological limits, for the purpose defined herein, or finished product microbiological criteria, yet to be fully defined by DOD for the products they purchase, may be used to verify that a supplier's control programs for controlling microbiological contamination are effectively designed and implemented. When there is evidence that the supplier's controls are poorly designed or implemented, it may be prudent to increase the frequency of microbiological testing; this testing may include testing against microbiological limits provided herein, finished product testing, environmental monitoring, and sanitation effectiveness monitoring. It seems reasonable to expect that appropriate food safety and quality programs are more likely under the following conditions:

- the food safety regulatory program in the supplier's country has been deemed equivalent to its U.S. counterpart,
- the supplier has developed, implemented, and documented appropriate preventive and pre-requisite food safety programs such as ensuring a safe and properly plumbed water supply, GAPs, GMPs, and SSOPs,
- the supplier has developed, implemented, and documented a process-oriented risk-based preventive food safety plan, including a HACCP system, that substantially complies with risk-based preventive controls regulations authorized by FSMA, and
- the supplier's food safety system has achieved third-party certification against standards fulfilling the requirements such as those specified in the Global Food Safety Initiative Guidance Document.

Pathogens Important to Public Health

It is somewhat easier to establish microbiological limits, and specifications, for certain pathogens because whenever there is a likelihood of pathogens being present, sampling and testing plans can be designed to require the absence of the pathogen at a given stringency of testing, *i.e.*, quantitative values need not be established.

The Committee considered where pathogens are reasonably likely to occur for each category of food. The pathogens may have resulted from process control failures (*e.g.*, contaminated raw materials and ingredients, inadequate processing conditions and insufficient interventions, failures in pre-requisite programs and preventive programs) or insanitary conditions (*e.g.*, failure in cleaning and sanitation, inferior facility and equipment design, poor personal hygiene). Combining these analyses with summaries on the causative agents of foodborne outbreaks allowed the Committee to prepare the microbiological limits for pathogens for the major food categories that may reflect loss of process control or insanitary conditions (3).

Indicators that Reflect Loss of Process Control or Insanitary Conditions

Indicator organisms typically used to reflect process control or insanitary conditions include those familiar to food manufacturers, e.g., APC, coliforms, *E. coli*, *Enterobacteriaceae*, *S. aureus*, pseudomonads, and yeasts and molds. The levels of indicator organisms which indicate loss of process control or insanitary conditions during processing are dependent upon factors such as the cleaning and sanitation procedures and products, the types of processes used, the sanitary design of equipment and the facility, and the food being manufactured.

One of the more difficult microbiological limits to establish to reflect loss of process control or insanitary conditions is that for Gram-negative bacteria, whether coliforms, fecal coliforms, *Enterobacteriaceae* or *E. coli*. Kornacki and others (13) provide an historical evaluation of these criteria for foods and their utility based on current knowledge. None of these Gram-negative bacteria accurately and consistently reflect fecal contamination of raw and processed foods nor are they useful or reliable as index organisms predicting the presence of pathogens. These criteria may be useful indicators of insanitary conditions and loss of process control; however these uses are dependent upon many factors such as the type of food, the extent and type of processing, the relationship between bacterial numbers and food quality, and the length of time between production and sampling and testing. Kornacki *et al.* also reviewed the testing methods and the many variables that affect the accuracy and utility of the results. For these reasons, whichever indicator microorganisms are used, they are generally considered guidelines for use. Based on this current review, in general, the indicator microorganisms of most value would be *Enterobacteriaceae*, followed by *E. coli*, coliforms and fecal coliforms.

DOD is at a disadvantage without data from suppliers defining their normal cleaning and sanitation practices, and their sanitation effectiveness monitoring program, as well as process control data measured by manufacturers throughout their production runs. Setting arbitrary quantitative limits for indicator organisms for a category of food products is guidance at best and may or may not be reflective of insanitary conditions or lack of process control. For this reason, the microbiological limits provided herein to DOD should be considered guidelines and a starting point for suppliers and DOD to evaluate the process controls and sanitary conditions under which the products were manufactured. The process flow diagrams indicating where bacterial numbers may increase during manufacturing provide some guidance to DOD on questions to ask of suppliers regarding where samples are taken, or process control measurements made, during processing and what corrective actions might be taken based on the results of such sampling and testing.

Comments on Microbiological Limits for Specific Food Categories

One of the limitations of microbiological limits as indicators of process control or insanitary conditions is the balance of statistical validity with practicality (Appendices K, L and M). Microbiological limits and sampling schemes are often dictated by common practice and are not based on statistical design. The guidance below is based on review of the available literature, expert opinion, and industry practice. Consequently, the limits discussed below should be

considered provisional starting points toward more formally designed microbiological limits for process control that are updated and revised over time as additional data are acquired.

The tables (Appendix J) presented in this document are intended to provide guidance on microbiological limits, proposed primarily for use by DOD for suppliers without documented and functioning food safety plans, that reflect effective process controls and sanitary conditions used to produce food products using good quality ingredients, validated pathogen intervention strategies and lethality steps, GMPs and GAPs. Microbiological populations in raw commodities are expected to be higher and more diverse than those in foods produced using a validated lethality process. The limits identified are on a “per gram” or “per ml” basis and typically assume a 25 g analytical unit unless otherwise described.

The microbiological limits are intended to help identify when a process is not in control so the manufacturer can investigate causes and implement corrective actions. The limits reported for indicator organism testing are not lot acceptance criteria. In some cases, the action to be taken after exceeding the limit may be to increase sampling to determine the source of contamination or to test for pathogens or other indicators of insanitary conditions. In cases where any microorganism or class of indicator organisms exceed regulatory limits, then the lot should be evaluated appropriately, and typically destroyed or diverted for reconditioning if appropriate. As an example, the FDA Dairy Compliance Policy Guide 527.300 (23) considers cheese made with pasteurized milk to be adulterated if the cheese contains 10^4 CFU/g *S. aureus* or *B. cereus* or 100 CFU/g *E. coli*; these lots should be rejected and additional investigation conducted. If enterotoxins produced by *S. aureus* or *B. cereus* are detected, the product also should be destroyed.

Enrichments (such as for pathogens in environmental sponge samples) may be performed on composite samples. However, with compositing, if samples are pulled from multiple locations or over the course of producing several lots of finished products, a positive result for the enrichment would implicate all locations and the lots manufactured during the sampling period. In contrast, enumeration data should be generated from a single sample analytical unit; pooling samples might dilute unacceptable or marginal populations with samples having low populations and thereby provide misleading results.

Assaying for APC to assess process control and sanitary conditions may be relevant for some RTE foods but not others. APC values used to assess process control and sanitary conditions during production should be low in RTE foods in which all components of the food have received a lethality step (e.g., pasteurization, cooking, roasting). When RTE foods contain some components that have received a lethality step, but then were further handled (e.g., sliced, assembled or mixed) before preparation of the final food product, APC levels would be expected to be moderately higher. In contrast, using APC to assess process control and sanitary conditions during the production of foods such as fresh fruits and vegetables, fermented or cultured foods and foods incorporating these, has little value as these foods would have an inherently high APC because of the normal microbiota present.

The presence of *E. coli* in RTE foods is undesirable because it represents poor hygienic (insanitary) conditions or inadequate heat treatment (lack of process control). Thus, *E. coli* should not be detected in RTE foods; generally, when microbiological specifications are established, a microbiological limit of <10/g or <3 MPN/g (the limit of detection of usual test methods) is typical for this microorganism. Levels exceeding 100/g are typically interpreted as a level of contamination that may be associated with the introduction of pathogens or conditions that allowed pathogen survival.

The Committee concurs with the common practices for environmental monitoring, *i.e.*, testing for *Listeria* spp. in wet, RTE-food processing environments, particularly for foods that support growth of *Listeria*, and for *Salmonella* in dry, RTE-food processing environments. *Salmonella* monitoring in warm, wet, RTE-food processing environments also may be appropriate depending upon the product and facility. If product contact surfaces (Zone 1) are tested, finished product should be held until results are confirmed negative; if testing demonstrates that the product contact surfaces are positive for the pathogen, investigational testing in finished product and corrective action is indicated. As of 2014, the U.S. maintains a standard of non-detectable *L. monocytogenes* for all RTE food products. Other countries may allow up to 100 CFU/g for *L. monocytogenes* in RTE foods that do not support growth (*e.g.*, frozen foods, those with pH <4.4, water activity (a_w) < 0.92, or pH < 5 and a_w < 0.94) (5, 6).

All dairy food categories listed below are presumed to be made with pasteurized milk to eliminate common vegetative bacterial pathogens. Therefore, the presence of any pathogens when testing for process control or sanitary conditions represents post-process contamination. *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* are considered adulterants in RTE dairy products. In the U.S., these dairy products are either regulated under the PMO Pasteurized Milk Ordinance (24) or microbiological standards are identified in the Dairy Compliance Guidelines (23). Other resources for microbiological specifications and guidelines include the Compendium of Methods for the Microbiological Examination of Foods (Milk and Milk Products (1) and Standard Methods for the Examination of Dairy Products (29) . Alkaline phosphatase level in pasteurized fluid bovine milk is limited to less than 2.0 micrograms phenol equivalent per gram in one or more subsamples whereas cheeses may have higher limits. Actionable limits for *S. aureus* and *B. cereus* are set to 10^4 CFU/g whereas limits for *E. coli* or coliforms are product specific.

The general recommendation for DOD procurement of any beef, pork or poultry product, whether raw or RTE, is to identify an establishment in the country which is authorized to ship that product to the U.S. and procure product from that establishment. This will ensure the establishment meets current FSIS performance standards and/or regulatory requirements. If such an establishment cannot be identified, the testing recommended in Appendix J may be used to determine the level of process control and sanitary conditions for establishments not currently authorized to ship the product to the U.S.

Microbiological testing of finished products that receive a lethality step, such as baking or cooking, may not be a good indicator of improper storage temperatures and hold times (process

controls) of ingredients or blends before the lethality step (such as extended runs between clean up). Certain ingredients or foods may support microbiological growth and production of heat stable toxins, such as those produced by *S. aureus* or *B. cereus*. Thermal treatments may inactivate the vegetative cells in the final product but the toxin may remain. As a result, the process must have validated microbiological control steps throughout the production to minimize the risk of toxin being present in the finished product.

Routine and Non-routine Testing

In setting the microbiological limits to be used by suppliers that do not have documented and functioning food safety plans, including HACCP systems, the Committee defined the recommended testing frequency as routine and non-routine. Specific time intervals cannot be set for each indicator organism, class of indicator organisms, pathogen, environmental monitoring, or in some instances, chemical hazard (e.g., mycotoxin). The frequency of routine and non-routine testing will be dependent upon numerous factors such as the production process, the product being produced, the sanitary design of the facility and the equipment used at the facility, the historical data generated by the supplier, the organism or class of indicator organisms, and the investigative reason for testing. General guidance on the definition of these frequencies is as follows.

Routine testing is defined as testing done at pre-determined intervals at sufficient frequency to establish process control or sanitary conditions. The sampling interval may be on a physical lot basis (e.g., 2,000 lb. combos for ground beef) or temporal basis (e.g., per shift, daily, weekly, monthly). Non-routine testing can be investigational, for verification, validation, surveillance, or for qualifying suppliers. Non-routine testing is less frequent and can be based on time intervals (e.g., weekly, monthly, quarterly) or based on other indicators of lack of process control or insanitary conditions. For example, if routine testing shows that samples of a pasteurized egg product exceed limits for *E. coli*, testing for *Salmonella* may be appropriate. If routine testing of a RTE food that can support growth of *L. monocytogenes* indicate contamination of the food with *Listeria* spp., additional testing for *L. monocytogenes* may be appropriate. When a supplier is manufacturing multiple- component foods (e.g., frozen desserts with inclusions, deli salads, sandwiches, entrees), routine or investigational sampling and testing may be focused on those components with the highest microbiological risk.

Plan of Action if Limits are Exceeded

The microbiological limits provided in Appendix J are useful to assess process control and insanitary conditions. The action taken by a supplier if indicator organisms in samples taken at the supplier location exceed the specified limits should be to investigate the cause of the high counts, implement corrective and preventive actions, and reevaluate the effectiveness of the actions after implementation. In the cases of a pathogen detected when there has been no additional lethality step, an evaluation of the finished product associated with the sample tested should occur to determine if the product should be rejected or, if appropriate, reworked or diverted for processing that will inactivate the pathogen. Products contaminated with heat-stable toxins typically will be destroyed as reconditioning likely will not eliminate the hazard.

If levels of indicator bacteria in samples assayed during distribution or at the point of sale exceed the limits provided in Appendix J, a more thorough investigation should be taken by DOD and the supplier to identify the cause of the higher counts. The investigation should note if the food was at the end of the marked shelf-life, is considered perishable, if the packaging was intact, and if the chill-chain was maintained during storage and distribution. Growth of spoilage microbes is expected to occur during extended storage of perishable items. The higher counts may have resulted from normal growth of spoilage microorganisms or temperature abuse rather than the lack of process control or sanitary conditions during manufacture.

Commodity Specific Comments on Microbiological Limits

Beverages – Bottled water (artisan, mineral, purified, sparkling, spring) – Appendix A, Flow Diagram A.1, Appendix J, Table J.1

The Committee recommends routine coliform testing for bottled water and ice to assess process control and sanitary conditions. In countries where additional microbiological regulations apply, testing for those organisms may be done periodically. A 2013 WHO Draft Report on regulations and standards for drinking water quality recommends routine testing for *E. coli* or thermotolerant coliforms to provide evidence that these microorganisms are undetectable in a 100-ml sample (WHO, 2013). Other indicators also were reviewed in the WHO Draft Report and the following recommendations were made. The presence of total coliforms immediately after treatment indicates inadequate treatment. *C. perfringens* (undetectable in 100 ml) can be used as an indicator of the effectiveness of filtration process to eliminate enteric viruses or protozoan oocysts (WHO, 2013). Enterococci (undetectable in 100 ml) may survive longer than *E. coli* and can be used as an indicator instead of *E. coli*. Total heterotrophic bacteria (limit of 100 CFU/ml at 22 or 20 CFU/ml at 37°C) can be used for operational monitoring of treatment and disinfection and assessing cleanliness of the distribution system. *Pseudomonas aeruginosa*, parasites and enteric viruses were not considered in the WHO report; although they may be required by individual country regulations.

Beverages – Ice, packaged – Appendix A, Flow Diagram A.2, Appendix J, Table J.2

Microbiological testing and limits will be similar to those for bottled water. In countries where additional microbiological regulations apply, periodic testing for the organisms listed in those regulations is appropriate.

Beverages – Juices and drinks, pasteurized, refrigerated – Appendix A, Flow Diagram A.3, Appendix J, Table J.3

The Committee recommends routine coliform testing for process control purposes. Fruit juices in the U.S. are subject to FDA regulations mandating HACCP and achievement of lethality against pathogens of significance (*E. coli* O157:H7, *Salmonella* spp.); thus, periodic testing for pathogens may be indicated (28). This category also includes low acid drinks such as bottled coffees, teas, and vegetable juices. For low-acid juices and drinks, the food safety plan should address the control of pathogenic sporeformers, such as *C. botulinum*. For products that

support the growth of pathogenic sporeformers and where cold-chain management cannot be guaranteed, alternative safety measures could be the inclusion of ingredients that inhibit growth (e.g., blending with acidic juice to reduce pH) or alternative processing such as ultra-high temperature processing to destroy spores. High levels of patulin can be produced in decaying or moldy apples, and thermal processing does not destroy the mycotoxin. Therefore, apple juice products should be tested for patulin (21) (U. S. Department of Health and Human Services, 2005)

Beverages – Shelf stable – Appendix A, Flow Diagram A.4, Appendix J, Table J.4

Process control of shelf-stable (commercially sterile) beverages is dependent upon control of formulation and verification and monitoring of CCPs rather than routine microbiological testing. If inspection observes indications of spoilage such as bulging containers, pH changes, and off-odors then further investigation should be done by DOD and the supplier. Methods for investigating failures in processing for commercial sterility are given in the Compendium of Methods for the Microbiological Examination of Foods (4). Shelf-stable apple juice products should be tested for patulin for the reasons described above for refrigerated juices (21) (U. S. Department of Health and Human Services, 2005).

Dairy – Butter, margarine – Appendix A, Flow Diagram A.5, Appendix J, Table J.5

Although whipped butter held under unrefrigerated conditions has been associated with outbreaks of *S. aureus* intoxication, the low moisture and high salt content, or lactic acid levels of many of these products, generally preclude microbiological growth. However, routine monitoring of sanitation and process control using indicators such as coliforms should be done. Products containing added seasonings, herbs, or spices may have additional testing requirements as the inclusion of unsafe adjunct ingredients has been linked to foodborne illness. Testing for *S. aureus*, *Enterobacteriaceae*, and yeast and molds is useful under special circumstances, such as the investigation of out-of-specification results. Due to listeriosis outbreaks linked to contaminated butter, routine environmental testing of Zone 2 and 3 surfaces for *Listeria* spp. should be done. Although not routinely tested, if Zone 1 environmental samples are found to be positive for *Listeria* spp., investigational testing of finished product should be undertaken.

Dairy – Cheese (hard) – Appendix A, Flow Diagram A.6, Appendix J, Table J.6

Although reported cases of foodborne illness have been linked to foods in this category, microbiological safety issues in hard cheeses made with pasteurized milk and active starter cultures are extremely rare. The presence of active cultures in these products makes the use of routine microbiological testing for APC impractical as a tool for evaluation of process controls and sanitary conditions. In contrast, routine testing for coliforms as an indication of sanitary conditions should be conducted. Testing for *S. aureus* or *E. coli* is useful under special circumstances such as validation, verification and investigation when production has occurred without adequate process control. Finally, routine environmental testing of the food production

environment for the presence of *Listeria* spp. is recommended as a verification step for sanitation programs.

Dairy – Cheese (soft, semi-soft, surface ripened) – Appendix A, Flow Diagram A.7, Appendix J, Table J.7

This category represents a broad range of cheeses. Routine environmental monitoring for *Listeria* spp. in the environment and coliforms in finished product should occur for all products in this category. For products in this category which support the growth of *L. monocytogenes* and have been implicated in illness such as soft cheeses with high pH values, in-plant monitoring for this pathogen may be appropriate (15). Testing for *S. aureus* and *E. coli* may be used when processing or insanitary conditions indicate a potential increased microbiological risk.

Dairy – Cultured, pH<4.8 – Appendix A, Flow Diagrams A.8a and 8b, Appendix J, Table J.8

Rapid acidification and low final pH of these products precludes growth of bacterial pathogens. The presence of active cultures in cultured dairy products make the use of most routine microbiological testing impractical as a tool for evaluation of process controls and sanitary conditions. Routine testing by suppliers for coliforms is recommended to assure compliance with pertinent U.S. regulations and guidance (24). Non-routine testing for *S. aureus* is advisable under limited conditions such as evaluating the impact of a slow fermentation processes. Mold and yeast testing may be applicable when producing cultured products without mold inhibitors or when products contain inclusions such as fruit puree that are known to carry spores. Finally, routine environmental testing of the food production environment for the presence of *Listeria* spp. is recommended as a verification step for sanitation programs.

Dairy – Cultured, pH>4.8 and < 5.4 – Appendix A, Flow Diagram A.9, Appendix J, Table J.9

The active starter culture and acid content present in these fermented products reduces the growth rate of bacterial pathogens; but because the pH is higher than the aforementioned cultured products with pH <4.8, prevention of post-pasteurization contamination is more critical. The presence of active cultures in these products makes the use of most routine microbiological testing impractical as a tool for evaluation of process controls or insanitary conditions. However, routine testing by suppliers for coliforms is recommended to assure compliance with pertinent US regulations and guidance (24) and routine environmental testing of the food production environment for the presence of *Listeria* spp. is recommended as a verification step for sanitation programs. Although typically not done, if Zone 1 environmental samples are positive for *Listeria* spp., finished product testing for *L. monocytogenes* should occur. Testing for *S. aureus*, psychrotrophic microorganisms, yeasts, and molds is useful under the special circumstances described above for Dairy – Cultured, pH<4.8, when investigating results exceeding microbiological limits, or during validation and verification efforts.

Dairy – Dried products (does not include dairy ingredients used to make infant formula) – Appendix A, Flow Diagram A.10, Appendix J, Table J.10

The low moisture content of dried dairy product precludes microbiological growth. However, routine monitoring of sanitation using coliforms and APC should occur. Furthermore, routine testing for *Salmonella* by suppliers should occur as these products have been implicated in cases of salmonellosis. Non-routine testing for *S. aureus* and *B. cereus* should be done under special circumstances such as during investigation of possible mishandling prior to drying, validation or verification efforts, or an investigation done in response to results indicative of process failures or insanitary conditions.

Dairy – Frozen desserts, Appendix A, Flow Diagram A.11, Appendix J, Table J.11

Dairy ingredients used in a dessert mix are pasteurized and will have low microbiological counts; frozen storage will control microbiological growth. Routine testing for coliforms by suppliers should occur to establish process control and monitor sanitation. Although APC can be used to monitor process control, inclusions, such as nuts, cookie dough and fruits, may result in higher populations than the base mix. Periodic testing for *Salmonella* may be indicated under special circumstances such as when lack of process control is suspected, the supplier is using inclusions which have been previously associated with outbreaks, or during validation or verification efforts.

Dairy – Milk and milk products (fluid) – Appendix A, Flow Diagram A.12, Appendix J, Table J.12

Fluid milk in the U.S. is produced under the PMO (24) which provides microbiological limits; when done, such as when there is a pasteurization issue, alkaline phosphatase must be <2.0 micrograms phenol equivalent per gram as an indicator of adequate pasteurization. Routine testing of APC and coliforms by suppliers should occur to ensure regulatory compliance, to help establish process control, and to assist with evaluating sanitary conditions. Routine environmental monitoring of Zone 2 and 3 surfaces for *Listeria* spp. is recommended.

Dairy – Processed Cheese – Appendix A, Flow Diagram A.13, Appendix J, Table J.13

This product is manufactured by heating cheese with water, emulsifier and other ingredients to kill vegetative pathogens; molten cheese may then be hot-filled into loaves or blocks and chilled and cut into individual slices for use; these cheeses are intended to be stored refrigerated. Shelf-stable hot-filled cheese spreads or cheese sauces must be formulated for safety to inhibit *Clostridium botulinum*. Cooling process cheese on casting belts or chill rolls may involve a relatively high degree of environmental exposure of the product. The presence of non-sporeforming microorganisms is indicative of post-process environmental contamination. Low levels of such contamination are inevitable in these cases. Consequently, process cheese producing facilities need to have robust environmental sampling and control plans for *Listeria* spp. and *Salmonella* spp. Formulae with low levels of salt in the moisture phase could potentially allow growth of enterotoxin producing *Staphylococcus* spp., principally *S. aureus*; likely originating from human contact. The presence of generic *E. coli* on process cheese is reflective of production in an insanitary environment.

Egg Products – Pasteurized, processed – Appendix A, Flow Diagram A.14, Appendix J, Table J.14

Pasteurized egg products and pasteurized shell eggs receive a lethality treatment during processing and may be used in dishes which are uncooked or lightly cooked. These products may be recontaminated during packaging, handling and storage. These products should be tested by suppliers routinely for *S. aureus*, coliforms, APC and *Salmonella* to verify process control. Periodically, suppliers may test these products for *B. cereus* and *Enterobacteriaceae*. Routine environmental testing for *Listeria* spp. and *Salmonella* is useful to evaluate sanitary conditions. If samples exceed the microbiological limits, further investigation and correction action should occur. Environmental monitoring of Zone 2 and 3 surfaces for *Listeria* spp. is recommended; if *Listeria* spp. are found, it may lead to testing of Zone 1 surfaces for *Listeria* spp. Finished product testing should occur for *L. monocytogenes* if *Listeria* spp. are detected on Zone 1 surfaces (indicative of insanitary conditions) or suspected illnesses are reported.

Egg Products – Shell eggs, raw – Appendix A, Flow Diagram A.15, Appendix J, Table J.15

Raw shell eggs are not pasteurized and are not intended for consumption without an additional lethality step, such as thorough cooking. Regulations in the U.S. require that high-volume producers ($\geq 50,000$ laying hens) test for *Salmonella* serotype Enteritidis to verify non-detection of this pathogen in the shell eggs (22). High-volume producers supplying shell eggs to DOD should test for *S. Enteritidis*. For other producers, the Committee recommends only periodic or investigational testing of raw shell eggs and no microbiological limits are provided. Testing for *E. coli*, coliforms or *Enterobacteriaceae* by suppliers may be useful to assess sanitary conditions or establish process control.

Grain-based Products – RTE, baked items, refrigerated or temperature/time controlled for safety (TCS) – Appendix A, Flow Diagram A.16, Appendix J, Table J.16

These products are prepared with a lethality step to eliminate pathogens; but the potential of recontamination during handling and the pH- a_w range (that can support microbiological growth during extended out-of-refrigeration storage) warrants microbiological testing. Routine monitoring of coliforms by suppliers should assess insanitary conditions (including post-process contamination). APC testing should not be conducted if the products include ingredients which are prepared using starter cultures (e.g., cheese, salami).

Grain-based Products – RTE, baked items, shelf stable or non-TCS – Appendix A, Flow Diagram A.17, Appendix J, Table J.17

When manufacturing these products, the dough or batter goes through a baking step which provides lethality against pathogens and pathogen growth is unlikely during storage due to reduced water activity. While routine microbiological testing by suppliers generally is unnecessary, environmental monitoring and in-process sample testing may be appropriate under special circumstances that may increase the microbiological risk (e.g., excessive water due to condensate or roof leaks) or when ingredients are added after the lethality step (e.g., dusting of bread surface with flour).

Grain-based Products – RTE, cereals – Appendix A, Flow Diagram A.18, Appendix J, Table J.18

RTE cereals are made from grains that go through a lethality step sufficient to eliminate pathogens of concern. Mycotoxin surveillance testing should be completed on incoming grains to ensure the grains meet the individual country's regulations. These RTE grain-based products do not support the growth of microorganisms due to the very low a_w . Routine microbiological testing of finished product by suppliers is not recommended; but routine environmental testing for *Salmonella* is useful to assess sanitary conditions. Non-routine testing for coliforms, *Enterobacteriaceae*, APC and *Salmonella* by suppliers is appropriate for verification purposes, qualifying lines, or when events occur during processing that may increase the microbiological risk (e.g., excessive water due to condensate or roof leaks). If vitamin-containing or other such solutions are sprayed atop cereals after heat-processing, and depending on the source and processing of these solutions, sampling and testing of these solutions may be a useful measure of process control.

Grain-based Products – RTE, cold pressed bars – Appendix A, Flow Diagram A.19, Appendix J, Table J.19

Cold-pressed bars are made from cooked grains, carbohydrate-based binders, and inclusions such as fruit, nuts and chocolate. Verification of the microbiological quality of ingredients used in the cold-pressed bar formula is important since the bars will not receive a validated lethality step during manufacturing. Recommendations for finished product and environmental testing by suppliers are the same as for RTE cereals above.

Grain-based Products – Non-RTE, dry, flour-based mixes – Appendix A, Flow Diagram A.20, Appendix J, Table J.20

These Non-RTE grain-based products harbor a complex and extensive microbiota and routine microbiological testing by suppliers does not provide useful data to indicate process control and sanitation (17). Flour is a minimally-processed commodity that is ground and sifted without any lethality step. These products should receive a lethality step to eliminate pathogens before consumption.

Grain-based Products – Non-RTE, pasta, dried or refrigerated – Appendix A, Flow Diagram A.21, Appendix J, Table J.21

Pasta is produced by combining flour and water and sometimes other minor ingredients. The microbiological profile may be similar to that of flour and routine testing by suppliers is not particularly useful. However, the manufacturing process must be controlled to minimize proliferation of naturally occurring microbiota after the introduction of moisture. Non-routine testing of in-process samples by suppliers may be useful in special circumstances (e.g., evaluation of potential growth and enterotoxin production by *S. aureus* during extended down time prior to drying or refrigeration). Although most of these products are intended to be cooked by consumers before consumption, some varieties, such as instant noodles, may be prepared with limited heating. Cooking of refrigerated pasta filled with meat or cheese may be sufficient to

cook the outer pasta, but not sufficient to provide a validated lethality step in the product interior. Verification testing of raw materials (to support the Certificate of Analysis) and periodic testing of product by suppliers for *Salmonella* may be appropriate; and environmental testing for *Listeria* spp. or *Salmonella* should occur to verify sanitary conditions.

Meals and Entrees – Non-RTE, Ready-To-Cook (RTC) meals, includes raw ingredients – Appendix A, Flow Diagram A.22, Appendix J, Table J.22

This category includes a wide range of multi-component (some raw), frozen or refrigerated food products which are expected to be cooked by the consumer or food service operation. Routine testing of these meals is not recommended; however manufacturers should be aware of the following points. Suppliers should assess the pathogens and indicator organisms associated with their products and sample and test if there is a reason to do so. Some of these meals and entrees may be improperly prepared by the consumer using conventional or microwave ovens and not undergo a validated lethality step. Pathogens of concern may vary depending on the specific food. For example, meals prepared with cooked rice may pose a greater risk for *B. cereus*; *E. coli* O157:H7 may be of concern for foods including raw, non-intact beef, and poultry products may contain *Salmonella*. Histamine testing may be appropriate when scombroid species are present.

Meals and Entrees – RTE, deli salads, sandwiches, heat-eat meals, sushi – Appendix A, Flow Diagram A.23, Appendix J, Table J.23

This category includes a wide range of multi-component, short shelf-life, refrigerated food products. They are expected to have diverse microbiological populations depending on the ingredients used, may include ingredients which are raw, such as fresh produce, and are frequently subjected to multiple handling steps which can introduce contamination. Routine testing by suppliers of in-process or finished products for *E. coli* and environmental testing for *Listeria* spp. and in some instances, *Salmonella* spp., should occur to assess process control and sanitary conditions. As with the non-RTE, RTC meals, other non-routine testing of indicator organisms and pathogens may be appropriate depending on the ingredients used and the type of finished product. Although not routinely done, if *Listeria* spp. is found in Zone 1 environmental samples, investigational testing for *L. monocytogenes* may be indicated.

Meals and Entrees – RTE sous vide, cook and chill – Appendix A, Flow Diagram A.24, Appendix J, Table J.24

Sous vide products are prepared with raw or partially cooked foods, which are vacuum packaged in an impermeable bag, cooked in the bag, rapidly chilled, and refrigerated with time-temperature combinations that inhibit pathogen growth. If the cook process does not provide at least a validated 6-log₁₀ reduction of non-proteolytic *C. botulinum* spores (7), validation data should be provided by the supplier to demonstrate that the process eliminates vegetative pathogens. Because of the lack of inhibitory barriers in typical sous-vide products and the concern for potential outgrowth of botulinum spores, strict adherence to refrigerated storage after treatment is extremely important. If a validated cook step is used and verified, no routine testing is recommended. In the absence of a validated cook process, testing for vegetative

microorganisms should be done by the supplier on post-cook samples to verify the thermal process. Testing for *E. coli* can serve as a verification of thermal processing; periodic testing of coliforms, *Enterobacteriaceae* and APC are useful for verification purposes. If cooling deviates from prescribed requirements such as those given in USDA Appendix B (18) , testing for *C. perfringens* may be useful as a part of the supporting documentation for safety. Routine testing for *C. perfringens* typically is not done.

Meat, Pork, Poultry Products –Non-RTE, beef and pork, raw, intact and non-intact – Appendix A, Flow Diagram A.25, Appendix J, Table J.25

These products include both intact (e.g., non-tenderized steaks, chops) and non-intact (e.g., whole muscle destined for ground product, trim, ground product, needle-tenderized steaks) raw beef and pork products. Under normal operating conditions, no routine testing is recommended. When it is necessary to meet a regulatory or customer requirement to confirm production is occurring with process control and sanitary conditions, suppliers should test for *E. coli* (typical for the U.S.) or *Enterobacteriaceae* (typical for the European Union). Those manufacturers supplying DOD with non-intact product should request that their suppliers (secondary suppliers) provide a Certificate of Analysis demonstrating that the raw materials have tested negative for *E. coli* O157:H7 and other STEC, if appropriate. Suppliers to DOD also may test for *Salmonella* to meet regulatory requirements or to provide evidence that they are meeting performance standards that indicate production has occurred under sanitary conditions; this testing may typically be done only for ground products.

Meat, Pork, Poultry Products –Non-RTE, poultry, raw – Appendix A, Flow Diagram A.26, Appendix J, Table J.26

These products include both intact (e.g., non-injected whole birds, non-injected parts) and non-intact (e.g., injected or “enhanced” or vacuum-tumbled poultry parts, ground poultry) raw poultry products. Under normal operating conditions, no routine testing is recommended. Production of these foods should include appropriate process controls to reduce pathogens to acceptable levels and to prevent pathogen growth. When it is necessary to meet a regulatory or customer requirement to confirm production is occurring with process controls and sanitary conditions, or under specific circumstances when an investigation is underway, suppliers may test for *Salmonella* and *Campylobacter* to verify process control and that pathogens are being reduced to acceptable levels. In this case, testing should be performed on the relevant product type such as raw poultry parts if they are the product type purchased. Testing for indicator organisms or classes of organisms such as generic *E. coli*, coliforms, *Enterobacteriaceae*, or APC, could provide additional information regarding maintenance of process control and sanitary conditions.

Meat, Pork, Poultry Products – RTE, cooked, perishable – Appendix A, Flow Diagram A.27, Appendix J, Table J.27

This group includes a spectrum of cooked beef, pork and poultry products which require strict refrigeration for shelf life and safety (e.g., deli meats, hot dogs). While process control is often monitored through routine testing of *E. coli*, potential contamination of *L. monocytogenes* is a

major concern and should be addressed by the supplier through routine environmental monitoring of Zone 2 and 3 surfaces for *Listeria* spp. Although not routinely tested, if Zone 1 environmental samples are positive, finished product testing for *L. monocytogenes* may be indicated. Non-routine testing of coliforms or *Enterobacteriaceae*, APC, *Salmonella*, and *C. perfringens* may be useful for additional verification of sanitary conditions, adequate cooling, or as periodic verification of process control.

Meat, Pork, and Poultry Products – RTE, fermented, dried – Appendix A, Flow Diagram A.28, Appendix J, Table J.28

These products (e.g., jerky, dried pepperoni, meat sticks) are characterized by having chemical/physical characteristics (e.g., a_w and pH) that ensure the products will not spoil or become unsafe when stored out of refrigeration throughout the manufacturer's specified shelf-life. However, it is essential that production of these foods include appropriate process steps to reduce pathogens to acceptable levels and prevent growth of pathogens or the formation of their toxins (e.g., cooking jerky with adequate humidity to prevent surface drying, active fermentation to inhibit growth of *S. aureus*, and a lethality step to eliminate low-infectious dose pathogens such as *Salmonella* and *E. coli* O157:H7) (8). Suppliers should use *E. coli* for routine monitoring; coliforms and *Enterobacteriaceae* may be appropriate for verification monitoring. Testing of products for bacteria, such as *Salmonella*, *E. coli* O157:H7 and *S. aureus* may be appropriate when process controls are suspect, e.g., failed fermentation or extended drying times.

Nuts and Nut Butters – RTE, not processed for lethality – Appendix A, Flow Diagram A.29, Appendix J, Table J.29

Raw nuts (not processed for lethality) may be contaminated with microbiota from orchards, the ground, or equipment and personnel during harvesting, shipping, processing, and handling. Because consumption of raw nuts has been associated with illness, suppliers should test in-process samples and finished products routinely for *Salmonella* and implement an environment testing program that includes testing for *Salmonella*. For certain nuts (e.g., peanuts, pistachios, Brazil nuts), routine testing for aflatoxin B1 should be done. Non-routine testing for *E. coli* and aflatoxin B1 (for those not tested routinely for aflatoxin B1) may be done to assess sanitary storage and production, and the quality of the raw nuts.

Nuts and Nut Butters – RTE, processed for lethality – Appendix A, Flow Diagram A.30, Appendix J, Table J.30

In this category, peanuts and tree nuts are processed for lethality (e.g., by dry roasting, oil roasting, or steam processing). Because nuts and nut butters have been associated with illness, routine environmental testing, testing in-process samples, and finished product testing for *Salmonella* should be done. For certain nuts (e.g., peanuts, pistachios, Brazil nuts), routine testing for aflatoxin B1 should be done. Non-routine testing for *E. coli* and aflatoxin B1 (for those not tested routinely) may be conducted to help assess sanitary storage and production, and the quality of the raw nuts used in manufacturing.

Produce –Fruits and vegetables, cut, frozen or refrigerated, minimally processed – Appendix A, Flow Diagram A.31, Appendix J, Table J. 31

Further processing of fresh fruits and vegetables may increase or decrease microbiological populations depending on GMPs, sanitary design of equipment, washing, blanching, or the use of antimicrobials. Routine testing by suppliers of product for *E. coli* and the environment for *Listeria* spp. should be done to assess process control and sanitary conditions. Periodic testing by suppliers of in-process or finished products for *Salmonella* or *E. coli* O157:H7 (or other appropriate STEC) may be pertinent depending on the commodity, geographic location and use of GAPs.

Produce –Fruits and vegetables, whole – Appendix A, Flow Diagram A.32, Appendix J, Table J.32

Fruits and vegetables are expected to have microbiota associated with them. Whole fruits and vegetables may be washed before introduction to commerce, but undergo no other lethality step. Environmental testing in the packing house for *Listeria* spp. and *Salmonella* should be done by the supplier to assess sanitary conditions, with the frequency dependent upon factors such as the commodity, geographic location and use of GAPs. Although not listed in Table J.32 nor routinely done, the DOD may consider testing (by the supplier or DOD) for *Cyclospora cayentanensis*, *Cryptosporidium parvum*, enteric viruses, or *Shigella* spp. as appropriate when there is knowledge or suspicion high risk farming and handling practices (e.g., where evidence of previous contamination exists, water contamination is likely, or contaminated fertilizer is used).

Produce –Mushrooms – Appendix A, Flow Diagram A.33, Appendix J, Table J.33

Mushrooms are generally commercially produced indoors on composted substrate. They are grown, harvested, sorted, graded, and packaged, and may or may not be sliced. No routine testing of product is typically conducted because populations of indigenous microbiota likely will be high. Routine monitoring and testing of the environment s by suppliers for *Listeria* spp. may be deemed appropriate by DOD to assess sanitary conditions and process control. Such testing would depend on factors such as the type of compost used, the water used, the harvesting techniques, the storage and handling conditions, and the intended end use.

Produce – Packaged salads and leafy greens – Appendix A, Flow Diagram A.34, Appendix J, Table J.34

Salad greens are expected to have microbiota that can originate from numerous sources such as irrigation water, insects, birds, animals, and post-harvest handling and processing. When salad greens are washed, particularly when a chemical such as chlorine is added to the wash water, some microorganisms can be physically washed off; however, the washing process also can contribute to cross contamination. Packaged salads and leafy greens generally have a limited shelf life. Suppliers can use testing for *E. coli* to assess process control and sanitary conditions. Environmental testing for *Listeria* spp. in processing facilities should be conducted to monitor sanitary conditions.

Produce – Vegetable sprouts – Appendix A, Flow Diagram A.35, Appendix J, Table J.35

These are sprouted vegetable seeds before true leaves emerge that may be consumed raw or cooked. Routine testing of in-process and finished products by suppliers for *E. coli* should be done as an indicator of process control and sanitary production. Appropriate testing of spent irrigation water for *Salmonella* and *E. coli* O157:H7 should be conducted to assess potential product contamination. Routine environmental monitoring for *Listeria* spp. also should occur to assess sanitary conditions.

Seafood – Raw – Appendix A, Flow Diagrams A.36a-e, Appendix J, Table J.36

Routine microbiological testing of in-process and finished products by suppliers is not recommended for raw (fresh or frozen) finfish or raw crustaceans for either quality or safety. Non-routine testing of in-process and finished products for coliforms and *Salmonella* may be done to verify proper sanitation and process control. A visual inspection for parasites is recommended if the product is intended for raw consumption. Alternatively, the supplier may verify that freezing treatments are applied to destroy certain parasites. For scombroid species, testing of finished product for histamine is recommended.

Seafood – RTE, fish, cold smoked – Appendix A, Flow Diagram A.37, Appendix J, Table J.37

Suppliers should conduct routine environmental testing for *Listeria* spp. to demonstrate that production is occurring under sanitary conditions. The supplier also should test in-process and finished products periodically for *L. monocytogenes* and *Salmonella* to demonstrate that the product is produced under sanitary conditions. The pH of pickled herring should be verified periodically. Scombroid species may contain histamine and products made from these species should be tested to verify that proper temperature control was maintained.

Seafood – RTE, cooked or hot smoked – Appendix A, Flow Diagram A.38, Appendix J, Table J.38

The supplier should apply a validated process that results in at least a 6-log₁₀ reduction of *L. monocytogenes*. When such a validated process is used, routine sampling of in-process and finished product for *S. aureus* and the environment for *Listeria* spp. should occur to verify that controls are in place to prevent recontamination. If required to further demonstrate that production is occurring under process control and sanitary conditions, the supplier could also test in-process and finished products for coliforms, APC, *Salmonella* and *L. monocytogenes*. If it is apparent that there is a potential for recontamination through mechanical or manual handling, testing finished products for *Salmonella* and *L. monocytogenes* should be done routinely. Scombroid species may contain histamine if temperature abused and fish decompose; finished products should be tested for histamine per FDA's guidance documents (26).

Seafood – RTE, raw molluscan shellfish – Appendix A, Flow Diagram A.39, Appendix J, Table J.39

Suppliers must demonstrate traceability that establishes that the product was harvested from approved waters in the U.S. or in countries (Canada, Mexico, New Zealand, South Korea) that have a Memorandum of Understanding with the U.S. Under these conditions, no routine microbiological testing of products is necessary by the supplier. Where the supplier is unable to prove the status of the harvest waters, or where contamination is suspected, the DOD should not accept the product. Non-routine in-process and finished product testing by suppliers on RTE, raw molluscan shellfish from approved waters to demonstrate process control and sanitary conditions may include analyses for APC, fecal coliforms, and *Vibrio parahaemolyticus* (or other *Vibrio* spp. if warranted). In addition, *Vibrio* control plans as outlined in the National Shellfish Sanitation Program (25) may be required if conditions warrant.

Spices and Herbs, Coffee and Tea – Appendix A, Flow Diagrams A.40.a, A.40.b, and A.40.c, Appendix J, Table 40

Harvested spices are expected to have a varied microbiota associated with them, including spore-forming bacteria and fungi. Also, when a dehydration process is performed outdoors there is the potential to acquire additional contamination. Suppliers should test in-process and finished products routinely for APC and *Salmonella* to assess process controls and sanitary conditions. The suppliers also should routinely test the environment for *Salmonella*. Non-routine testing of finished products by suppliers, when deemed necessary, to assess process control and sanitary conditions may include testing for *B. cereus* (or other toxigenic *Bacillus* spp.), *E. coli*, coliforms, mold and yeasts, and *E. coli* O157:H7 (or other STEC as appropriate).

OTHER INDICATORS OF PROCESS CONTROL AND SANITARY CONDITIONS

There are microbiological by-products, enzymes, products of decomposition (including those detected through visual observation), and other analytes that may reflect lack of process control or insanitary conditions. The following are examples of some of these indicators.

- Histamine in scombroid fish at high levels indicates possible temperature abuse, lack of sanitary conditions, and decomposition of these fish.
- The presence of non-microbiological alkaline phosphatase in milk is an indication that the milk has been inadequately pasteurized. Under these conditions microbiological pathogens endemic to raw milk may survive and result in milk-borne illness.
- Peroxidase testing is used to indicate that blanching of fresh vegetables has been adequate. Typical blanching temperatures (195 – 205°F for 3 minutes) would likely be sufficient to provide a lethality step eliminating vegetative pathogens.
- The presence of aflatoxin or other mycotoxins is indicative of significant growth of molds. The presence of aflatoxin or other mycotoxins may render the food unacceptable for human consumption or for use in further food processing.
- Gas formation causing swollen product containers would be indicative of spoilage and potential pathogen growth. Similarly, slime formation, visible mold growth, discoloration and product leakage from a container would be indicative of spoilage or potential growth of pathogens. Changes in product viscosity may be indicative of microbiological proteolysis or

starch hydrolysis; such activity may be the result of post-processing contamination and temperature abuse, or under processing.

- Peroxide values and concentrations of free fatty acids in nuts exceeding tolerance limits would be indicative of poor storage conditions, extended age or temperature abuse. In such situations, these changes would not indicate microbiological spoilage or growth, but oxidation that impacts quality.
- When free fatty acid concentrations in milk exceed tolerances, this is indicative of hydrolytic rancidity associated with poor raw material control and potential post-process contamination.
- Any signs of pests or pest infestation indicate contaminated packaging materials, poor storage conditions within a plant or distribution center, pest contamination within a transport container or at the location of sampling. These products should be considered compromised and unacceptable.
- Development of acidity (measured by pH or titration) is critical to the safe production of many fermented products such as cheeses, and fermented sausages. Fermentation of these products by harmless starter organisms retards or prevents the growth of pathogenic bacteria like *E. coli*, *Salmonella* and *L. monocytogenes*. However, in other products acid development is undesirable, e.g., flat sour defect in canned food resulting from undesirable microbiological growth. Undesirable fermentation can result in expression of purge in RTE meat products.

GLOSSARY

Term	Acronym /Symbol	Definition
Acceptance number	C	Indicates the maximum number of non-conforming analytical units (two-class sampling plans) or marginally acceptable analytical units (three-class sampling plans) that can result in lot acceptance.
Aerobic plate count	APC	The enumeration of colony forming units of mesophilic aerobic and facultative anaerobic organisms on an appropriate non-selective medium.
Analyte		Target for assay detection, isolation or quantification, e.g., <i>Salmonella</i> .
Analytical portion		The relevant quantity – mass, volume or area – of the food product that is being tested in each analytical unit. The analytical portion is less than or equal to the sample unit amount. For example, a 1 ml analytical portion of diluted homogenate may be analyzed from a 25 g sample unit.

Analytical unit		A single unit of food, from which a predetermined analytical portion is removed and tested for microorganisms. All or part of the sample unit may be used as the analytical unit, or multiple sample units may be composited into a single analytical unit for presence/absence testing.
Attributes sampling plans		Attributes sampling plans are used when the measured characteristics are qualitative or categorical. Microbial presence/absence data and quantitative concentration data categorized into numerical ranges are classified as attributes.
Bernoulli process		A Bernoulli process is a random process the result of which can only take one of two values, e.g., presence/absence.
Binomial distribution		The discrete probability distribution of the number of "successes" in a sequence of n independent Bernoulli (yes/no) trials, each of which yields success with constant probability (p)
Certificate of Analysis		A document attesting to the quality and purity of a product lot.
Certificate of Conformance		A document issued by a competent authority that the product meets required specifications.
Colony forming units	CFU	The number of single or clumped multiple cell aggregates giving rise to colonies recovered on a solid medium.
Consumer's risk	B	The probability of accepting a non-conforming lot. A false negative or type II error.
Control limits, lower and upper	LCL and UCL	The control limits delineate the expected extent of natural variability in the process. Conventionally defined as ± 3 standard deviations about the mean, but can be adjusted based on the desired false alarm rate.
Count		The number of colony forming units recovered from an analytical portion
Criterion/criteria		See microbiological criterion
Critical Control Point	CCP	The point in food manufacturing at which effective control can be exercised over a hazard.
Cumulative distribution function	CDF	Describes the probability that a random variable X will be found to have a value less than or equal to x: $F(x) = P(X \leq x)$.
Department of Defense	DOD	United States Department of Defense

Design prevalence		The prevalence that the sample is designed to detect with a specified probability. May or may not be the assumed prevalence of an attribute in a population from which samples are drawn.
Empirical cumulative distribution function	ECDF	The cumulative distribution function associated with the empirical (observed) measure of a sample. The non-parametric estimator of the CDF.
Empirical distribution function	EDF	Synonymous with empirical cumulative distribution function
Environmental monitoring program	EMP	A program wherein equipment and facility sites are tested routinely for non-pathogens or pathogens to determine the extent to which these microorganisms are present and could likely contaminate food products manufactured in the facility.
Exponential distribution		The probability distribution that describes the time between events in a Poisson process, <i>i.e.</i> , a process in which events occur continuously and independently at a constant average rate.
Exponentially weighted moving average	EWMA	A curve smoothing technique applied to time series data that exponentially down weights older observations.
False alarm rate	FAR	The expected rate of false positives, <i>e.g.</i> , indicating a loss of process control when the process actually remains under control
G-chart		A control chart used to monitor very low prevalence contamination. Tracks the interval (number of samples) between positives.
Good Manufacturing Practices	GMP	Those hygienic practices described in the Code of Federal Regulations, <i>e.g.</i> , 21CFR 110.
Guidelines		Advisory criteria used to inform food operators and others of the microbiological content expected in a food when best practices are applied.
High-event period		A production period when the observed prevalence likely exceeds the expected or design prevalence

Homogeneous (statistical)		Statistically, a volume of production is considered homogenous relative to a given characteristic (e.g., concentration of the microorganism) if the characteristic follows the same probability distribution throughout the volume (e.g., lognormal with fixed mean μ and fixed standard deviation σ). In contrast to a homogeneous (uniform) spatial distribution.
Individuals Chart (i-chart)		Control chart for individual measurements
In-process samples		Refers to sampling of food products or ingredients that have not completed a manufacturing process by a supplier
Insanitary		This word is used synonymously with unsanitary in this document. It refers to conditions where lack of appropriate hygienic conditions has resulted in unsatisfactory microbiological contamination.
Lognormal distribution		A continuous probability distribution of a random variable whose logarithm is normally distributed.
Lot		A predefined quantity of food product, produced under similar, or uniform, conditions so that the units in the lot are similar in their microbiological status. In lot acceptance sampling, the quantity of food product represented by the samples.
Mean time between positives	MTBP	The average number of samples between positives
Microbiological criterion		The specification of a microbiological criterion includes the selected microorganism(s); the microbiological limits; the sampling plan defining the number of sample units to be taken (n), the size of the analytical unit, and where appropriate, the acceptance number (c); and the analytical methods.
Microbiological limit		Microbiological limits are those levels above which might be indicative of loss of process control or insanitary conditions and may lead to further investigation with corrective or preventive actions.
Microbiological limit for marginally acceptable concentration	m	Delimits acceptable and marginally acceptable concentrations. Used in 3-class sampling plans

Microbiological limit for unacceptable concentration	M	Marks the limit beyond which the level of contamination is hazardous or unacceptable Used in 2- and 3-class sampling plans
Mixture distribution		The probability distribution of a random variable whose values can be interpreted as being derived from multiple underlying probability distributions
Most probable number	MPN	An estimated quantitative concentration measurement developed using serial dilutions and detection methods.
Negative		When the target organism is not detected in the analytical unit, then the analytical unit is commonly referred to as "negative."
Nonparametric		Makes no assumptions about the probability distribution of the random variable
Non-routine testing		Non-routine testing can be investigational, for verification, validation, surveillance, or for qualifying suppliers. Non-routine testing is less frequent and can be based on time intervals (e.g., weekly, monthly, quarterly) or based on other indicators of lack of process control or insanitary conditions.
Normal distribution		A continuous probability distribution that is symmetric about the mean (μ), with approximately 95% of values lying within ± 2 standard deviations (2σ) of the mean.
Operating characteristic curve		Describes the probability of accepting a lot as a function of lot quality
Parametric		Assumes that the data have come from a theoretical probability distribution defined by its parameters
P-Chart		A process control chart that monitors the proportion of non-conforming analytical units observed in a sample of size n, applicable for moderate prevalence levels.
Plan of action	POA	Pre-determined plan of action, such as corrective action plan
Poisson distribution		Describes the probability of a given number of events occurring in a fixed interval of time and/or space if the events occur independently with a constant average rate

Positive		When the target organism is detected in the analytical unit, then the analytical unit is commonly referred to as "positive."
Prevalence		The proportion of analytical units that contain the target microorganism. The observed prevalence depends on the analytical unit size and needs to be referenced to an analytical unit size, <i>i.e.</i> , prevalence of positives in X grams
Process capability	C_p	The ability of a process to meet specification limits.
Process control		Maintaining the output of a specific process (<i>e.g.</i> , food manufacturing, storage and distribution system) within a desired range.
Producer's risk	A	The probability of rejecting a conforming lot. A false positive or type I error.
Quantile		The value associated with a percentile of the cumulative distribution function. If $p(X \leq A) = B$, A is the quantile value and B is the percentile of the CDF.
R-Chart		Range Chart used to monitor process variability for continuous numerical data.
Routine		Routine testing is defined as testing done at pre-determined intervals at sufficient frequency to establish process control or sanitary conditions. The sampling interval may be on a physical lot basis (<i>e.g.</i> , 2,000 lb. combos for ground beef) or temporal basis (<i>e.g.</i> , per shift, daily, weekly, monthly). The frequency of testing should be determined based on potential risks and performance of the system.
Ready-to-eat food	RTE	Food that is in a form that may be safely eaten without additional preparation to achieve food safety
Sample		A subset of units from the lot or production process, selected in some predetermined manner.
Sample size	n	The number of samples units drawn to collect a sample
Sample unit		A single unit of food of a predetermined sample unit amount (mass, volume, or area). All or part of the sample unit may be used as the analytical unit, or multiple sample units may be composited into a single analytical unit for presence/absence testing.
Sampling plan		Defines the number of sample units to be taken (n), the size of the analytical unit, and where appropriate, the acceptance number (c).

Specification limits, lower and upper	LSL and USL	Boundaries that define acceptable product
Specifications		Specifications are part of a purchasing agreement between a buyer and a supplier of a food and may be advisory or mandatory according to use.
Standard operating range	SOR	A process is considered under statistical control when its output varies as expected within a standard operating range (SOR) of variation. This refers to common cause variation and represents the random variation inherent in a process.
Standards		Standards are mandatory criteria incorporated into a law or ordinance (normally pathogen oriented)
Statistical control		A process is considered under statistical control if it is stable over time and the observed variation is due to common, chance causes inherent to the process and there is no between-lot variation. Statistical control means only that the process output is predictable and is distinct from the capability of a process to meet specifications.
Statistical Process Control	SPC	A formal approach that uses statistical methods to monitor and control a process.
Temperature/time control for safety	TCS	A food that requires time/temperature control for safety to limit pathogenic microorganism growth or toxin formation. For a further description of TCS foods, refer to FDA 2013 Food Code at http://www.fda.gov/downloads/Food/GuidanceRegulation/RegulatoryInformation/FoodCode/UCM374510.pdf
Unit operations		A single manufacturing or supply chain step, e.g., blanching vegetables, slicing meat, loading a trailer.
Unsanitary		This word is used synonymously with insanitary in this document. It refers to conditions where lack of appropriate hygienic conditions has resulted in unsatisfactory microbiological contamination not conducive to or promoting health; dirty or unhygienic.
Validation		The body of scientific evidence that demonstrates a process or procedure is effective in producing the outcome for which it was intended
Variables sampling plans		Variables sampling plans are used when the measured characteristics are expressed on a continuous numerical scale, e.g., concentration data.
Verification		Those activities, other than monitoring, that establish the validity of a food safety plan and that the food safety system is operating according to the plan.

Water activity	a_w	A measurement between 0.00 and 1.00 defining the amount of moisture available for microbiological or chemical activity. Deionized water has an a_w of 1.00 under standard conditions. Microbes are not known to grow below a_w 0.60.
Worldwide Directory		Worldwide Directory of Sanitarily Approved Food Establishments for Armed Forces Procurement, 2012

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Appendix A. Schematic Flow Diagrams of Production of Various Food Categories and Bottled Water

These generic process flow charts are intended to provide DOD auditors with potential steps in the manufacturing process where microbiological counts could increase with loss of process control or development of insanitary conditions. In addition, the flow charts illustrate where there are lethality steps that reduce numbers of indicator organisms and pathogens if present.

Steps for receiving and storing packaging materials were omitted to simplify the creation – and use – of the process flow diagrams. It is expected that a DOD-approved food processing plant would have appropriate control and documentation of these functions, either as part of product-specific HACCP plans, or as preventive and pre-requisite programs such as Standard Operating Procedures for receiving and storage. It was recognized that a finished food product could move through many storage and distribution facilities as part of the supply chain. The final two steps were denoted “store finished product” and “distribute finished product” to simplify the creation and use of the process flow diagrams.

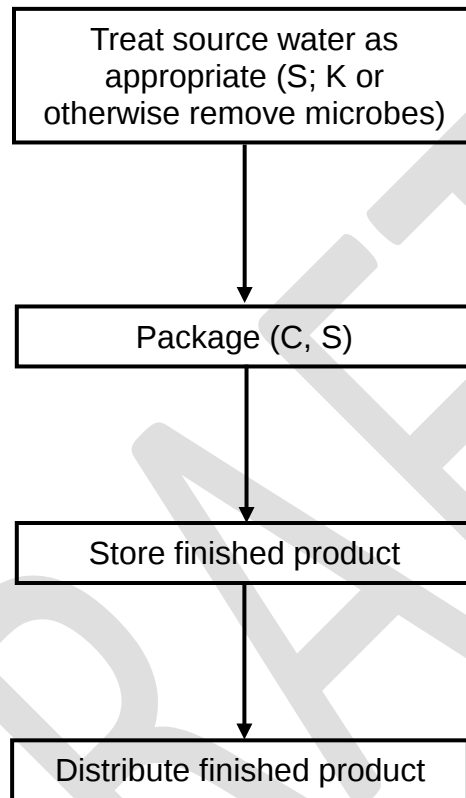
The intent was to include those steps relevant of the manufacturing process relevant to microbial aspects of food rather than to include all possible aspects or combinations of receipt, processing, storage, and distribution of production. The Committee assumes that DOD personnel will be able to recognize the specific steps observed at a food processing plant from among the general manufacturing steps shown on the process flow diagrams.

Steps may be followed by any of the following designations:

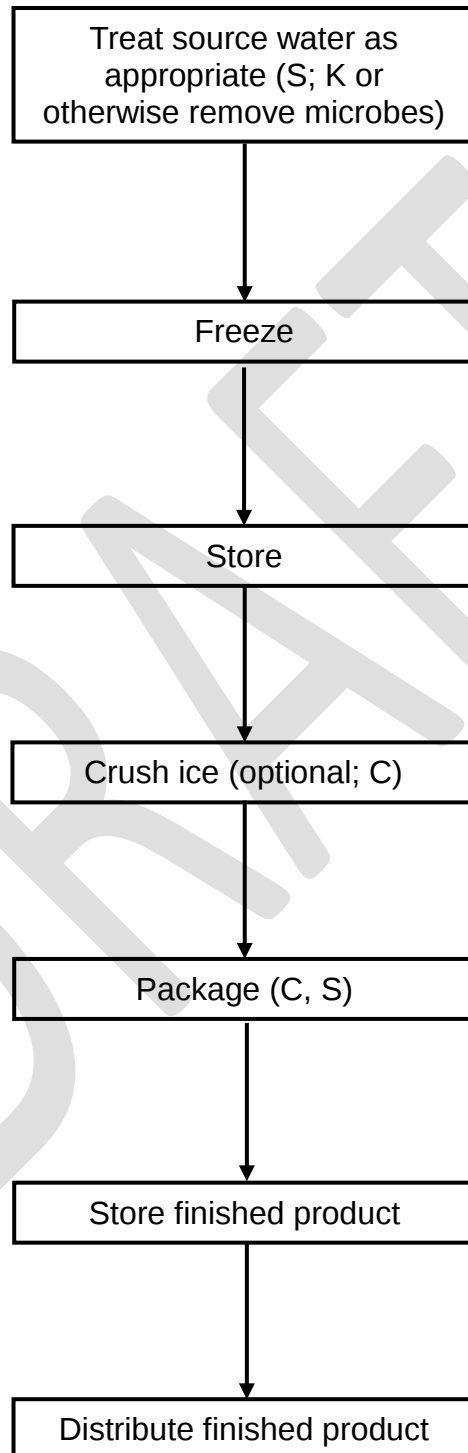
- C, a step at which significant contamination may occur when adequate process controls are not in place;
- G, a step in the process where growth of microorganisms can occur;
- K, a step where there is a pathogen kill step; and
- S, a point where sampling and testing by the supplier is recommended for verification or investigation.

Programs for minimizing contamination at the identified steps include Good Agricultural Practices, Sanitation Standard Operating Procedures, Good Manufacturing Practices, and purchasing specifications. DOD personnel should use the process flow diagrams to review the general steps to manufacture the food product under evaluation. From the process flow diagram, DOD personnel should determine the step(s) at which verification sampling should be done by the supplier. When analysis of verification samples indicates that the supplier may have shortcomings in process or sanitation control, DOD personnel should use the process flow diagram to determine steps at which contamination might occur or steps at which a failure to achieve the expected destruction of bacteria may be occurring.

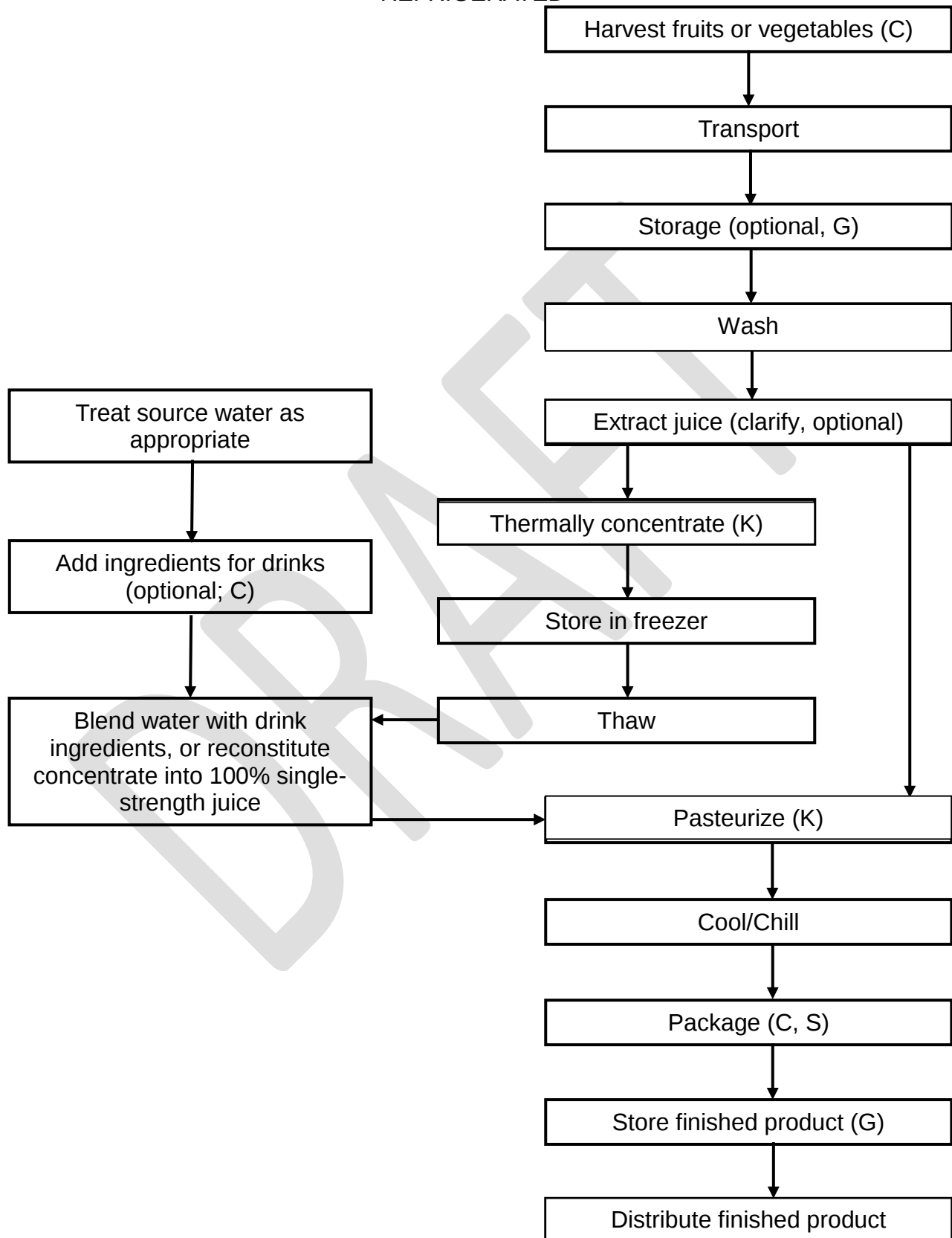
Flow Diagram A.1. BEVERAGES – BOTTLED
WATER (ARTESIAN, MINERAL, PURIFIED,
SPARKLING AND SPRING WATER)



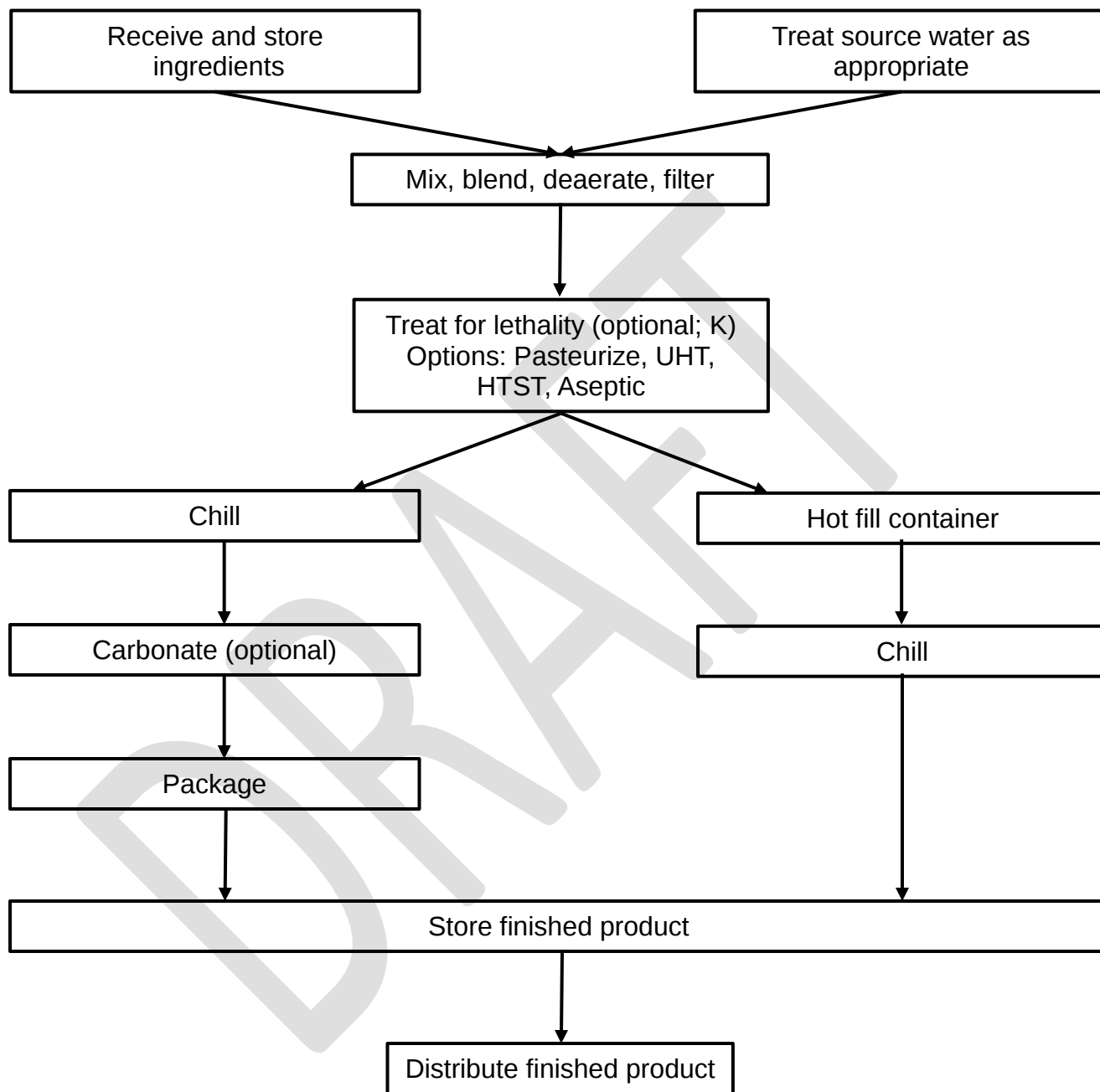
Flow Diagram A.2. BEVERAGES – ICE, PACKAGED



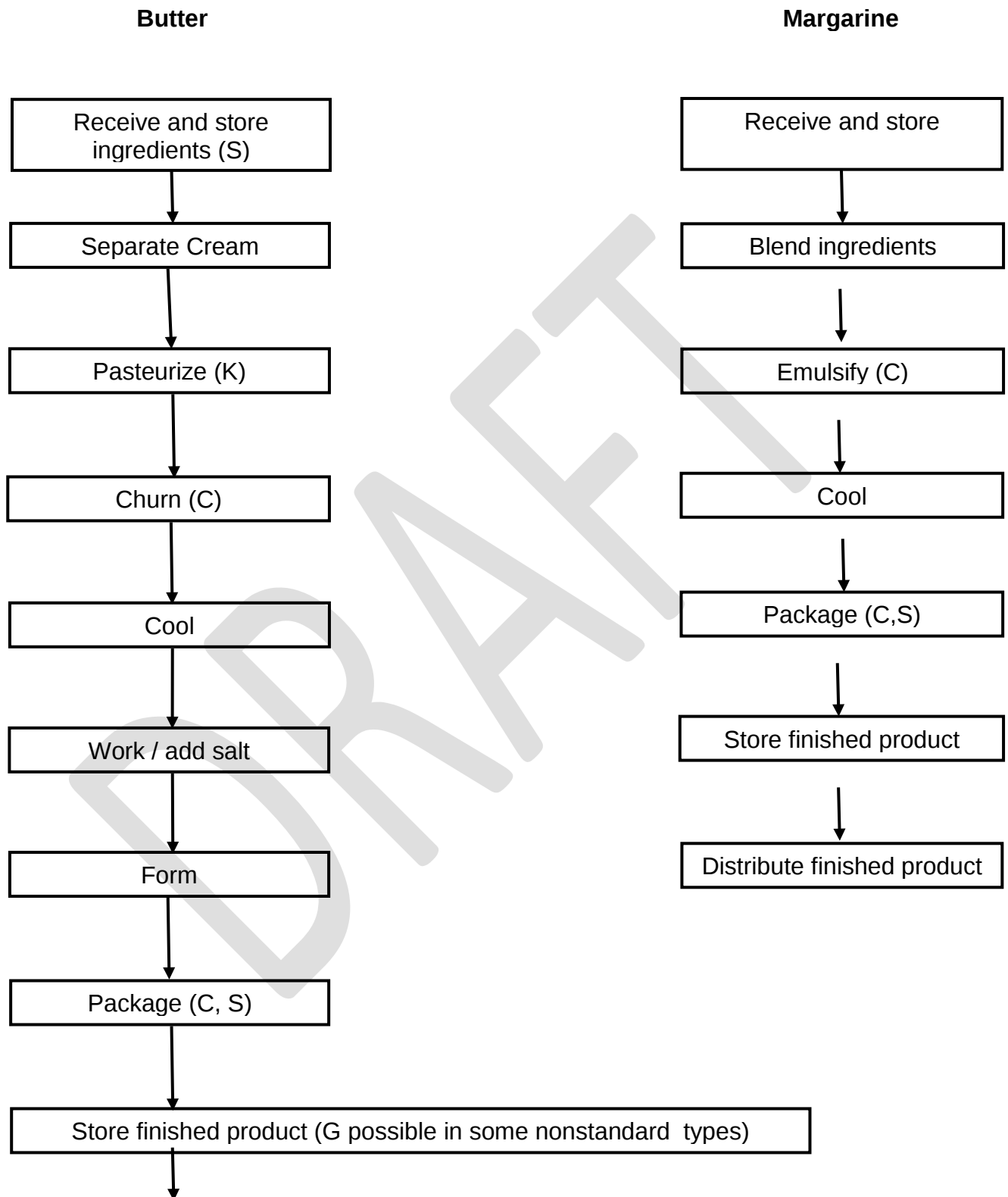
Flow Diagram A.3. BEVERAGES – JUICES AND DRINKS, PASTEURIZED, REFRIGERATED



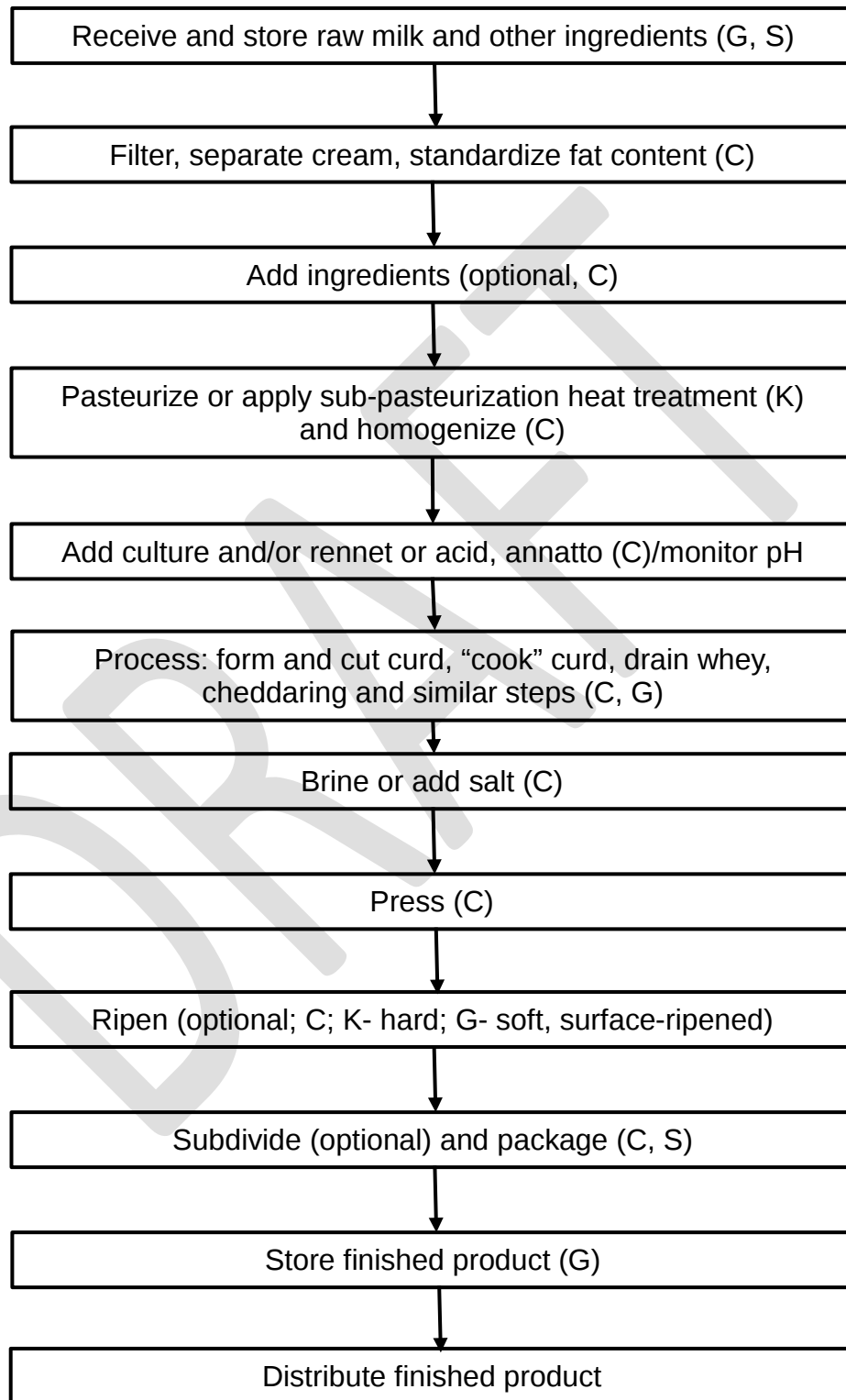
Flow Diagram A.4. BEVERAGES – SHELF STABLE



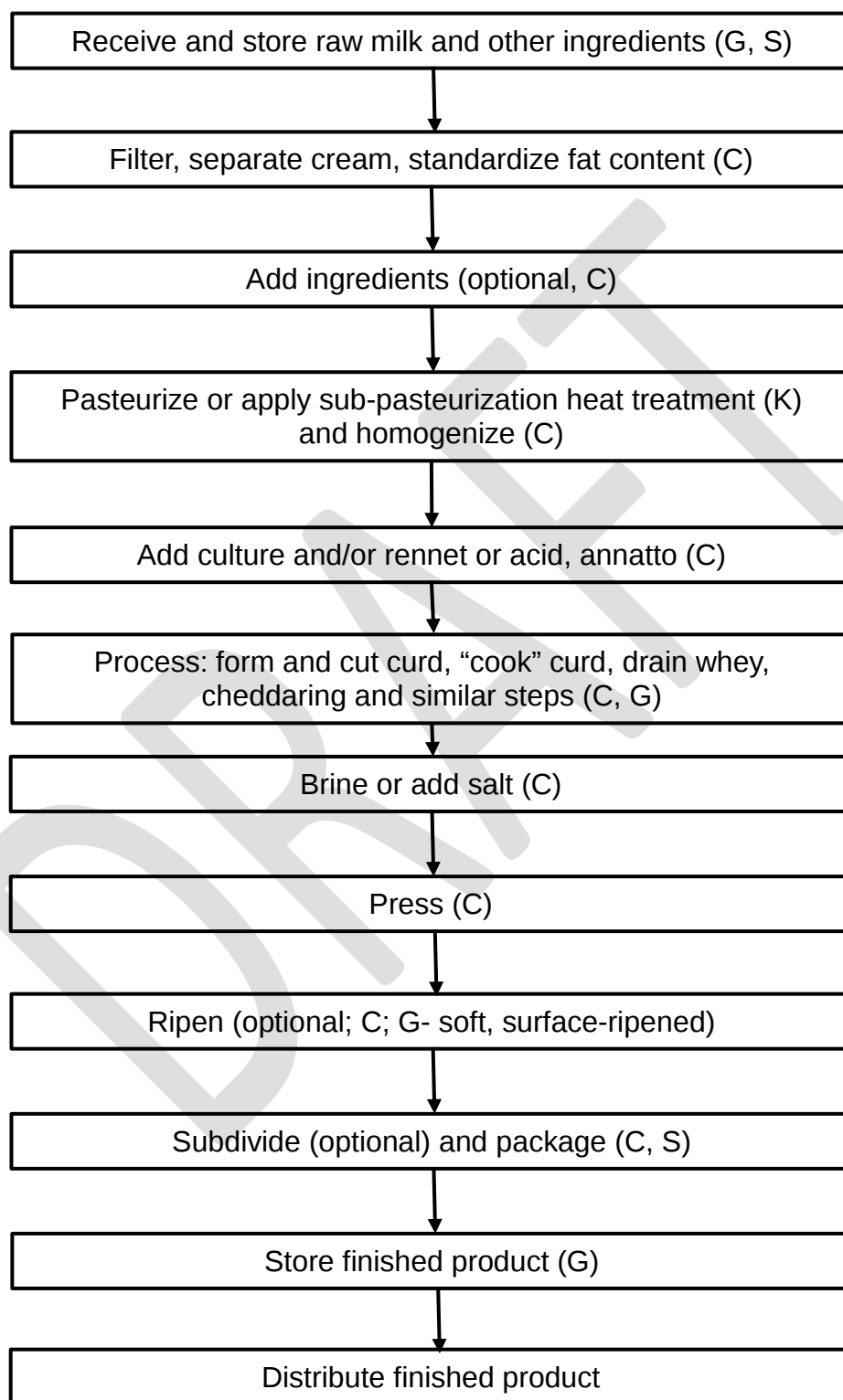
Flow Diagram A.5. DAIRY – BUTTER, MARGARINES



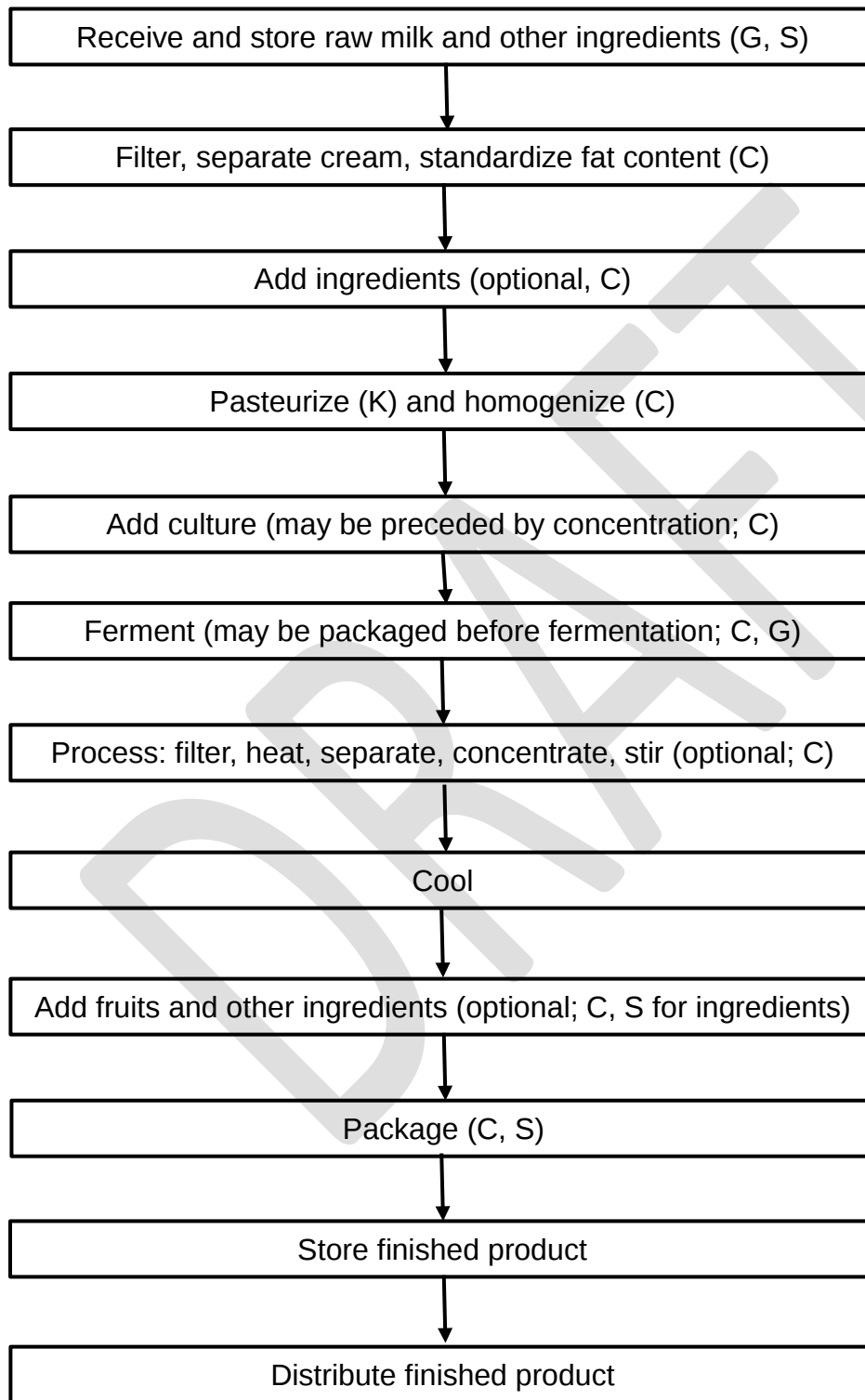
Flow Diagram A.6. DAIRY – CHEESE (HARD)



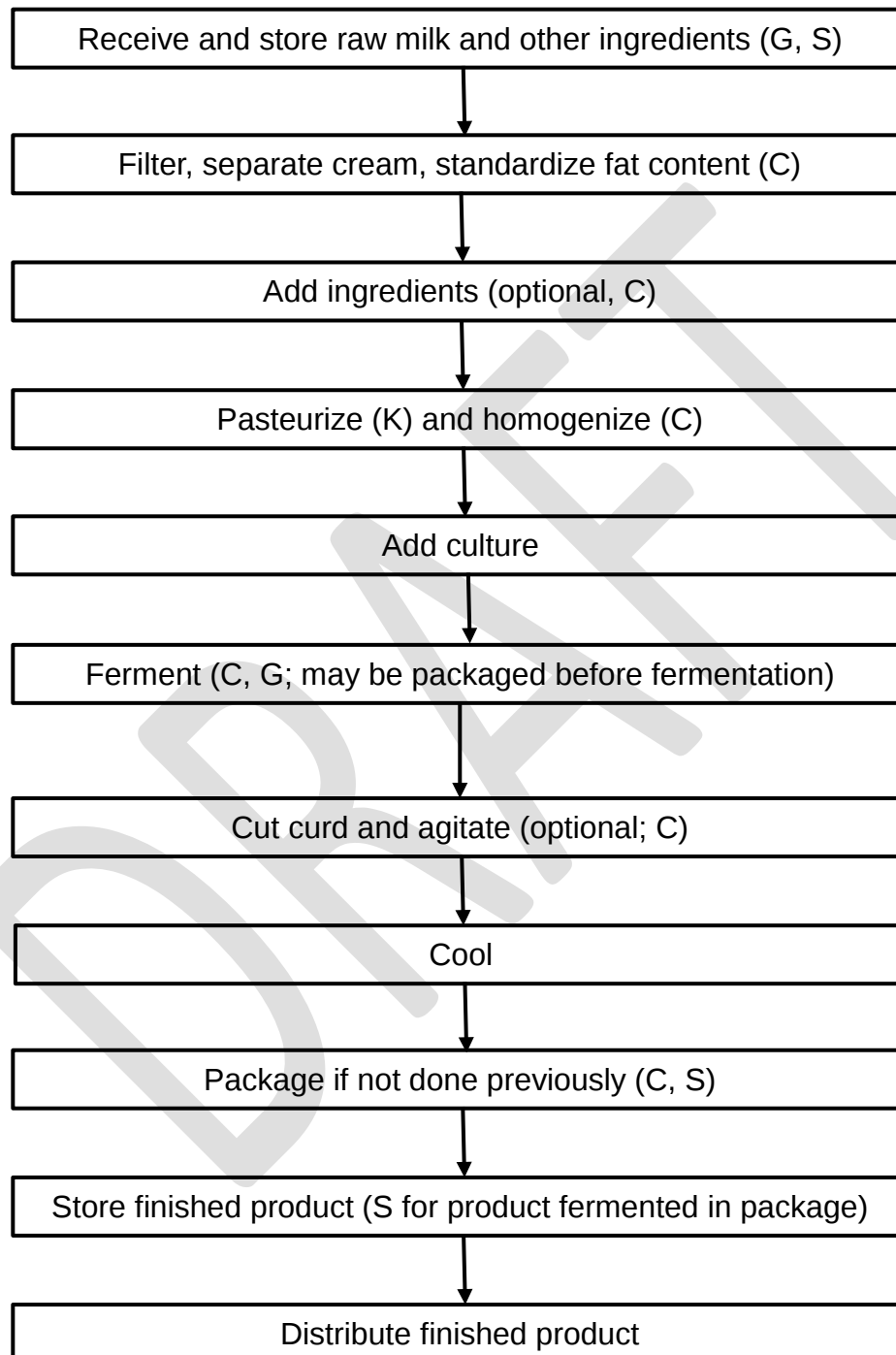
Flow Diagram A.7. DAIRY – CHEESE (SOFT, SEMI-SOFT AND SURFACE-RIPENED)



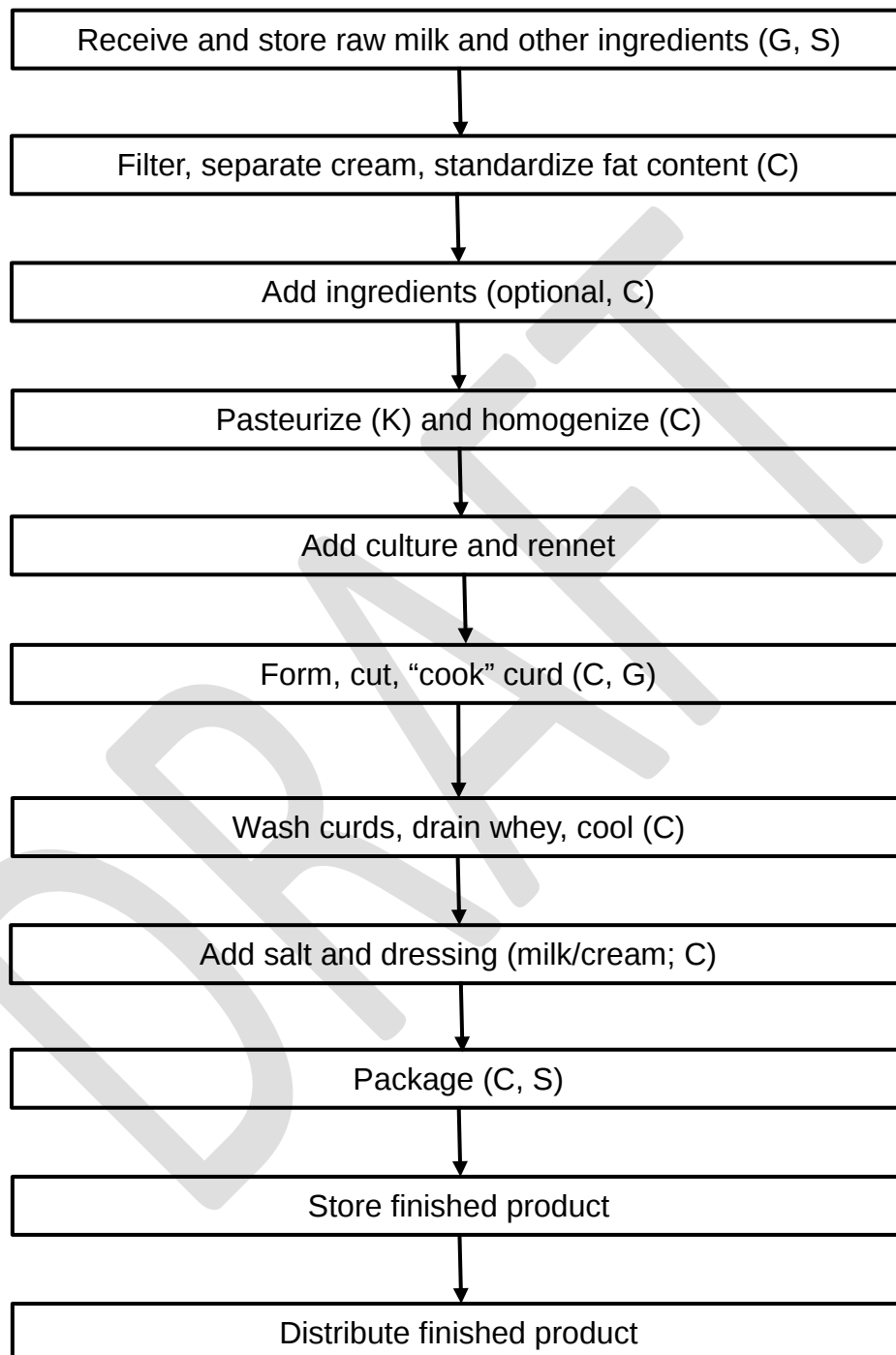
Flow Diagram A.8.a. DAIRY PRODUCTS Cultured pH<4.8 (Example – Yogurt)



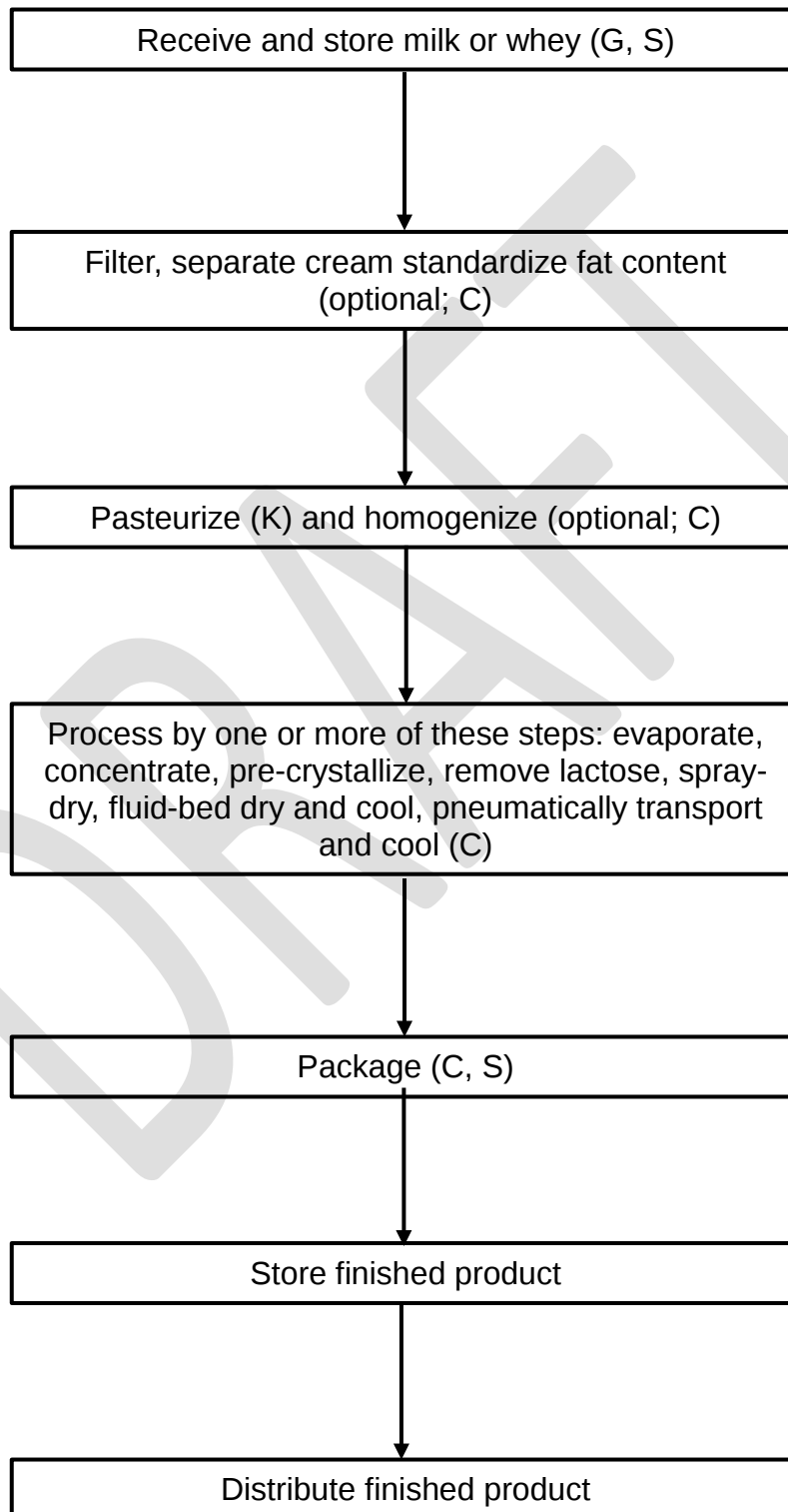
Flow Diagram A.8.b. DAIRY – CULTURED, pH<4.8 (Example – Sour Cream Buttermilk, etc.)



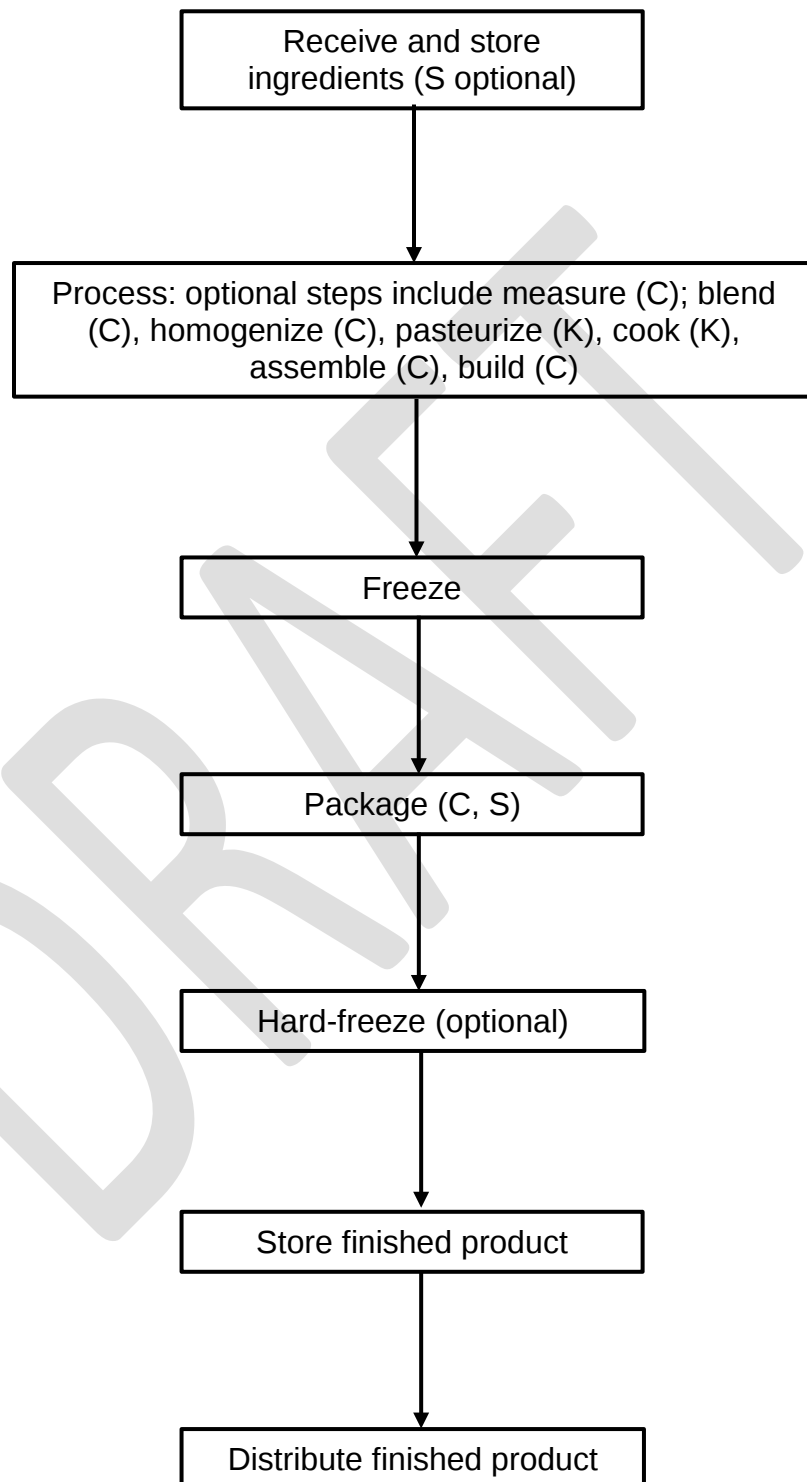
Flow Diagram A.9. DAIRY – CULTURED, pH>4.8 AND <5.4 (Example – Cottage Cheese)



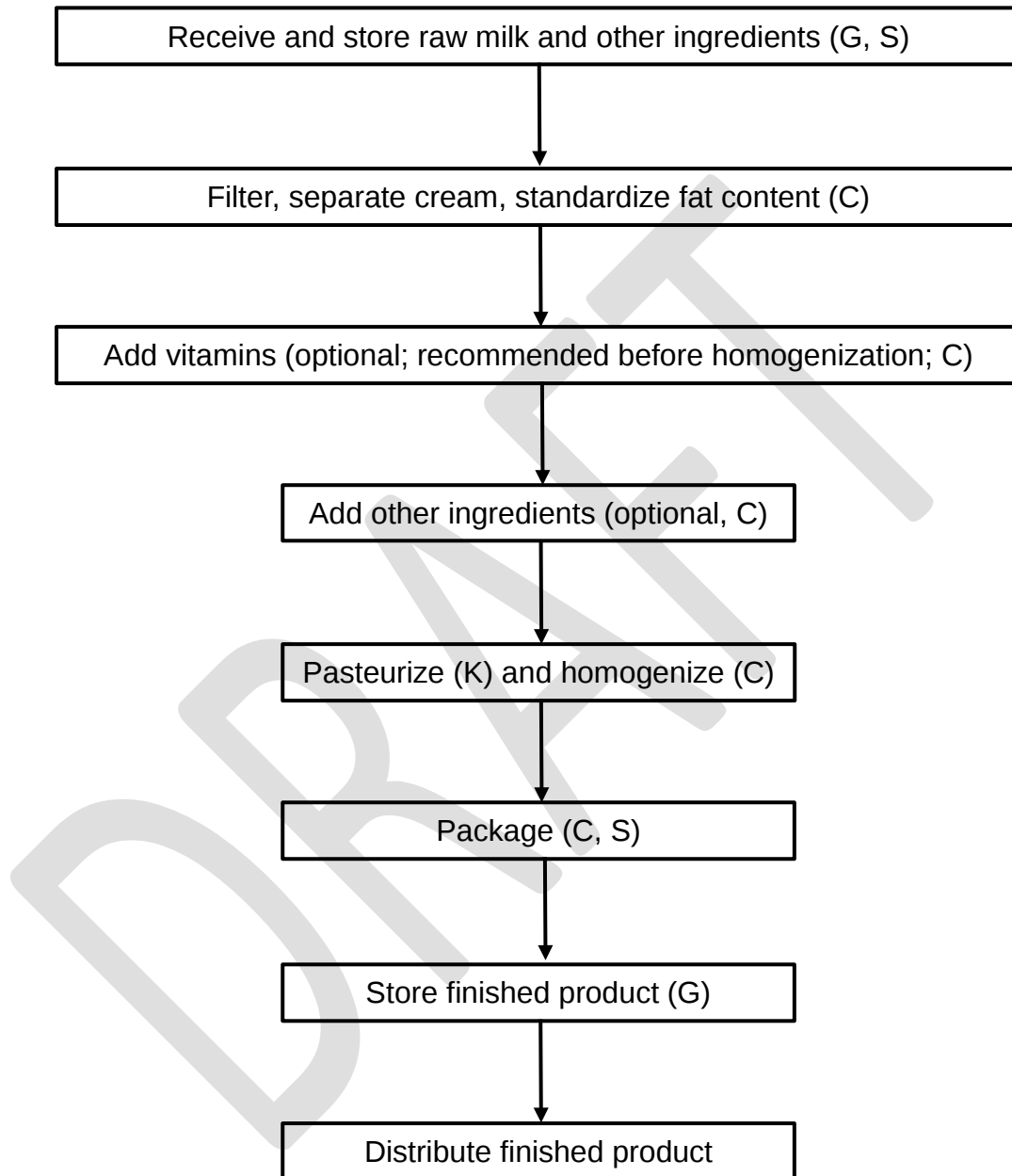
Flow Diagram A.10. DAIRY – DRIED PRODUCTS
(does not include dairy ingredients used to make infant formula)



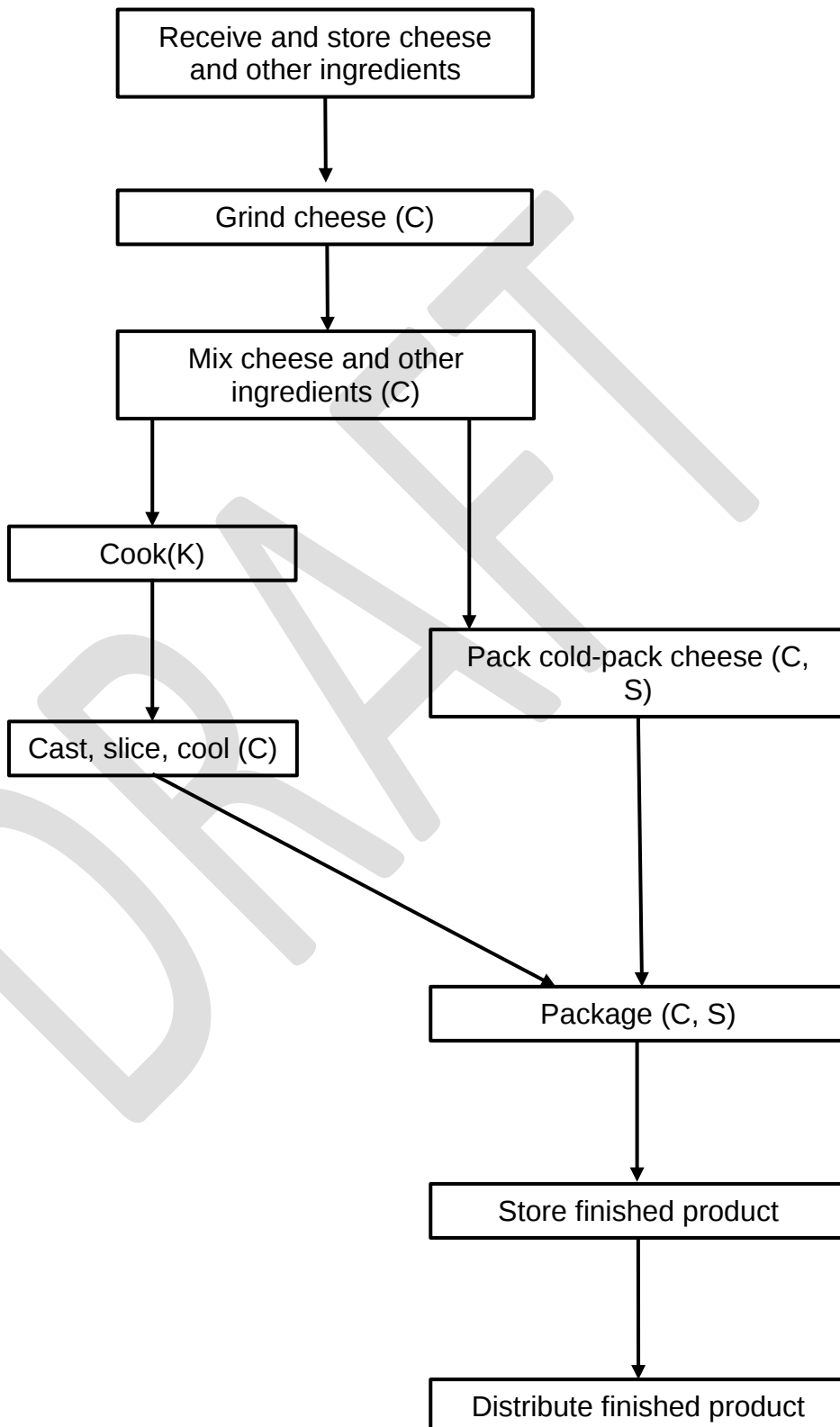
Flow Diagram A.11. DAIRY – FROZEN DESSERTS



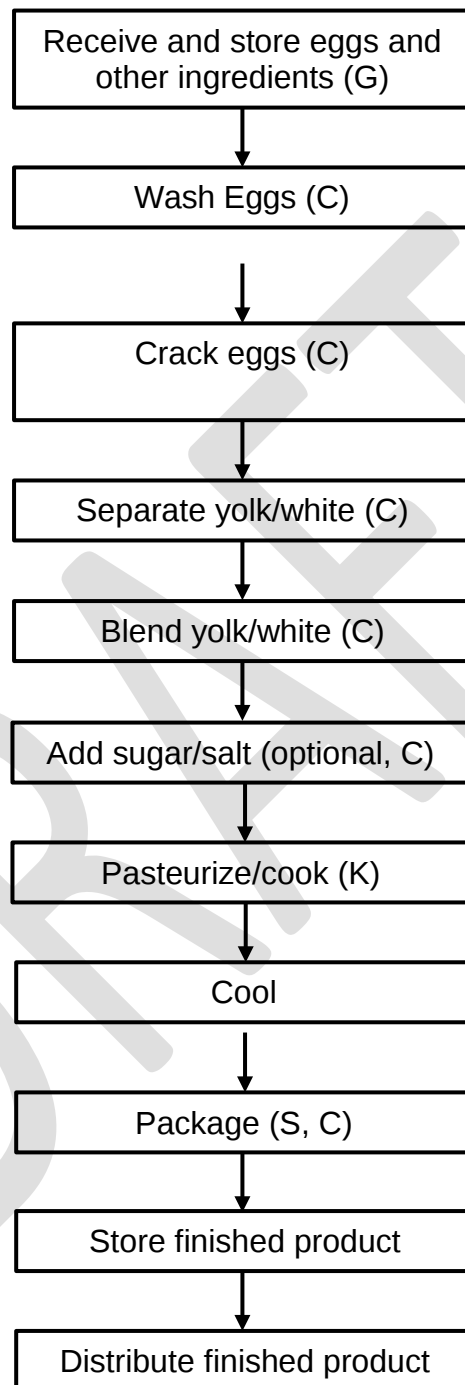
Flow Diagram A.12. DAIRY – MILK AND MILK PRODUCTS (Fluid)



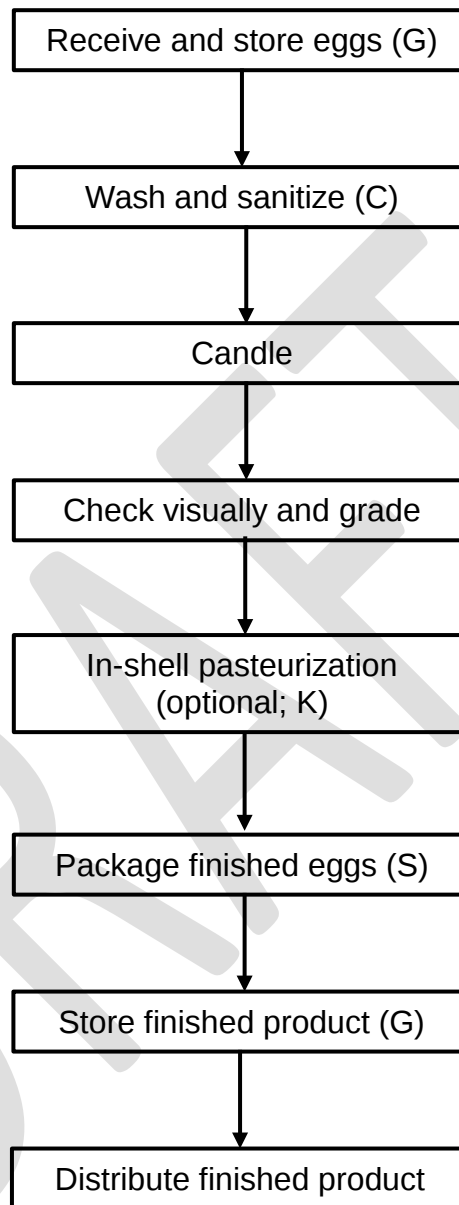
Flow Diagram A.13. DAIRY – PROCESS CHEESE



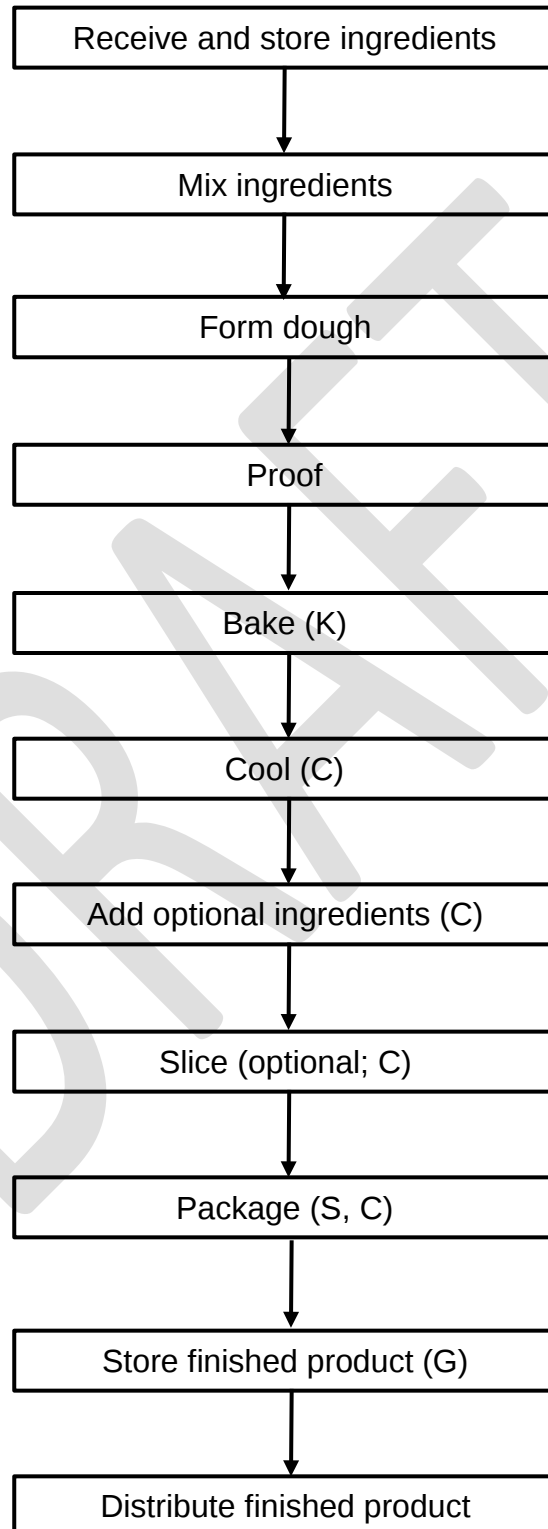
Flow Diagram A.14. EGG PRODUCTS – PASTEURIZED, PROCESSED



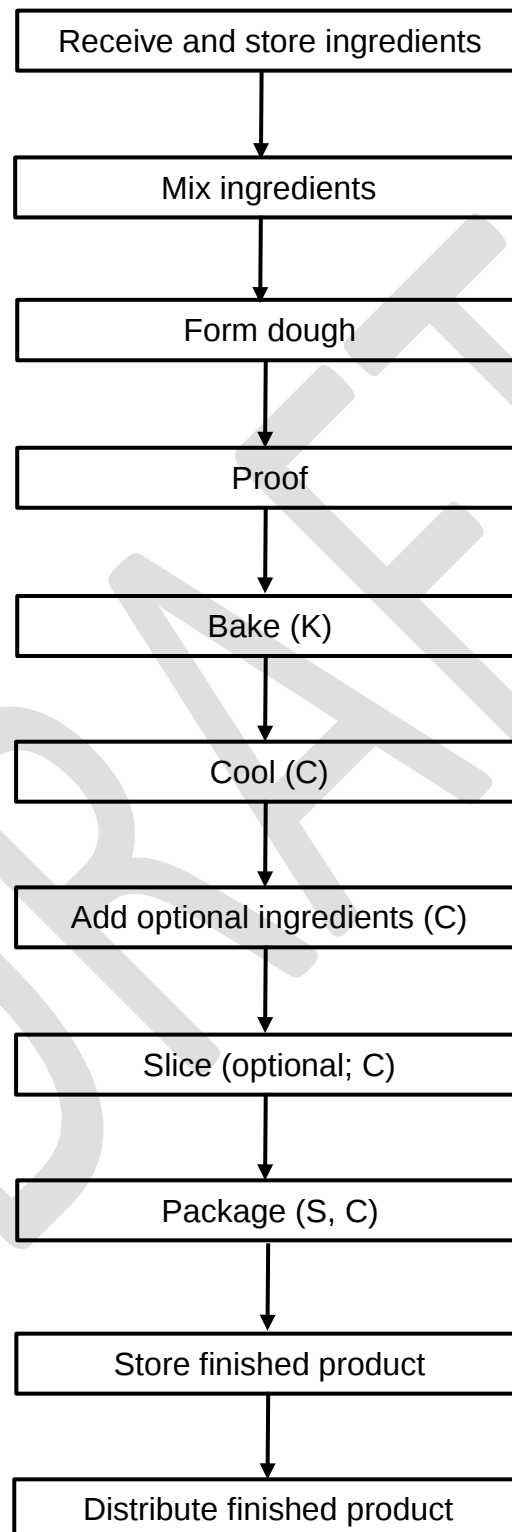
Flow Diagram A.15. EGG PRODUCTS – SHELL EGGS RAW



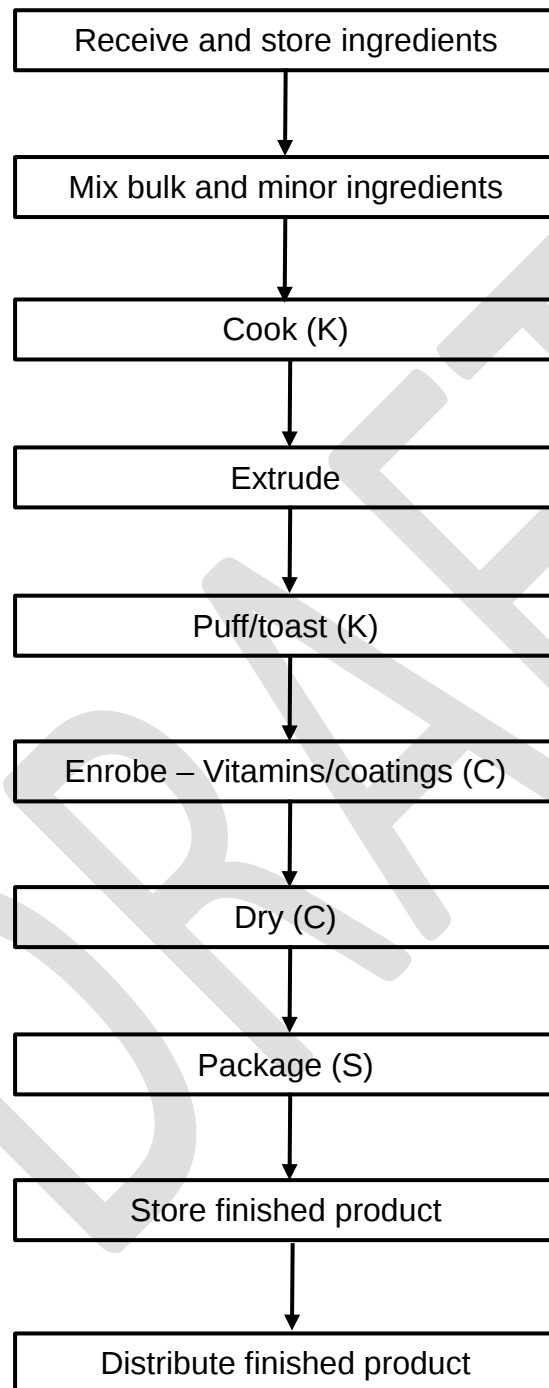
Flow Diagram A.16. GRAIN BASED PRODUCTS – BAKED ITEMS, RTE,
REFRIGERATED OR TCS



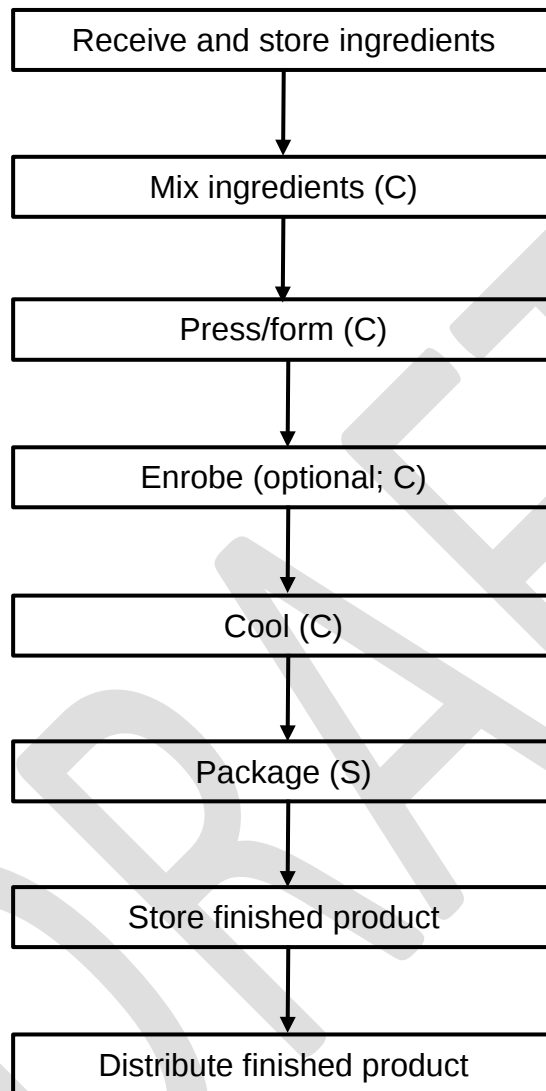
Flow Diagram A.17. GRAIN BASED PRODUCTS – BAKED ITEMS, RTE, SHELF STABLE, NON-TCS



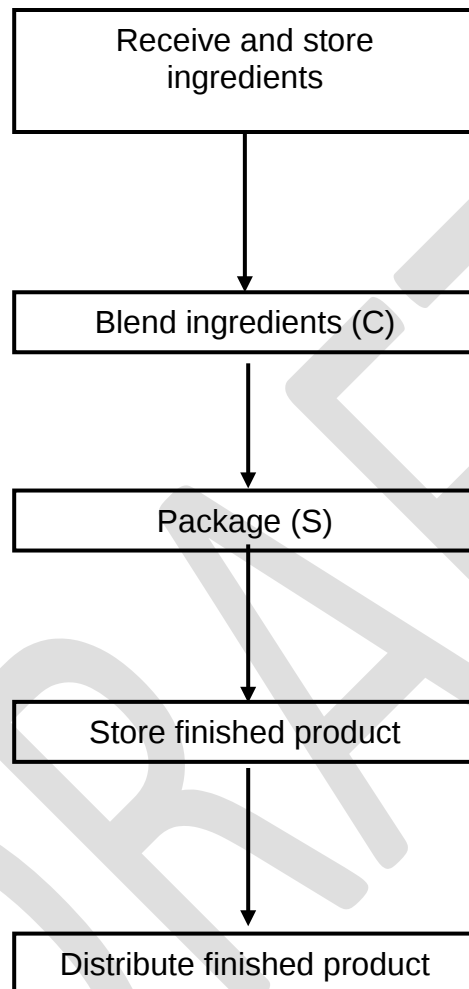
Flow Diagram A.18. GRAIN BASED PRODUCTS – RTE, CEREALS



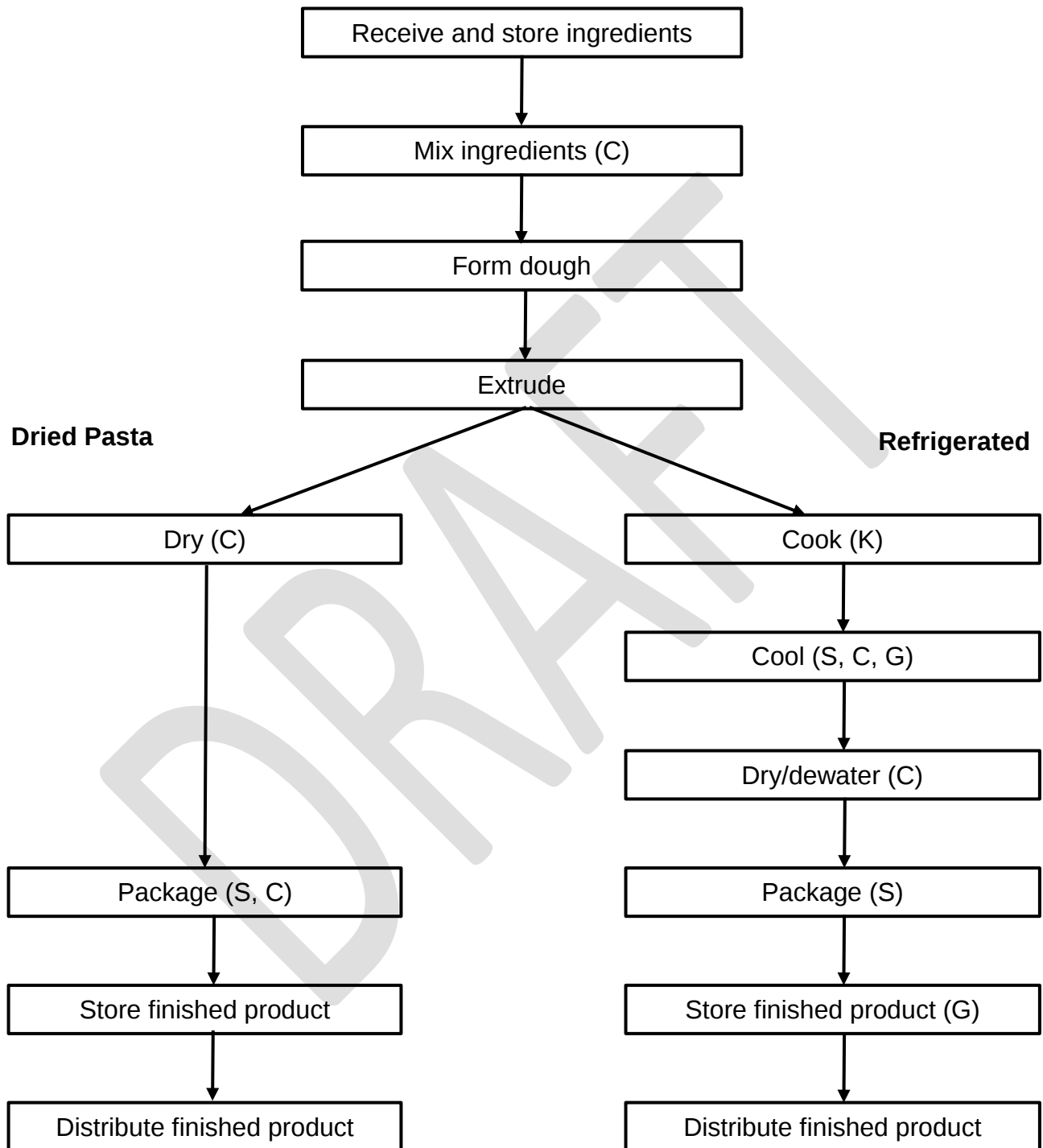
Flow Diagram A.19. GRAIN BASED PRODUCTS – RTE, COLD PRESSED BARS



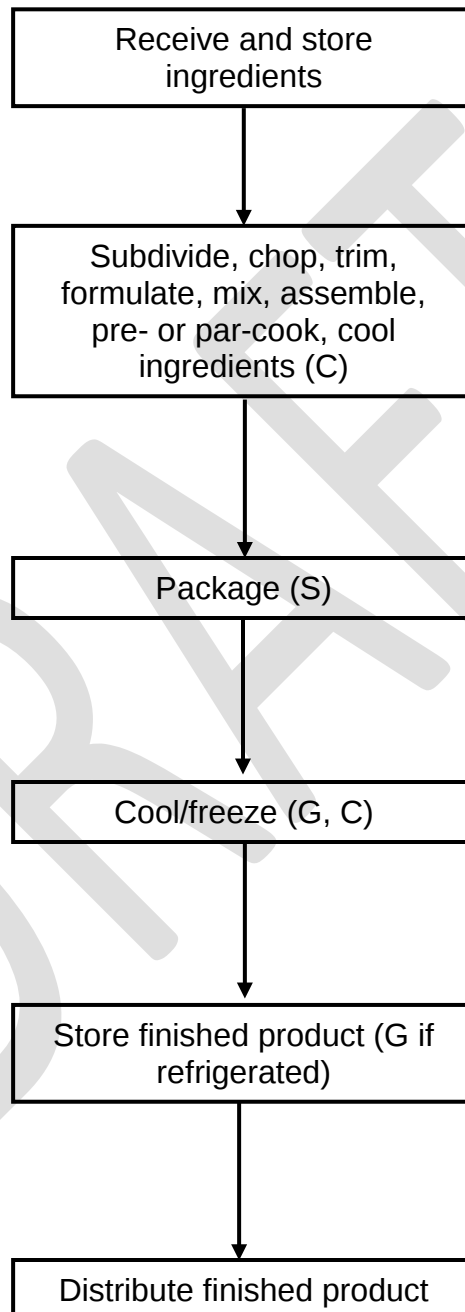
Flow Diagram A.20. GRAIN BASED PRODUCTS – NON-RTE, DRY FLOUR BASED MIXES



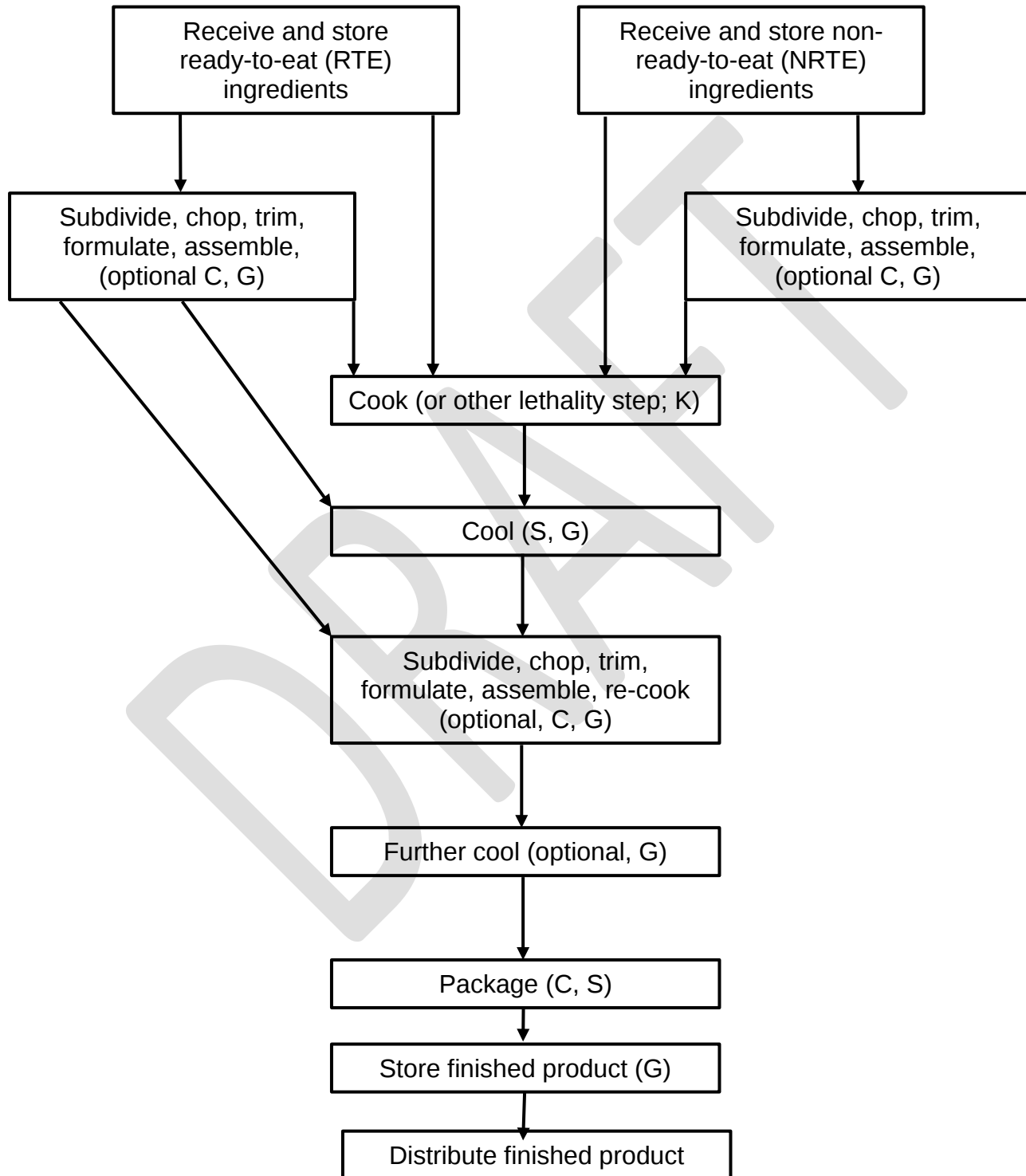
Flow Diagram A.21. GRAIN BASED PRODUCTS – NON RTE, PASTA, DRIED OR REFRIGERATED



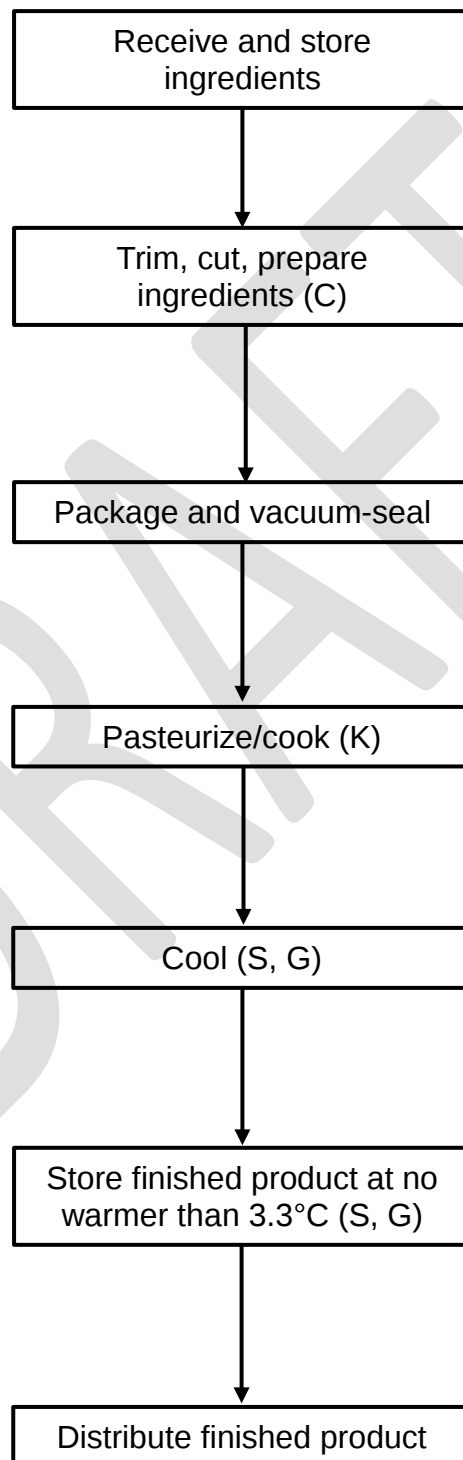
Flow Diagram A.22. MEALS AND ENTRÉES – NON-RTE, READY TO COOK (RTC)
MEALS, INCLUDES RAW INGREDIENTS



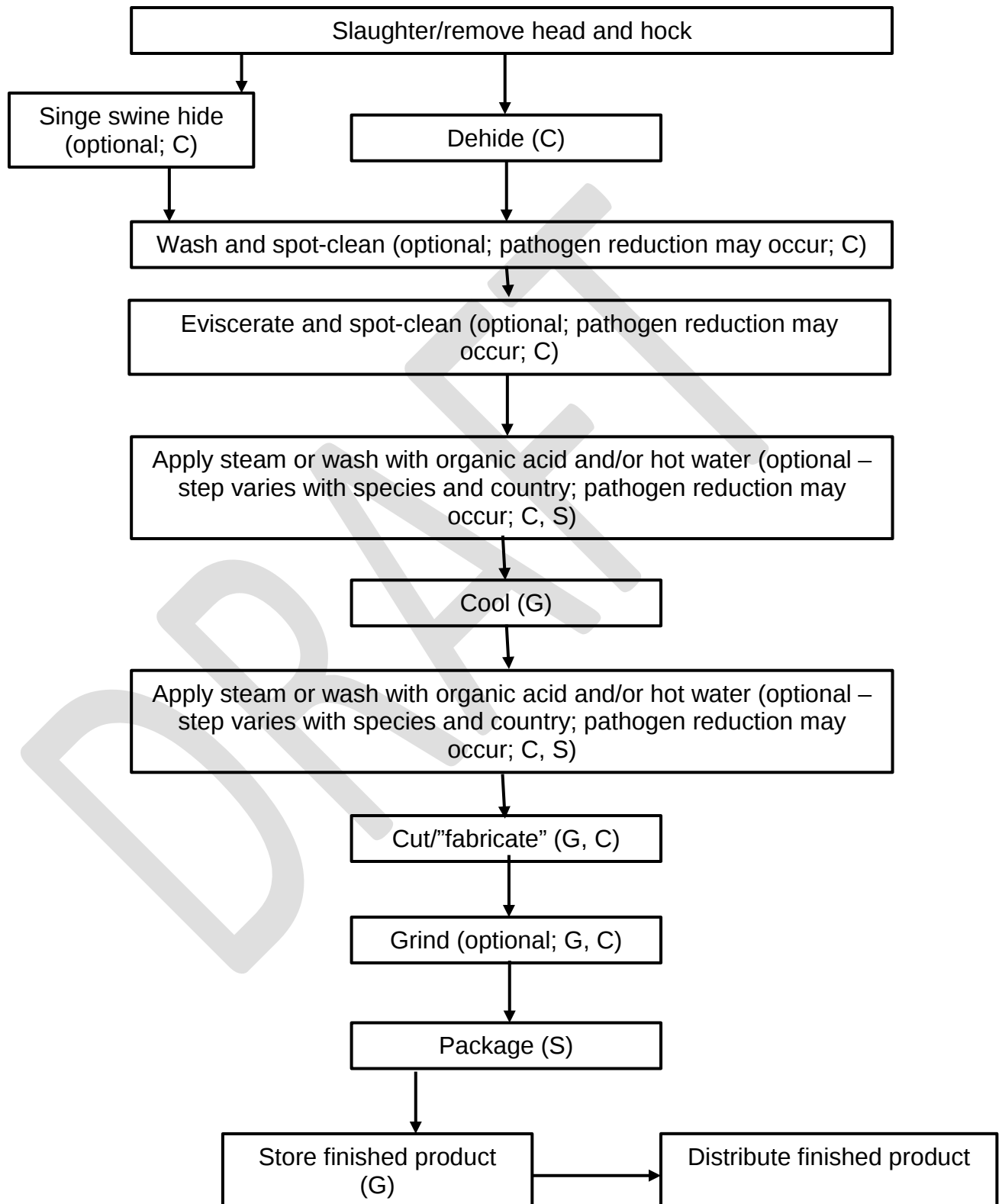
Flow Diagram A.23 MEALS AND ENTRÉES – RTE, DELI SALADS, SANDWICHES
HEAT-EAT MEALS, SUSHI



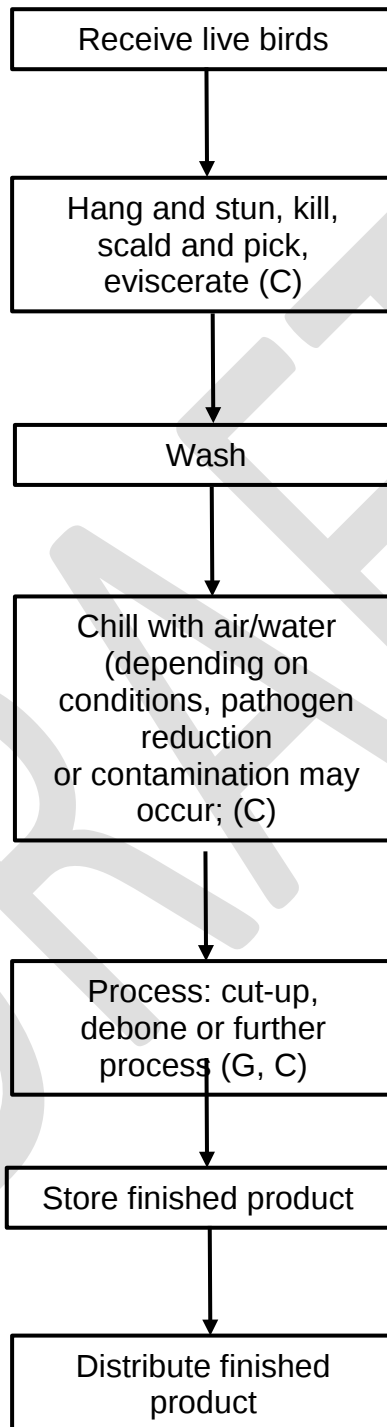
Flow Diagram A.24. MEALS AND ENTRÉES – SOUS VIDE, COOK AND CHILL



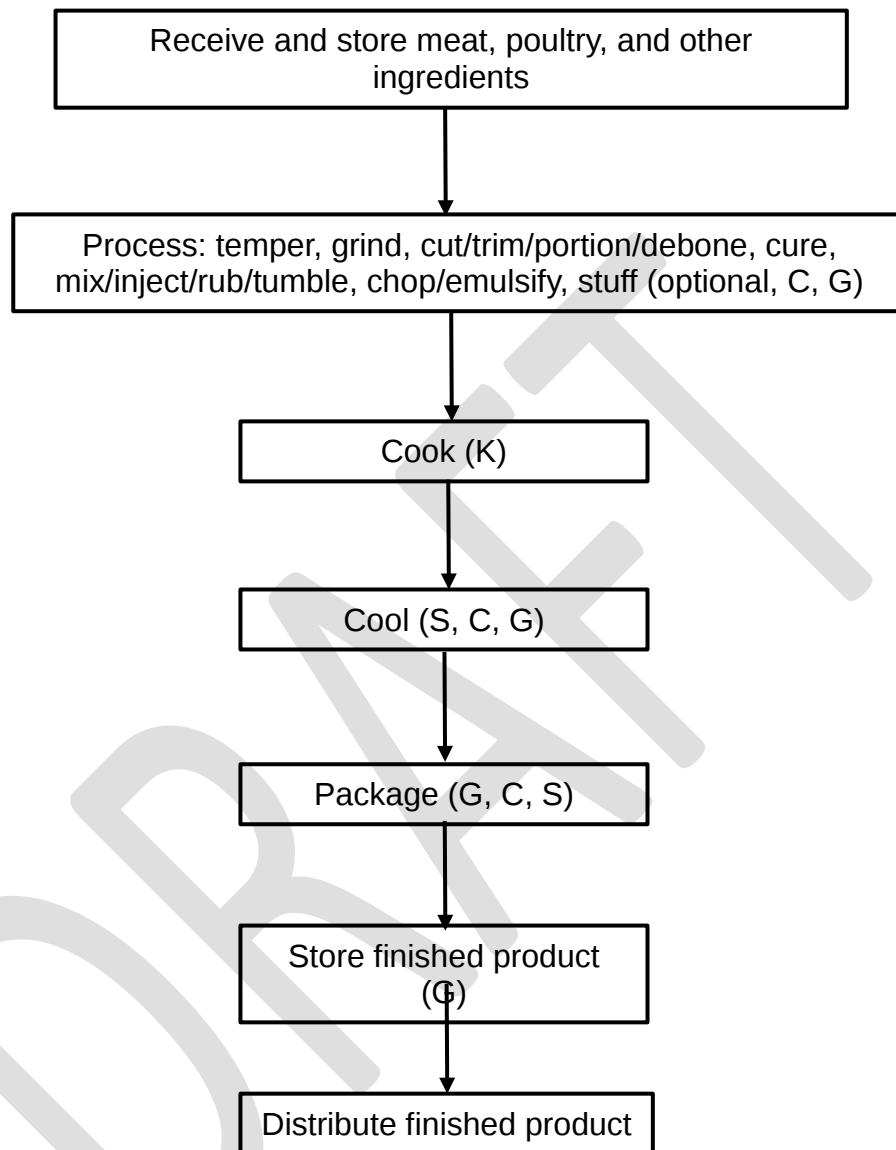
Flow Diagram A.25. MEAT, PORK AND POULTRY PRODUCTS – NON-RTE, BEEF AND PORK RAW, INTACT AND NON-INTACT



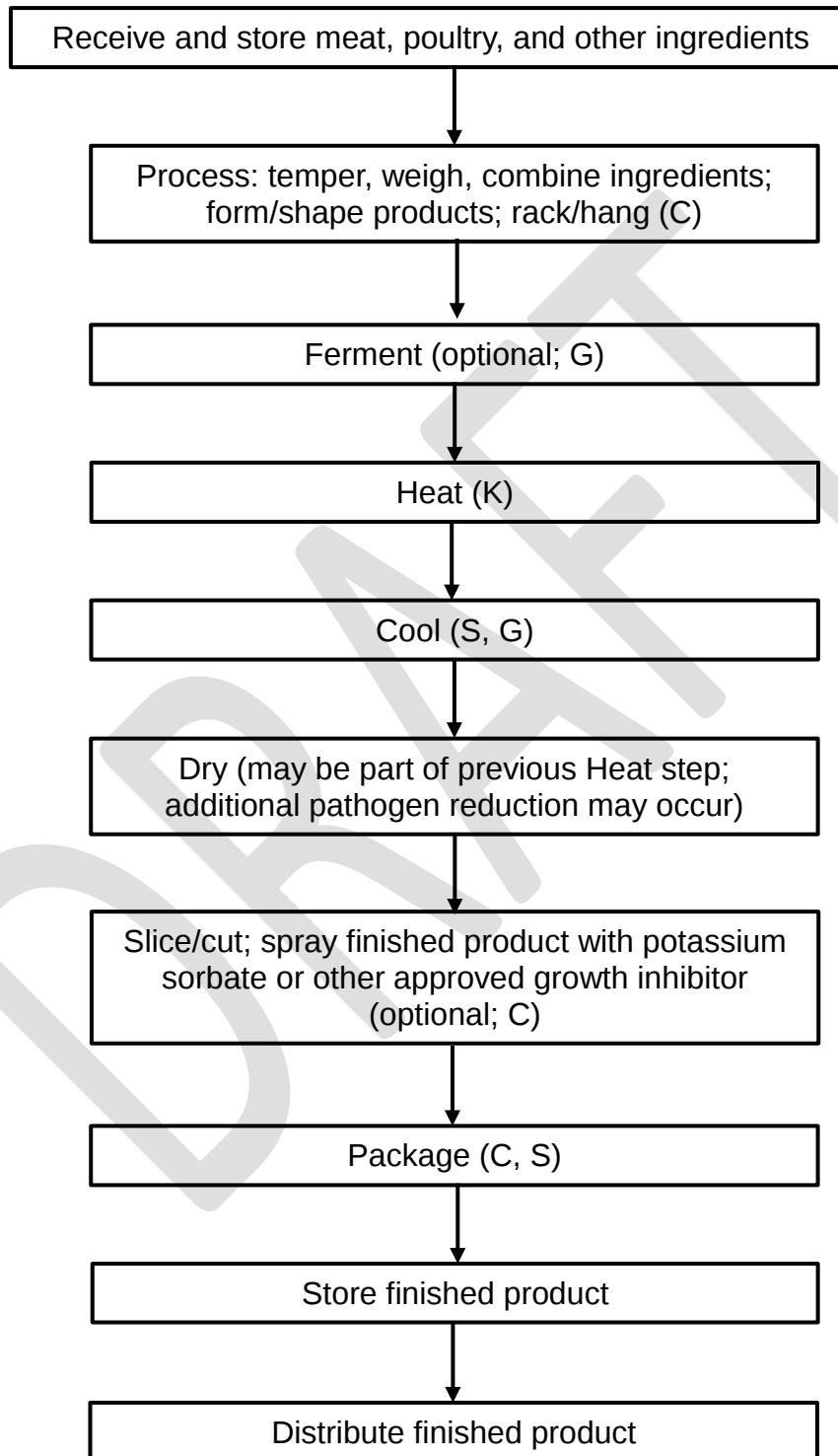
Flow Diagram A.26. MEAT, PORK AND POULTRY PRODUCTS – NON-RTE,
POULTRY, RAW



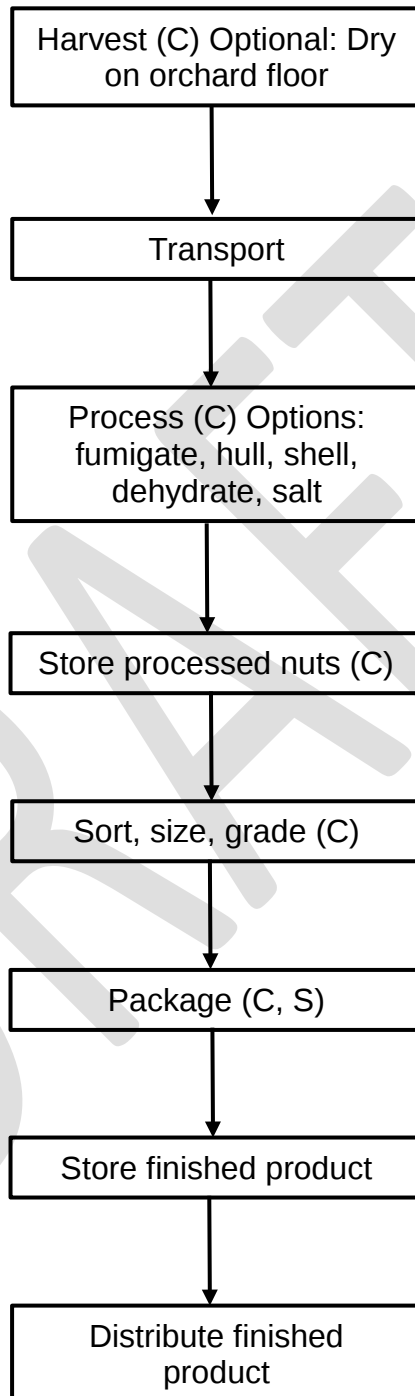
Flow Diagram A.27. MEAT, PORK AND POULTRY PRODUCTS – RTE, COOKED PERISHABLE



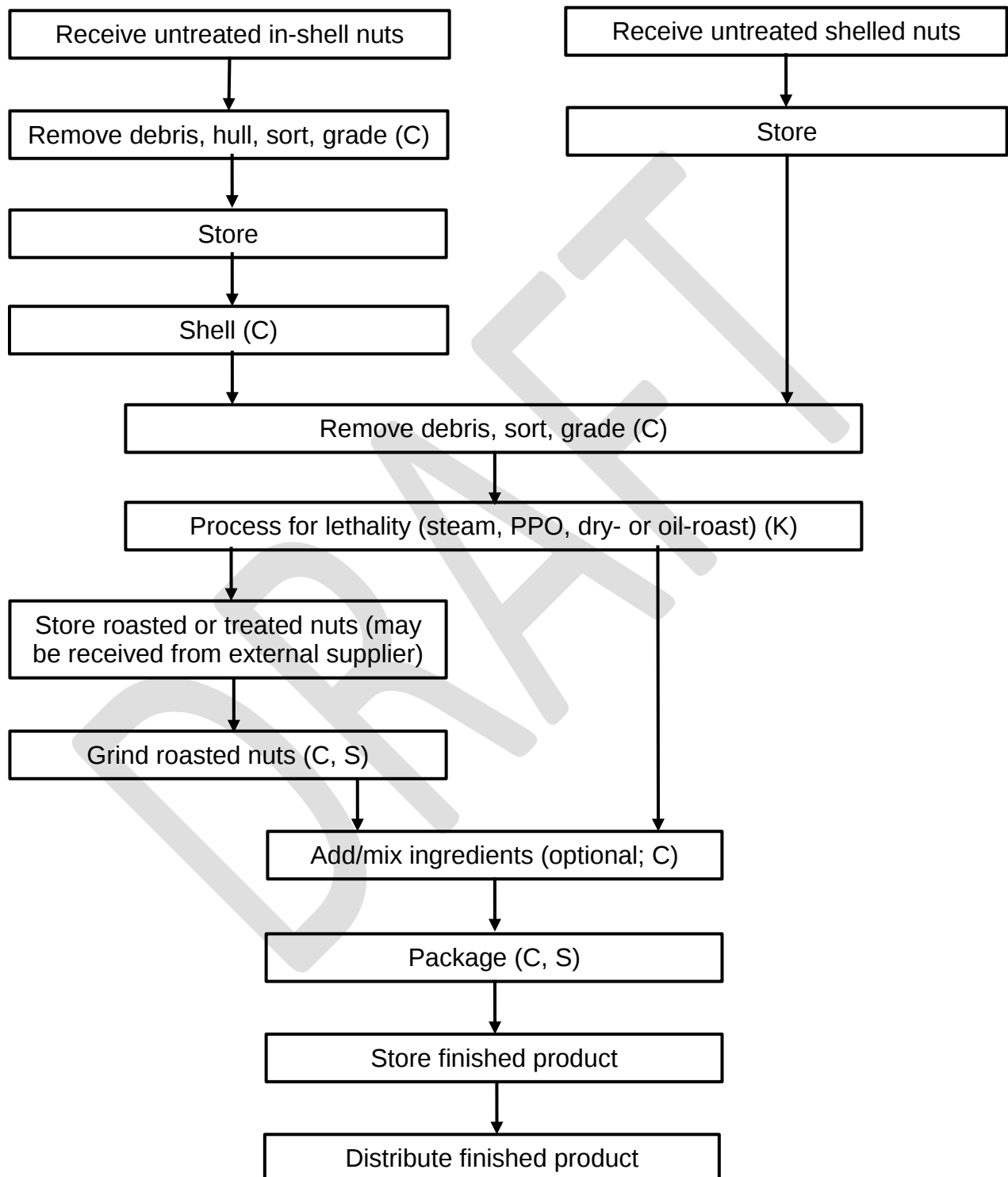
Flow Diagram A.28. MEAT, PORK AND POULTRY PRODUCTS – RTE
FERMENTED AND DRIED, DRIED



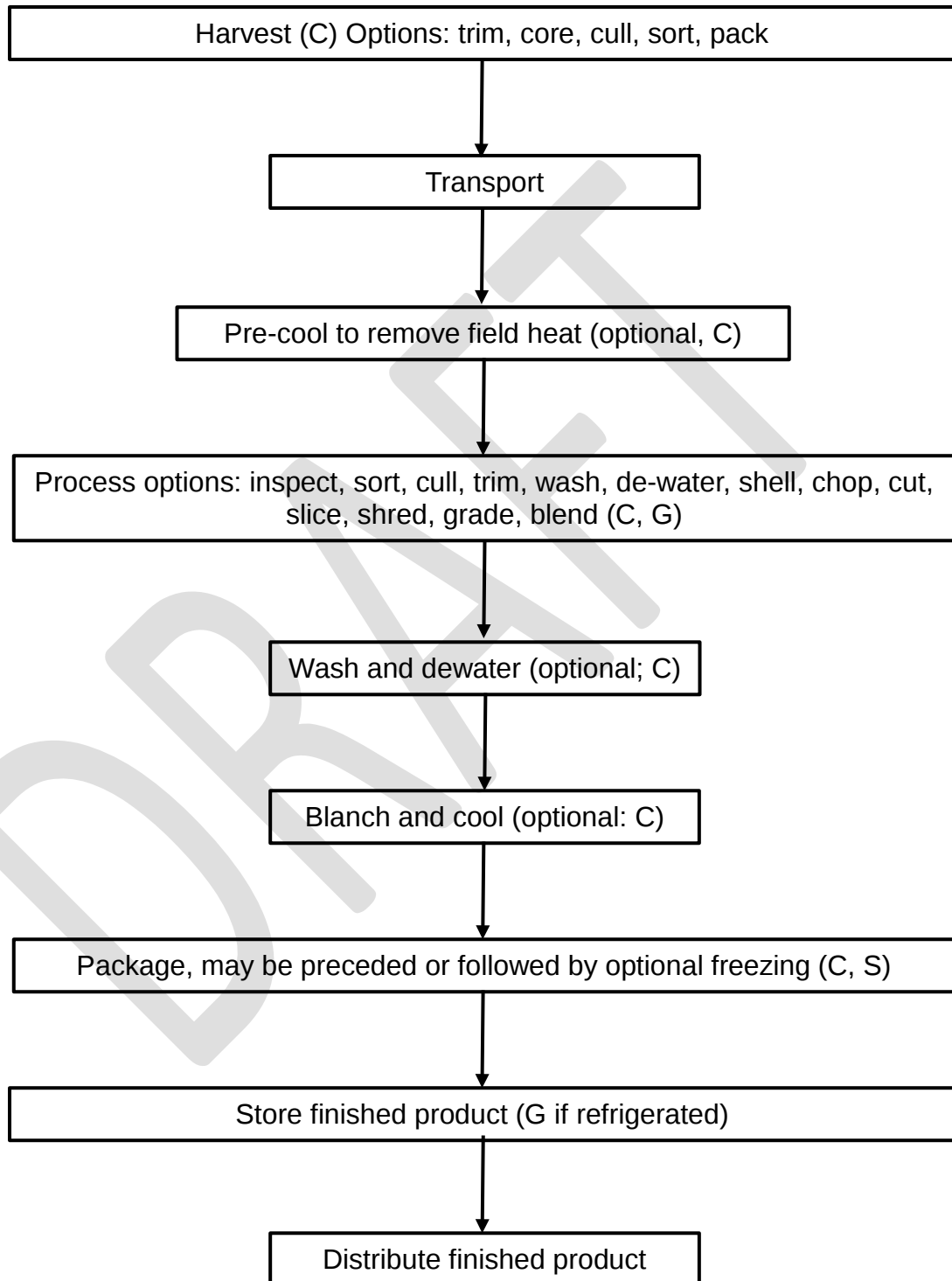
Flow Diagram A.29. NUTS AND NUT BUTTERS – NUTS, RTE,
NOT PROCESSED FOR LETHALITY



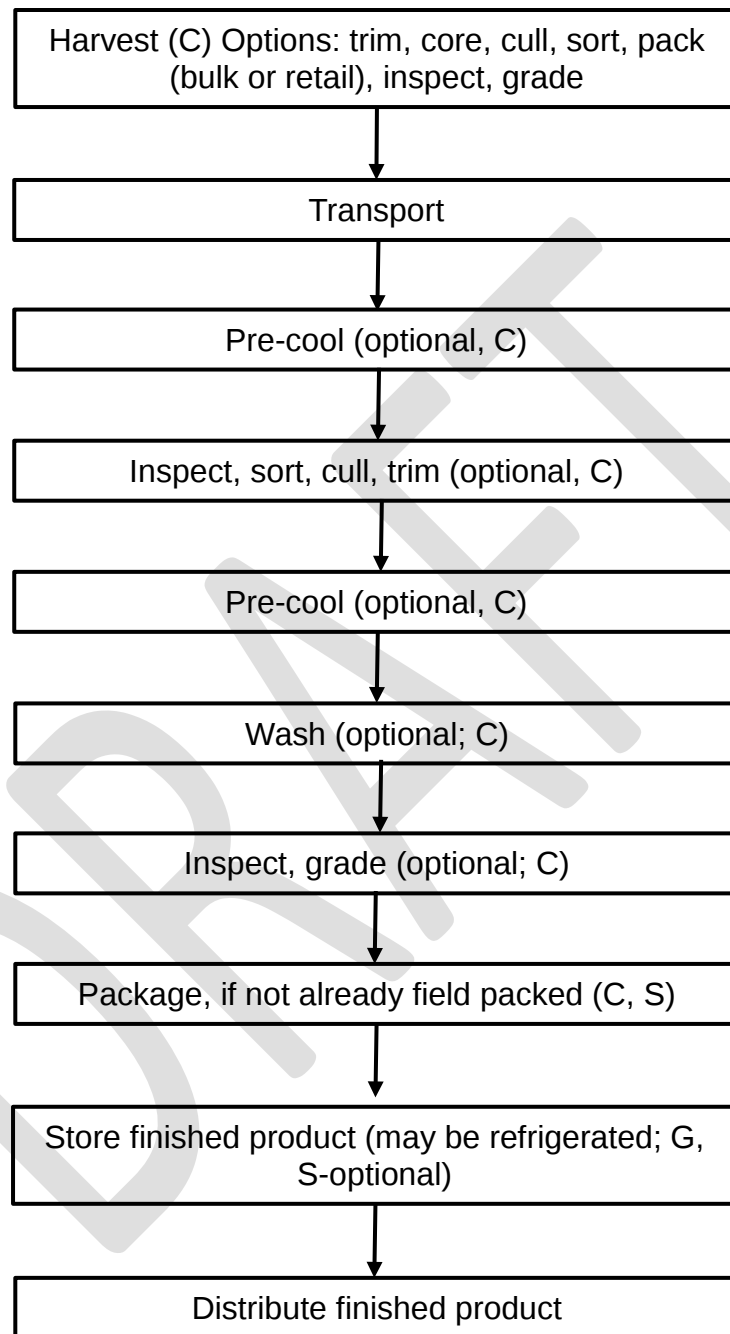
Flow Diagram A.30. NUTS AND NUT BUTTERS – RTE, PROCESSED FOR LETHALITY



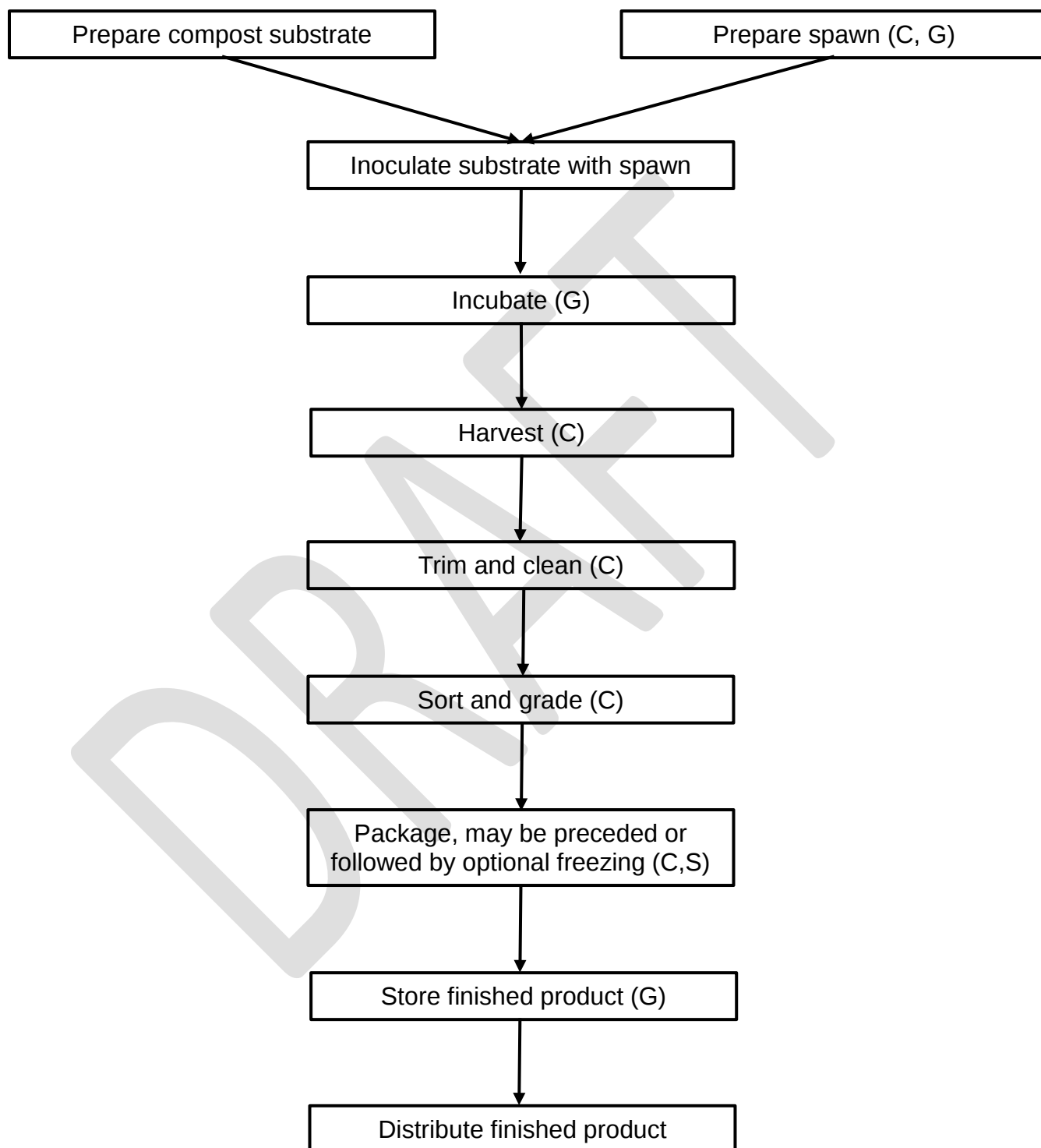
Flow Diagram A.31. PRODUCE – FRUITS AND VEGETABLES, CUT FROZEN, OR REFRIGERATED, MINIMALLY PROCESSED



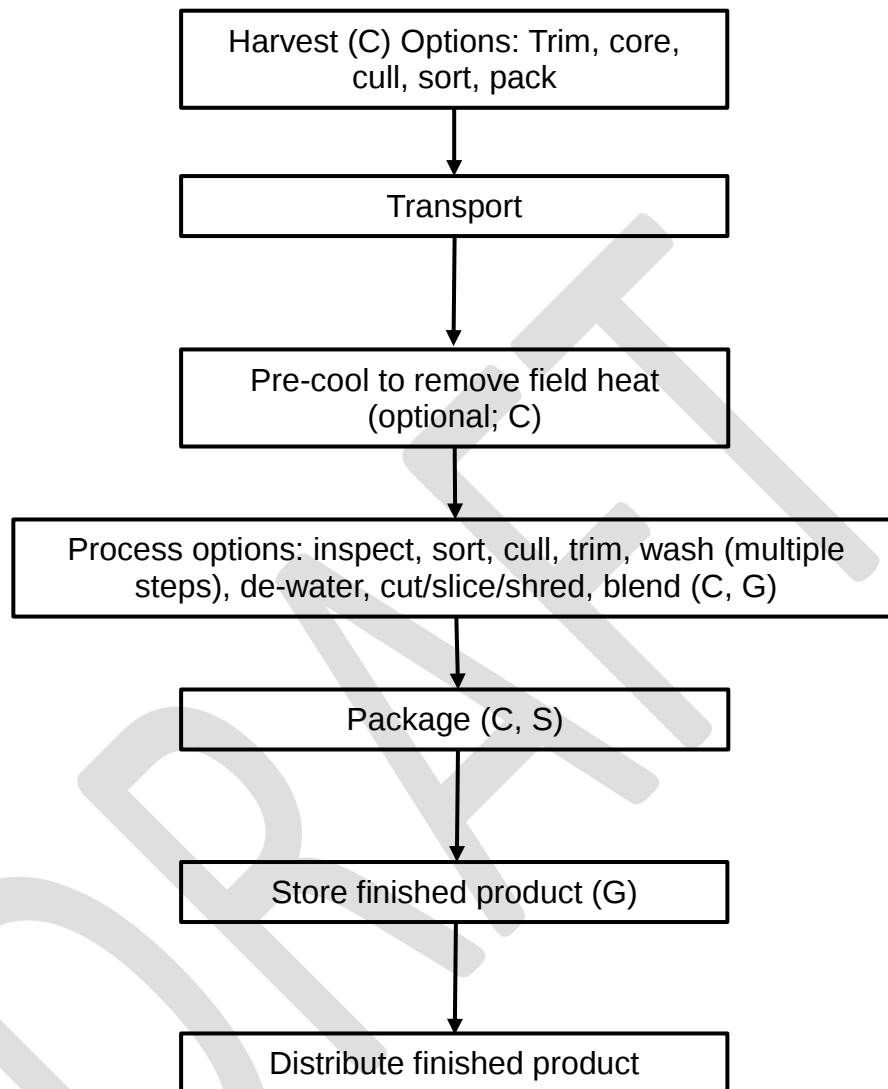
Flow Diagram A.32. PRODUCE – FRUITS AND VEGETABLES, WHOLE



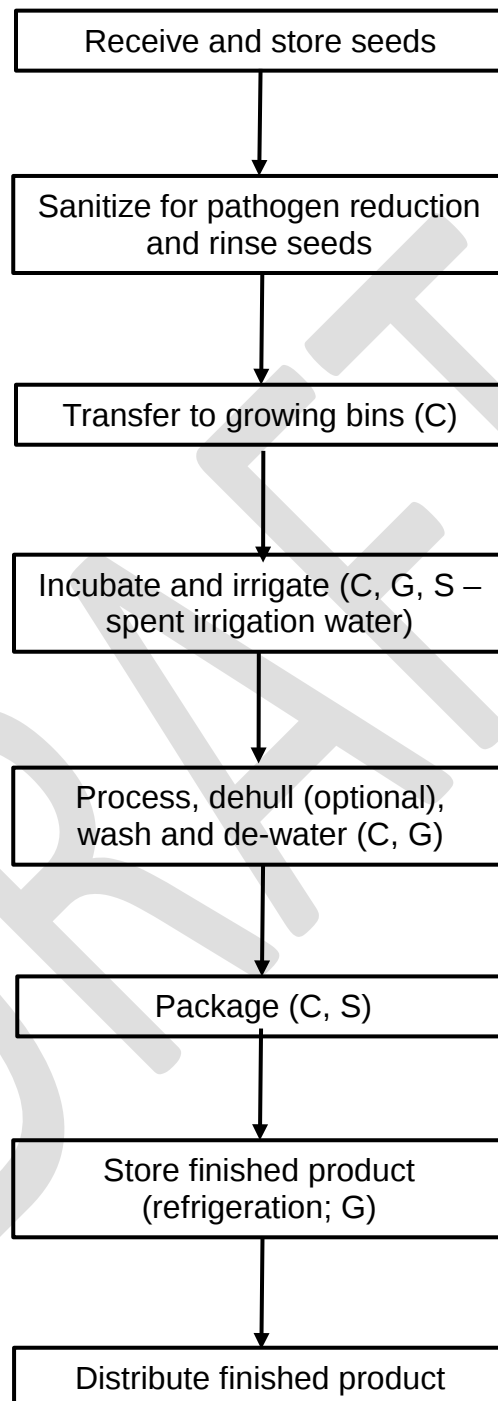
Flow Diagram A.33. PRODUCE – MUSHROOMS – FRESH OR FROZEN,
WHOLE, SLICED, NOT CANNED OR MARINATED



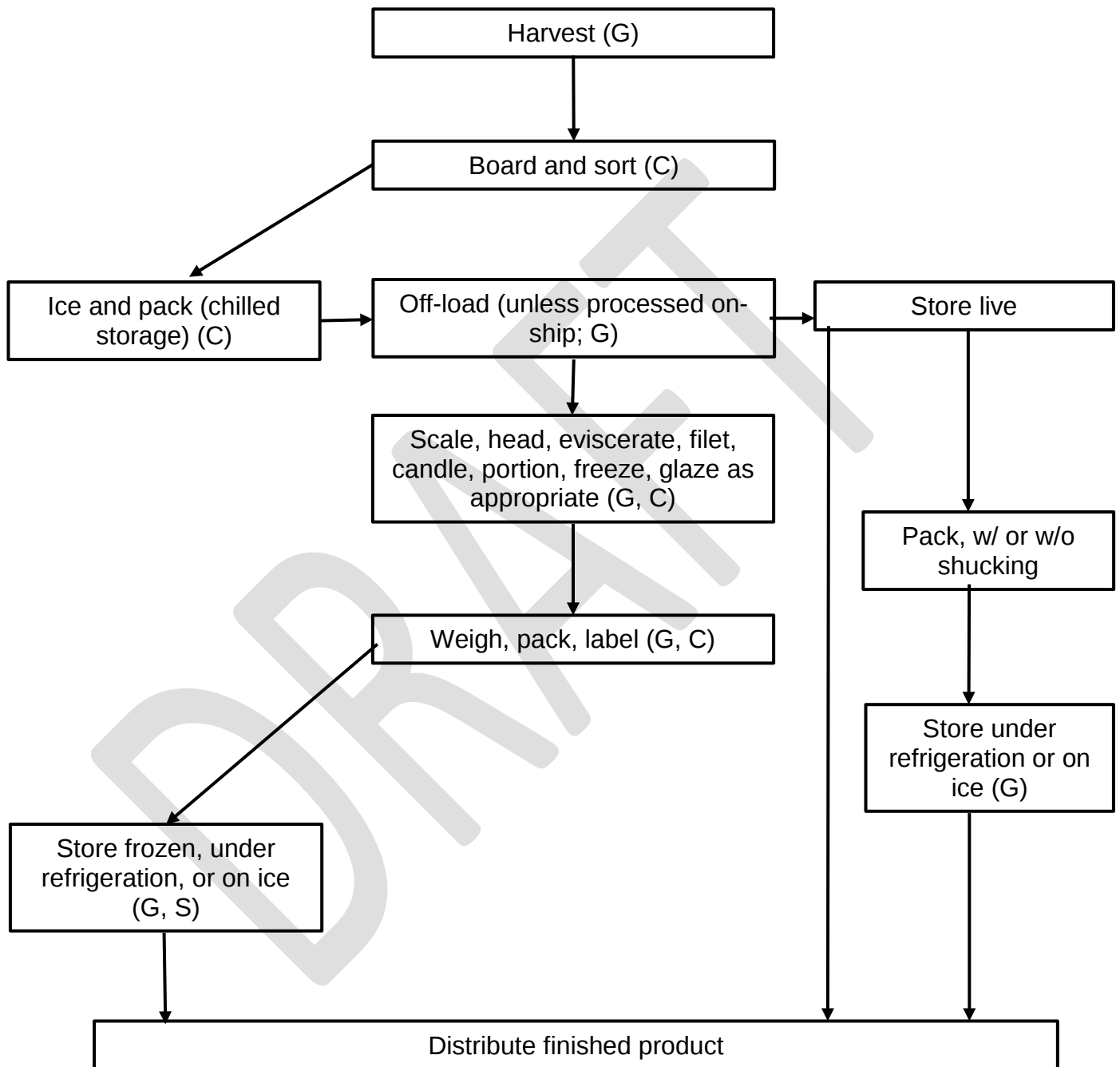
Flow Diagram A.34. PRODUCE – PACKAGED SALADS AND LEAFY GREENS



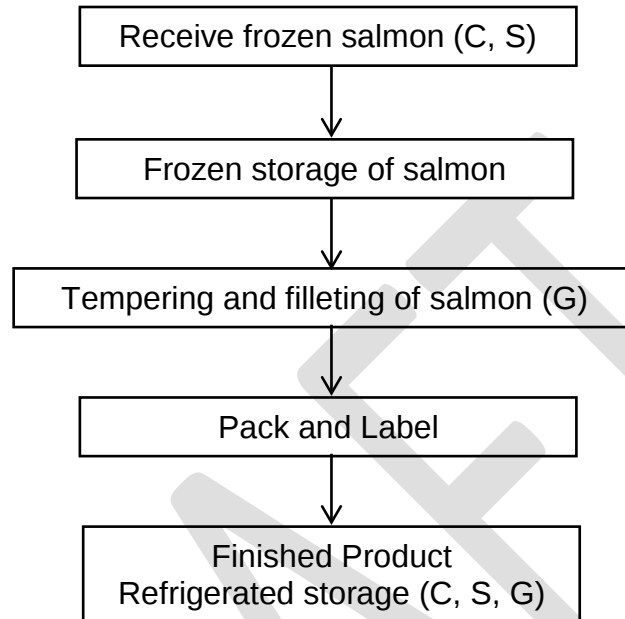
Flow Diagram A.35. PRODUCE – VEGETABLE SPROUTS



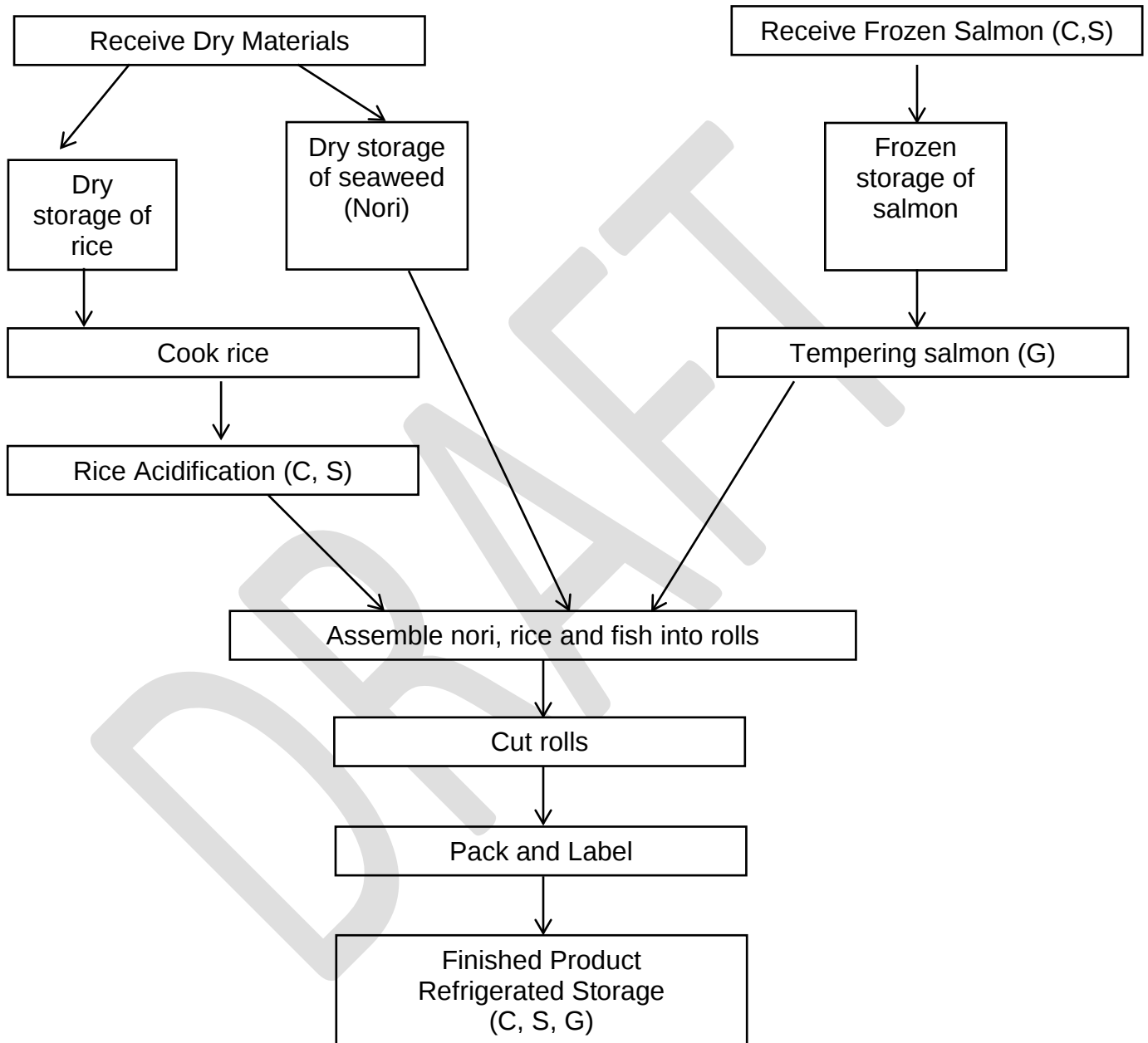
Flow Diagram A.36a. SEAFOOD – NON-RTE, RAW



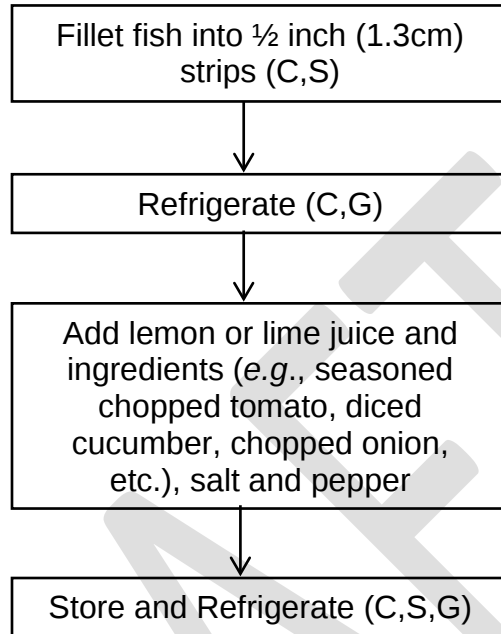
Flow Diagram A.36b. SEAFOOD –RAW
Wild Salmon Shashimi



Flow Diagram A.36c. SEAFOOD –RAW
Wild Salmon Sushi

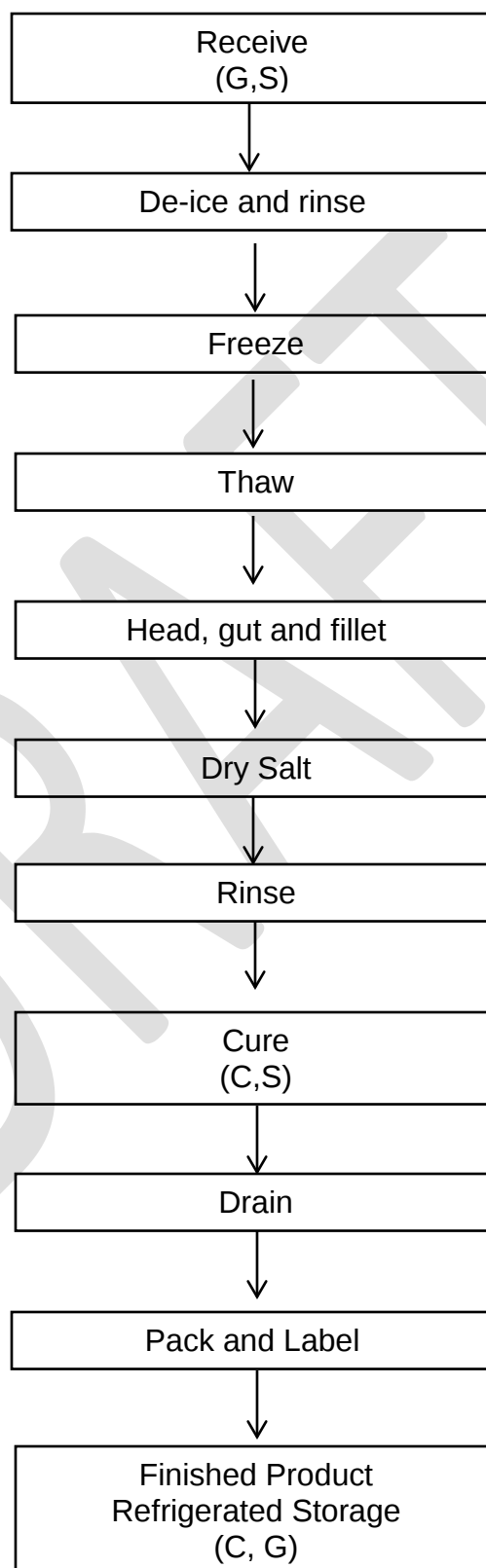


Flow Diagram A.36d. SEAFOOD –RAW
Ceviche

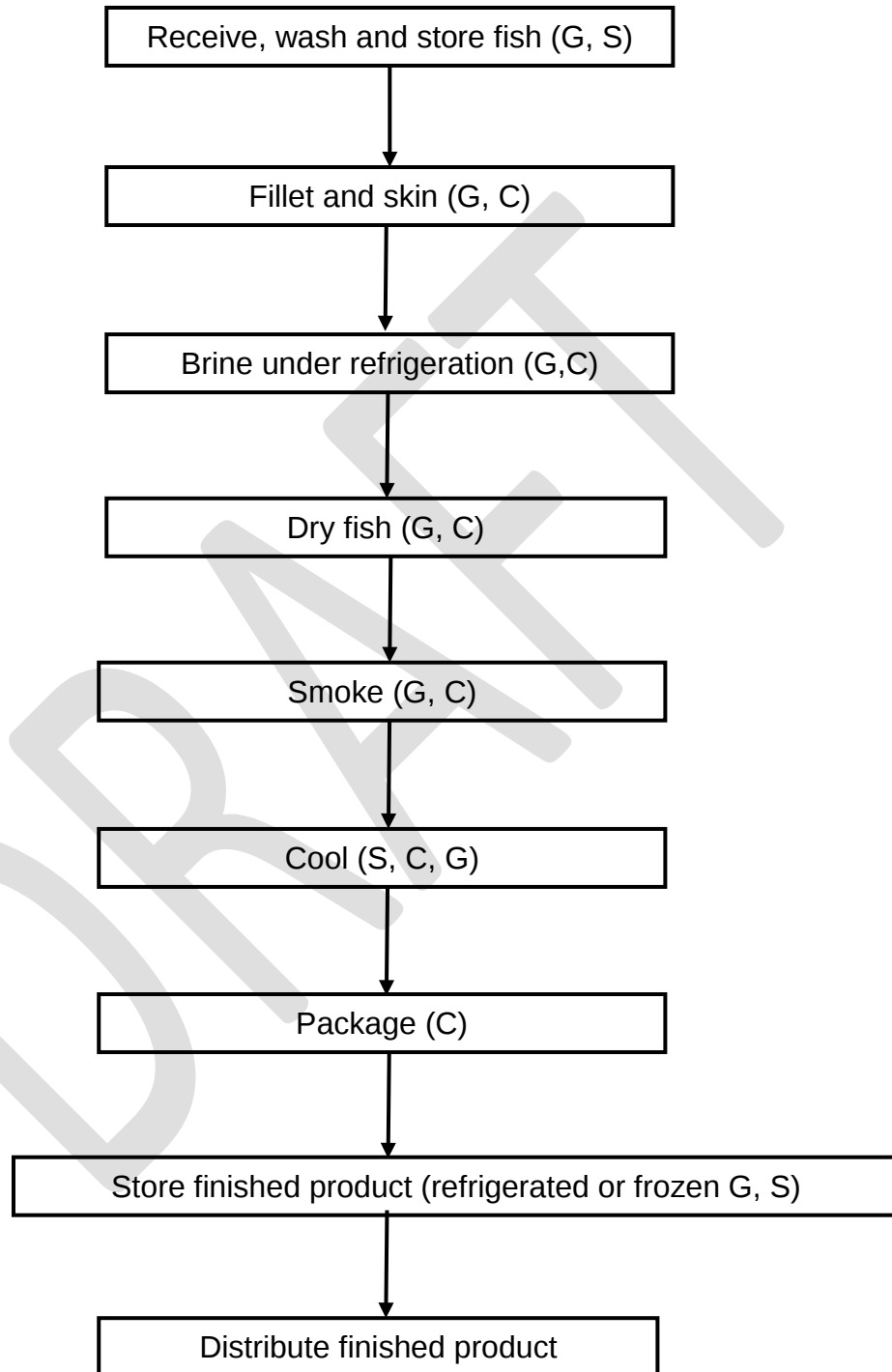


Flow Diagram A.36e. SEAFOOD –RAW

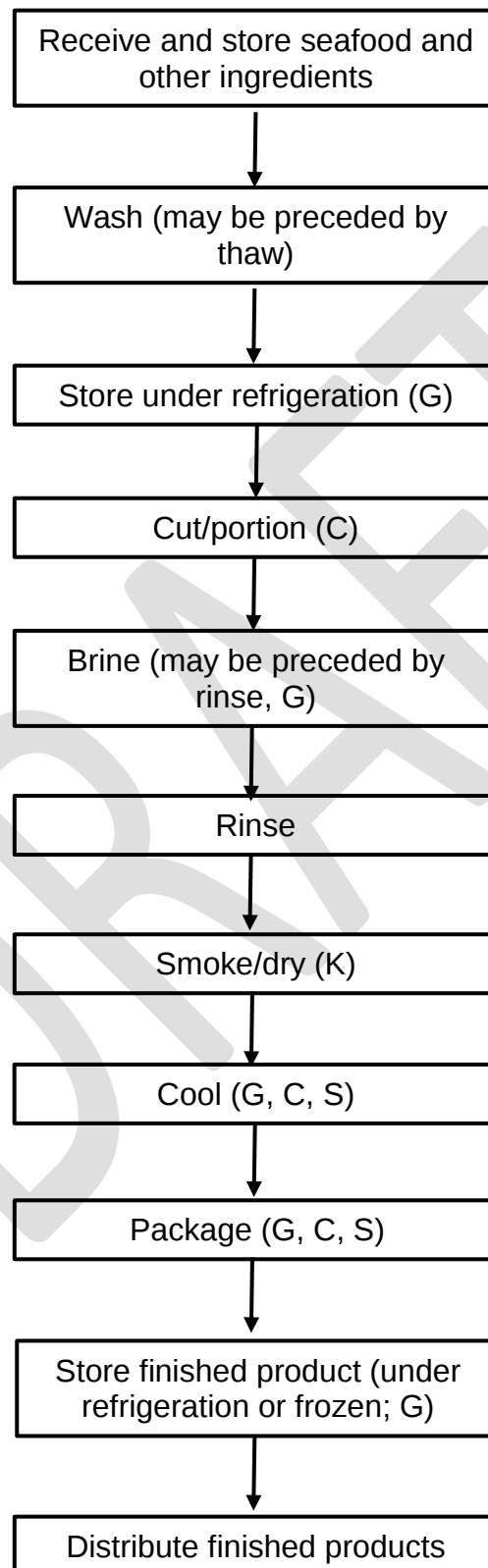
Pickled Herring Fillets



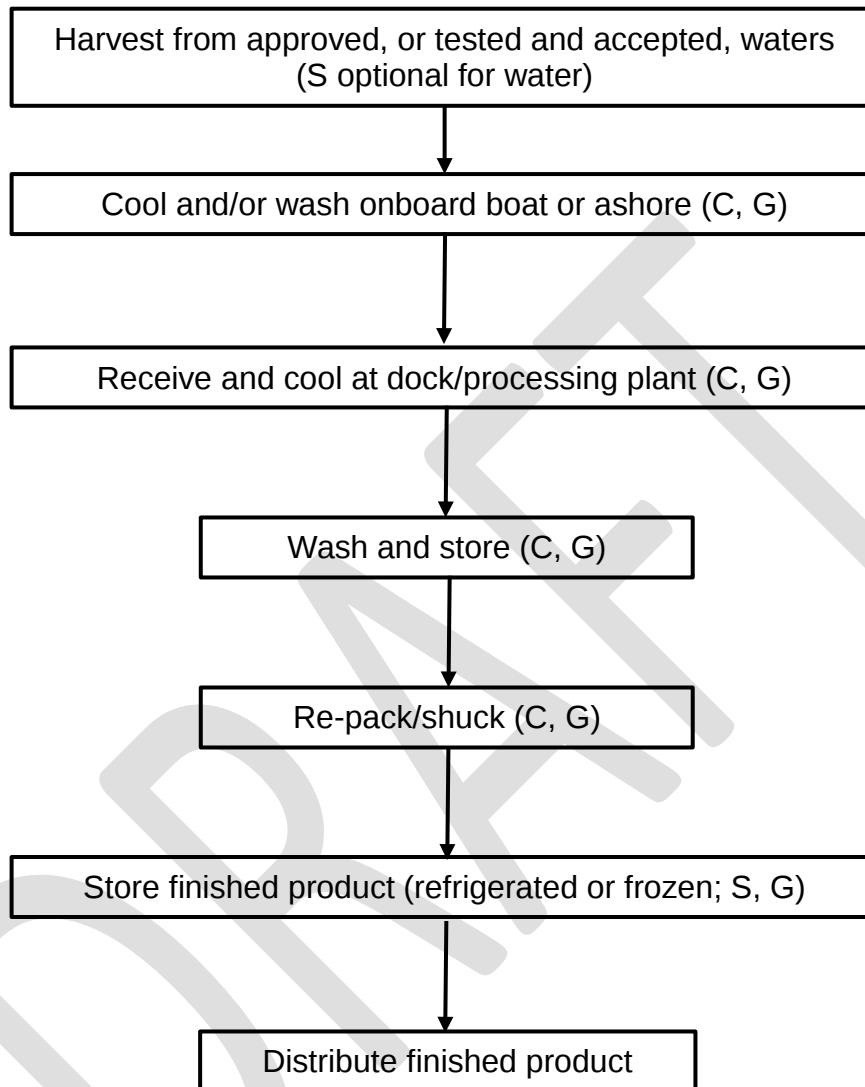
Flow Diagram A.37. SEAFOOD – RTE FISH, COLD
SMOKED



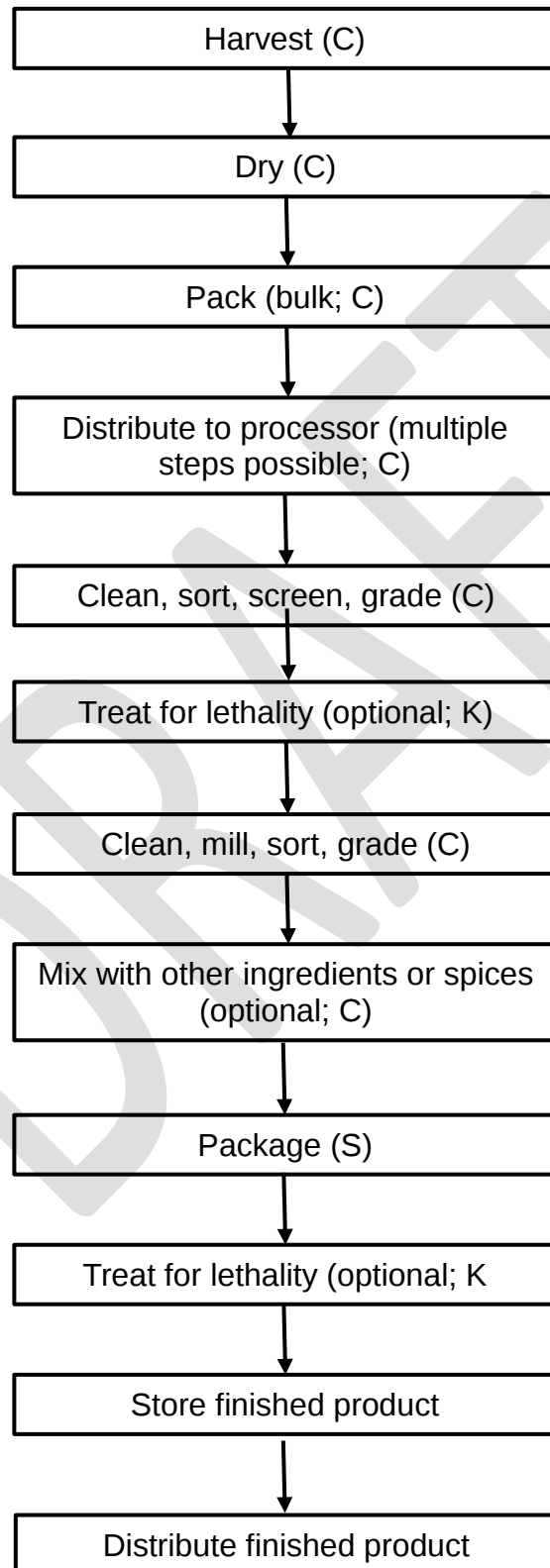
Flow Diagram A.38. SEAFOOD – RTE, FISH OR CRUSTACEAN, COOKED OR HOT SMOKED



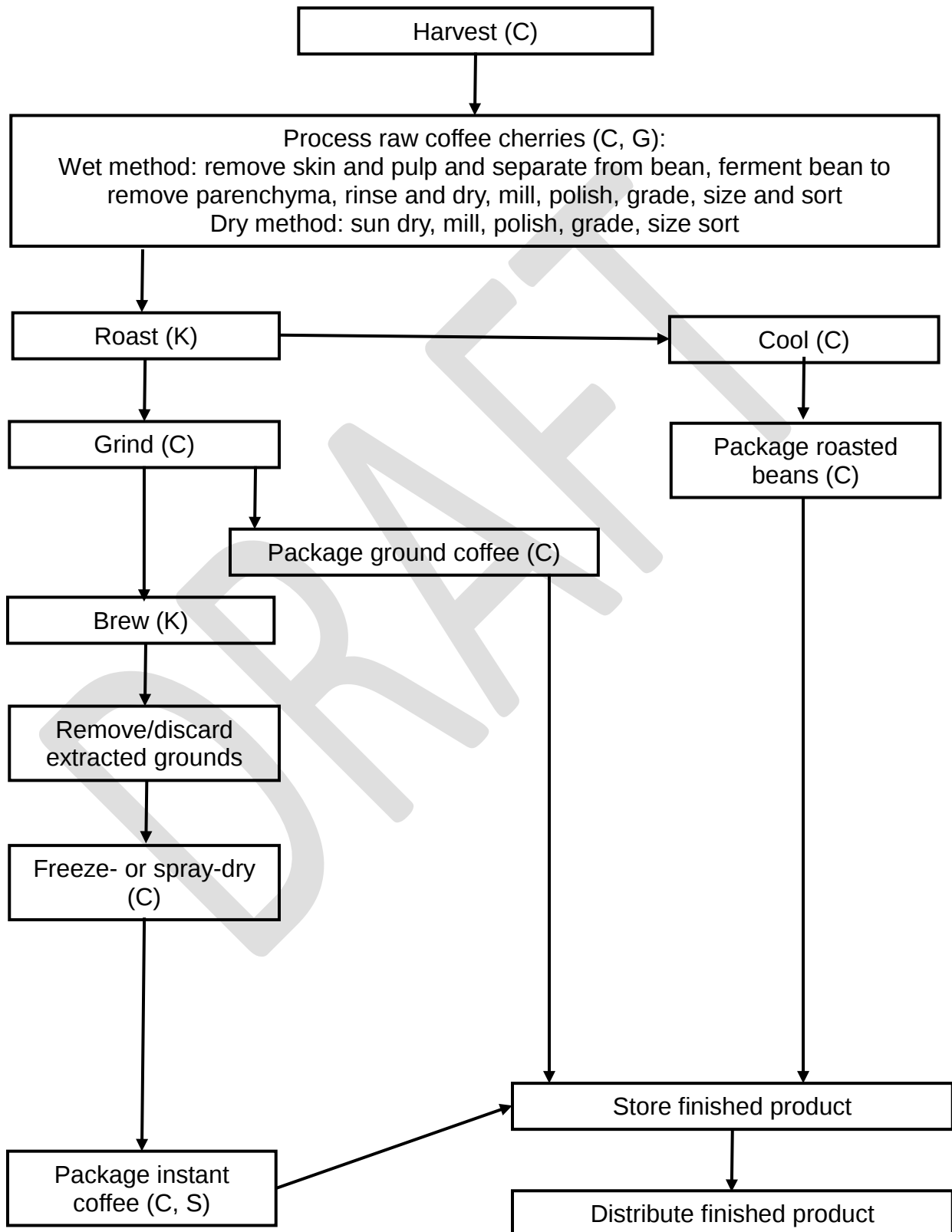
Flow Diagram A.39. SEAFOOD – RTE, RAW MOLLUSCAN SHELLFISH



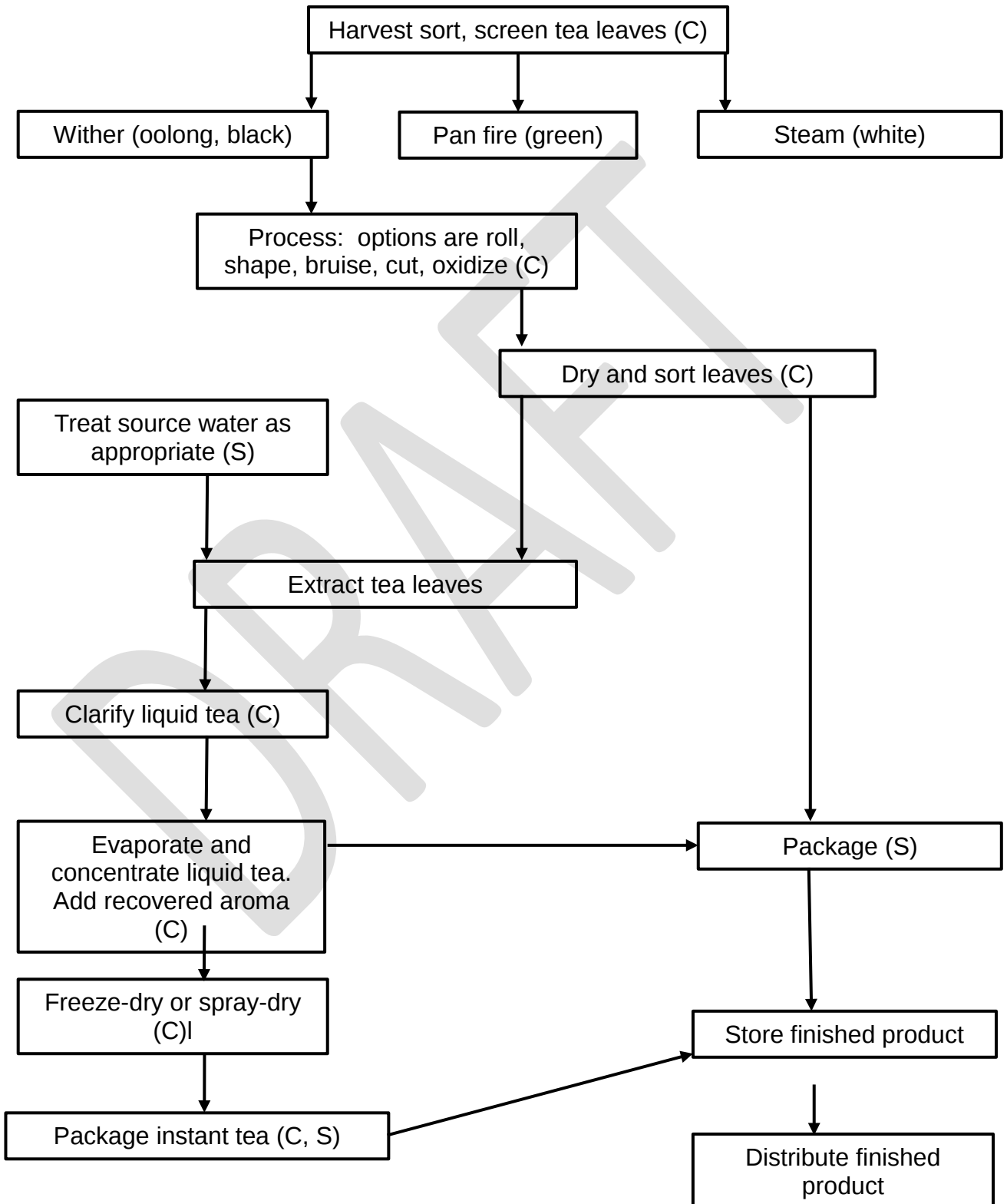
Flow Diagram A.40a. SPICES AND HERBS



Flow Diagram A.40b. BEVERAGES – COFFEE



Flow Diagram A.40c. BEVERAGES –



Appendix B: Statistical Process Control (SPC) Charts

Control charts are plots of process data collected over time used to determine if a process is in statistical control. It is important to note that there is a difference between a process being in statistical control and meeting specifications. A process is considered under statistical control if it is stable over time and the observed variation is due to common, chance causes inherent to the process (e.g., background noise due to normal variation in ambient temperature and humidity) and there is no between-lot variation. A process is considered out of statistical control if shifts in the process central tendency (e.g., mean), variability, or both result from uncommon sources associated with special or assignable causes (e.g., equipment malfunction, a change in raw materials, or failure of a laboratory procedure). A food process being under statistical process control does not imply its capability with respect to meeting microbiological specifications. The ideal situation is when a process is both under statistical control and is capable of manufacturing products that meet specifications. However, a process can be in statistical control and not capable of satisfying specifications. For example, the process consistently generates substandard product. Alternatively, a process can be out of statistical control but capable of satisfying specifications. For example, the process is designed to be robust to deviations from the norm, such that it meets specifications despite high variability. Given seasonal and other sources of variability beyond a supplier's control, the latter situation may be particularly relevant to food production processes.

SPC charts can be classified as control charts for variables (e.g., average and range charts) or control charts for attributes (e.g., p charts). Microbiological food safety characteristics can be classified as variables or attributes. Microbiological concentration data expressed on a continuous numerical scale are classified as variable data. Microbiological presence/absence data or concentration data classified into numerical ranges (e.g., $m < x \leq M$) are classified as attribute data.

Montgomery (2) cautions: "It is not possible to give an exact solution to the problem of control chart design, unless the analyst has detailed information about both the statistical characteristics of the control chart tests and the economic factors that affect the problem. A complete solution would require knowledge of the costs of investigating and possibly correcting the process in response to out-of-control signals, and the costs associated with producing a product that does not meet specifications. Given this kind of information, an economic decision model could be constructed to allow economically optimum control." However, such detailed information is not generally available for even a small subset of food production processes, and the available information is subject to considerable uncertainty, variability, and disagreement (e.g., regarding consumer health impacts). Therefore, this discussion is limited to some general guidelines that will aid in SPC chart design rather than identifying optimal designs.

Control Charts for Variables

Control of variable characteristics requires managing both the central tendency and variability. Measures of central tendency include the mean (μ) and median. Measures of variability include the standard deviation (σ) and the range (R). The $\bar{x}$ chart is used to monitor control of the process average. Process variability can be monitored with a control chart for the standard deviation (s chart) or the range (R chart). Due to its simplicity, the R chart is widely used.

Suppose that the microbiological concentration data (y) from a food process are lognormally distributed such that the log-transformed data ($x = \log_{10}(y)$) are normally distributed with mean μ ($\log_{10}$ cfu/g) and standard deviation σ ($\log_{10}$ cfu/g). Estimates of μ and σ are based on an initial process capability study conducted when the process is considered under statistical control. Let k = number of lots (subgroups) sampled and n = number of samples per lot (subgroup). As a rule of thumb, Shewhart (3) suggested that “a sequence of not less than twenty five samples of size four” is the minimum requirement for concluding that a process is in a state of statistical control (e.g., 4 samples per lot from 25 lots).

Let $\bar{x}_1, \bar{x}_2, \dots, \bar{x}_k$ be the geometric ($\log_{10}$) sample means from each lot (subgroup). The estimate of process average (μ) is the grand mean ($\bar{\bar{x}}$):

$$\bar{\bar{x}} = \frac{\sum_{j=1}^k \bar{x}_j}{k}$$

The sample range (R) is the difference between the largest and smallest observations within each lot (subgroup): $R = x_{max} - x_{min}$. The average sample range ($\bar{R}$) provides an estimate of the process standard deviation (σ):

$$\bar{R} = \frac{\sum_{j=1}^k R_j}{k}$$

$$\hat{\sigma} = \frac{\bar{R}}{d_2}$$

Where d_2 is the expected mean of R/σ . Table B.1 provides calculated d_2 values for $n=2-25$.

Table B.1. Factors for Control Charts for Variables Assuming a Normal Distribution

n	d2	d3	A2	D3	D4
2	1.128	0.853	1.880	0.000	3.267
3	1.693	0.888	1.023	0.000	2.575
4	2.059	0.880	0.729	0.000	2.282
5	2.326	0.864	0.577	0.000	2.114
6	2.534	0.848	0.483	0.000	2.004
7	2.704	0.833	0.419	0.076	1.924
8	2.847	0.820	0.373	0.136	1.864
9	2.970	0.808	0.337	0.184	1.816
10	3.078	0.797	0.308	0.223	1.777
11	3.173	0.787	0.285	0.256	1.744
12	3.258	0.778	0.266	0.283	1.717
13	3.336	0.770	0.249	0.307	1.693
14	3.407	0.763	0.235	0.328	1.672
15	3.472	0.756	0.223	0.347	1.653
16	3.532	0.750	0.212	0.363	1.637
17	3.588	0.744	0.203	0.378	1.622
18	3.640	0.739	0.194	0.391	1.609
19	3.689	0.733	0.187	0.404	1.596
20	3.735	0.729	0.180	0.415	1.585
21	3.778	0.724	0.173	0.425	1.575
22	3.819	0.720	0.167	0.435	1.565
23	3.858	0.716	0.162	0.443	1.557
24	3.895	0.712	0.157	0.452	1.548
25	3.931	0.708	0.153	0.459	1.541

By convention, statistical process control limits are based on $\mu \pm 3\sigma$, where 99.7% of values sampled from a normal distribution lie within a “Six Sigma” interval centered on the mean. Tabled values for factors for control charts for variables are typically based on the 3σ convention.

The $\bar{x}$ chart monitors between-lot variability in the process mean. The equations for constructing 3σ upper and lower control limits on the $\bar{x}$ chart are as follows:

$$UCL_{\bar{x}} = \bar{\bar{x}} + A_2 \bar{R}$$
$$LCL_{\bar{x}} = \bar{\bar{x}} - A_2 \bar{R}$$

where $A_2 = \frac{3}{d_2\sqrt{n}}$. Table B.1 provides A_2 values for $n = 2$ to 25. When 3σ control limits are used and the process is under statistical control, the probability of a sample mean being outside the $\bar{x}$ control limits simply due to random chance (the false-alarm rate (FAR)) is 0.3%. Note that the control limits are based on within-lot variability (σ) only (2). Thus the conventional limits treat any between-lot

variability ($\sigma_{\bar{x}}$) as indicating a lack of control, rather than a source of variation that may be intrinsic to the process. Achieving negligible between-lot variability may not be feasible in some food production processes. Even for relatively small sample sizes, the sampling distribution of the sample mean is approximately normal even if the underlying data are not; although the limit of quantitation presents a potential complication for microbiological data if the proportion of negative results is large (1). Montgomery (2) addresses monitoring processes with a high proportion of data such as those that fall outside the detection limit or are too numerous to count.

The R chart monitors within-lot variability. The equations for constructing 3σ upper and lower control limits on the R chart for process variability are as follows:

$$\begin{aligned} UCL_R &= D_4 \bar{R} \\ LCL_R &= D_3 \bar{R} \end{aligned}$$

where $D_4 = 1 + \frac{3d_3}{d_2}$, $D_3 = \max\left[0, 1 - \frac{3d_3}{d_2}\right]$, and $d_3 = \frac{\sigma_R}{\sigma}$. Table B.1 provides d_3 , D_3 , and D_4 values for $n = 2$ to 25. Even for normally distributed data, the sampling distribution of R (with the standard deviation, σ_R) is non-negative and positively skewed. Therefore, the symmetric 3σ control limits for R are only approximate, and the actual FAR depends on n and the underlying distribution. In food safety, the concern would typically be about excessive variation (UCL_R), but insufficient variation (LCL_R) may indicate a problem with sampling or analytical procedures.

The data used to construct $\bar{x}$ and R charts also provide information about process capability. The two-tailed process capability index (C_p) is defined in terms of the upper and lower specification limits (USL and LSL):

$$C_p = \frac{USL - LSL}{6\sigma}$$

Note, however, that the equation for C_p only considers process variability. It implicitly assumes that the process is centered on a mean of $(USL - LSL)/2$. Compare to an upper-tail C_p :

$$C_p = (USL - \mu) / (3\sigma)$$

Further details about the statistical basis for control charts for variables are available in standard texts (e.g., (2)). On-line calculators for control charts for variables are available from a variety of sources (e.g., <http://www.sqconline.com/>).

When establishing control limits based on an initial process capability study, it may be reasonable to remove a few extreme sample values due to assignable causes from the dataset to better represent common cause variation of a stable

process under statistical control. However, extreme values may simply be random outliers, and identifying an assignable cause for each extreme value may not be possible. Similarly, apparent patterns in small datasets (e.g., a sequence of extreme values or trends) may be simply due to random variation. If the initial data indicate that process variability is not in statistical control, then the control limits on the $\bar{x}$ chart may not be meaningful. Therefore, beginning the analysis with the R chart can be useful. It is customary to treat the control limits obtained in the initial phase as provisional and to update and revise the control limits over time as additional information is acquired (Appendix I) and the process matures.

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2. Montgomery, D. C. 2005. Introduction to Quality Control. 5th ed. John Wiley & Sons, Hoboken, NJ.
3. Shewhart, W. A. 1986. Statistical Method from the Viewpoint of Quality Control. Dover Publications.

Appendix C: Process Control for Attributes (*p* charts)

There are a variety of process control charts for attributes. The *p* chart is widely used; it charts the fraction of non-conforming analytical units over a sampling sequence. The *p* chart is based on the binomial distribution, which assumes that there are only two possible outcomes for each observation (conforming or non-conforming), the proportion of non-conforming analytical units (*p*) is constant, the samples are independent (e.g., defects do not cluster), and a fixed sample size (*n*). The sample proportion non-conforming ($\hat{p}$) is the ratio of the number of non-conforming analytical units (*d*) observed in a sample of size *n*:

$$\hat{p} = \frac{d}{n} \quad (\text{eq. C.1})$$

For the binomial distribution, the mean and variance of the sampling distribution of $\hat{p}$ are:

$$\mu_{\hat{p}} = p \quad (\text{eq. C.2})$$

and

$$\sigma_{\hat{p}}^2 = \frac{p(1-p)}{n} \quad (\text{eq. C.3})$$

respectively.

Assuming a constant sample size, the estimated average proportion non-conforming ($\bar{p}$) across lots (subgroups) is:

$$\bar{p} = \frac{\sum_{i=1}^m d_i}{mn} = \frac{\sum_{i=1}^m \hat{p}_i}{m} \quad (\text{eq. C.4})$$

where *m* = number of lots (subgroups) sampled and *n* = number of samples per lot (subgroup).

Conventionally, *p* chart control limits are based on a symmetric $\pm 3\sigma$ interval using a normal approximation to the binomial (1):

$$UCL = \bar{p} + 3\sqrt{\frac{\bar{p}(1-\bar{p})}{n}} \quad (\text{eq. C.5})$$

$$LCL = \bar{p} - 3\sqrt{\frac{\bar{p}(1-\bar{p})}{n}} \quad (\text{eq. C.6})$$

Note that equations C.5 and C.6 assume that $\bar{p}$ represents the desired target value of the proportion non-conforming for the process. In food safety, concern would normally focus on exceeding the upper control limit (UCL); however, observations below the lower control limit (LCL) could indicate problems with sampling and analytical procedures, or it could represent an opportunity on how to improve process quality. On-line calculators are available for computing conventional 3 sigma control limits for *p* charts (e.g.,

<http://www.sgconline.com/control-chart-calculator-attributes-discrete-data>).

It should be noted that the further the target value of *p* is from 0.5, the larger the sample size required for the normal approximation to be reasonable. As a general rule, the normal approximation is reasonable if $np \geq 5$ and $n(1-p) \geq 5$. In many food safety applications where the target value for *p* is substantially less

than 0.5, the sample size required for the normal approximation would be costly. More generally, even if an exact binomial method is used to calculate control limits, practical application of p charts is limited to cases where the target value for p is not very small (Table C.1). The sample size should be large enough to provide a reasonably high degree of confidence of observing at least one non-conforming unit (1). For example, if the target value for $p = 0.01$ and $n = 5$, the conventional upper control limit is 0.14 (eq. C.5). Consequently, observing a single non-conforming unit in the sample ($\hat{p} = 1/5 = 0.2$) would suggest a lack of process control.

Table C.1. Sample size requirements for p chart control limits

p target	n_1	n_2	n_3
0.01	500	299	230
0.02	250	149	114
0.03	167	99	76
0.04	125	74	57
0.05	100	59	45
0.10	50	29	22
0.20	25	14	11
0.30	17	9	7
0.40	13	6	5
0.50	10	5	4

n_1 = minimum sample size required for normal approximation

n_2 = sample size required for 95% confidence of observing at least one non-conforming unit

n_3 = sample size required for 90% confidence of observing at least one non-conforming unit

Standard texts provide additional details on constructing and interpreting p charts (e.g., (1)).

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1. Montgomery, D. C. 2005. Introduction to Quality Control. 5th ed. John Wiley & Sons, Hoboken, NJ.

Appendix D. High-Event Period Process Control

A high-event period may be defined as a production period when the observed prevalence likely exceeds the expected or design prevalence. Here prevalence refers to an attribute – either presence/absence or concentration in a range (e.g., CFU /g > M). The application of numerical criteria for identifying a high-event period is intended for cases where the prevalence is impracticably low for p-charts.

Suppose that the prevalence (p) is constant such that the number of positive test results (x) out of n independent samples follows a binomial distribution. Then we can determine combinations of x and n that are unlikely to occur by chance if the true prevalence is no more than the design prevalence. A sampling period would proceed until the testing results indicate a high-event period, or non-conformance with the design prevalence. After appropriate action is taken in response to the non-conformance, a new sampling period begins.

Tables D.1 and D.2 present the limits of conforming sample results for a false alarm rate (FAR) of 5% and 1%, respectively. From Table D.1, for example, if the number of positive test results observed is $x = 4$ out of $n < 198$, then there is less than a 5% chance of observing the data if the true prevalence is 1%. A 5% FAR might be appropriate for cases of low sampling frequency because a long sampling period may elapse before a producer receives an indication that the process is out of control.

Table D.1. High-Event Period Criteria for 5% False Alarm Rate

Positive Test Results (x)	Design prevalence (p)									
	0.005	0.01	0.015	0.02	0.025	0.03	0.035	0.04	0.045	0.05
	Samples (n) for given design prevalence									
1	71	35	24	18	14	12	10	9	8	7
2	164	82	55	41	33	27	23	21	18	16
3	274	137	91	69	55	46	39	35	31	28
4	395	198	132	99	79	66	57	50	44	40
5	523	262	175	131	105	88	75	66	59	53
6	658	329	220	165	132	110	95	83	74	67
7	797	399	266	200	160	134	115	101	90	81
8	940	471	314	236	189	158	135	119	106	95
9	1086	544	363	273	218	182	156	137	122	110
10	1235	618	413	310	248	207	178	156	139	125

Similarly from Table D.2, if the number of positive test results observed is $x = 4$ out of $n < 129$, then there is less than a 1% chance of observing the data if the

true prevalence is 1%. A 1% FAR might be appropriate for cases of high sampling frequency because a sampling period of limited duration would elapse before a producer receives an indication that the process is out of control.

Table D.2. High-Event Period Criteria for 1% False Alarm Rate

Acceptance Number (c)	Design prevalence (p)									
	0.005	0.01	0.015	0.02	0.025	0.03	0.035	0.04	0.045	0.05
Samples (n) for given design prevalence										
1	30	15	10	7	6	5	4	4	3	3
2	88	44	29	22	18	15	13	11	10	9
3	165	83	56	42	34	28	24	21	19	17
4	257	129	86	65	52	44	37	33	29	27
5	359	180	120	90	73	61	52	46	41	37
6	468	235	157	118	95	79	68	60	53	48
7	583	292	195	147	118	99	85	74	66	60
8	704	353	236	177	142	119	102	90	80	72
9	829	415	278	209	167	140	120	105	94	85
10	957	480	320	241	193	161	139	122	108	98

Appendix E. Control Charts for Very Low Prevalence

Here 'very low prevalence' is taken to mean that less than 2% of the samples taken are found positive for the analyte. The testing is typically limited to presence/absence methods, and the finding of a positive is sufficient to implicate the underlying lot.

The statistic of interest for this scenario is the prevalence proportion of positive results, or, equivalently, the 'mean time between positives' ('MTBP').

E. coli O157:H7 Testing of Ground Beef

Lots of ground beef are tested for *E. coli* O157:H7. While positives may result in rejected lots, this testing can also be used for process control.

The observed prevalence depends on the analytical unit size. In order to be meaningful, the prevalence needs to be referenced to an analytical unit size, i.e., a prevalence of positives in X gram samples (e.g., 325 g). Guidelines for this example require that prevalence of positives should average no higher than 1 in 500 samples or 0.2%, or a MTBP of 500 samples or more (the LSL). Because the prevalence is so low, the nonparametric method is probably not viable for this scenario, as a long sampling history would be required to find even a 99th percentile. For this example we therefore use a parametric approach.

The normal operations are modeled as a Poisson process, with the MTBP following an exponential distribution, which has a standard deviation equal to the mean. Hypothetical data for 325-gram samples from several years indicate a process MTBP of 690 sampling units with a standard deviation of 730, not much different from 690, supporting the use of an exponential model.

A parametric 'g-Chart' is shown in Fig. E.1, with an upper control limit calculated as:

$$\begin{aligned} \text{UCL} &= \text{MTBP} + 3 \sqrt{\text{MTBP} (\text{MTBP} + 1)} & (\text{eq. E.1}) \\ &= 2762 \end{aligned}$$

In the example, the LCL is zero. The factor '3' is based on the 3 sigma convention and corresponds to the 99.9th percentile (Alternatively, the exact exponential distribution quantiles could be used).

Also plotted is the exponentially weighted moving average (EWMA) of the MTBP data, with a smoothing constant of 0.1 and starting value $\text{EWMA}_0 = 690$:

$$\text{EWMA}_{k+1} = \text{EWMA}_k + 0.10 (\text{MTBP}_{k+1} - \text{EWMA}_k) \quad (\text{eq. E.2})$$

The EWMA smooths the rough curve and is helpful in visualizing the drift of the MTBP average.

The g-Chart helps define operational conditions that lead to relatively stable low prevalence. However, it has the limitation that it is an individual data trend chart and the LCL is absent. If the data line moves above the UCL, this suggests maintenance of this state of operations would result in lower prevalence, and should be investigated to see how this lower prevalence could be sustained. Also, if the data line exhibits a strong non-random pattern, this suggests a systematic cause, which should be investigated. Figure E.1. shows a saw-tooth appearance ('up' followed by 'down'), indicating negative autocorrelation.

Finally, we can supplement the control limits with a 'runs test', e.g., if a run of 11 or more MTBP data consecutively fall *below* the mean MTBP, then 'abnormal' operations are detected, and an assignable cause should be sought (for the exponential distribution, the mean is the 63rd percentile, so $0.63^{11} = 0.6\%$ FAR). The longest run observed below the mean MTBP is 7 samples here, which falls within expectations for normal operations. As an alternative, a 'center-line' at 0.693 MTBP could be added, for which results under statistical control are equally likely to fall on either side. A run of 7 or more results on the either side of this center line represents detection of an abnormal change in the process that should be investigated for an assignable cause, i.e., for the exponential distribution, the median = $0.693 \times \text{mean}$, so $0.5^7 = 0.8\%$ FAR.

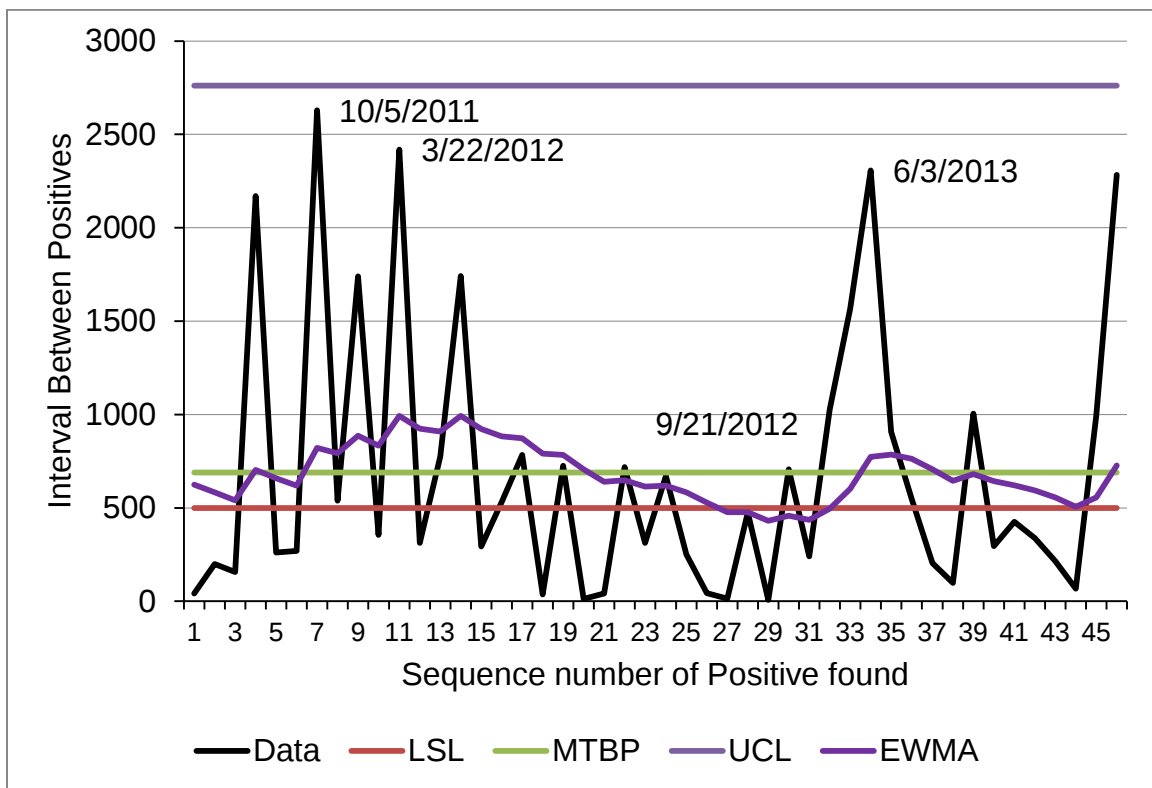


Figure E.1. g-Chart for hypothetical *E. coli* O157:H7 MTBP data.

Appendix F. Control Chart for Low Prevalence with Quantification

This scenario corresponds to prevalence (i.e., presence/absences followed by quantification of positive samples, or above or below limit of quantification) in the range 2% to 10% where samples provide quantitative estimates. These data can be used to create two different control charts, the g-Chart showing time between positive results, and an individuals chart with adjusted quantiles. As in Appendix E, the g-Chart helps define operational conditions that lead to relatively stable low prevalence. The individuals chart detects data that may indicate the presence of assignable causes that can support further investigation. The sampling can still be interpreted as the output of a Poisson process, and the prevalence and MTBP estimated. However, it is also assumed that positive results now occur often enough that they are routinely quantified.

The prevalence from history may be estimated as

$$p = (\# \text{ positives}) / (\text{total} \# \text{ samples}) \quad (\text{eq. F.1.})$$

and the MTBP as $1/p$, or as the average of the between-positive sampling intervals as before.

The g-Chart will still be useful for maintaining normal prevalence (MTBP).

If the observed counts (not concentration) in quantitation are below 100 and a single dilution is used, the counts may be modeled as Poisson distributed. If the observed counts are 10 or more typically, or multiple dilutions or a most probable number (MPN) technique is used, the counts (or estimates in the case of MPN) may be modeled as normally distributed after a logarithmic transformation.

Processes that are in control may be characterized by a constant expected prevalence with incidental modest contamination. A Poisson distributed contamination may arise from isolated contamination events such as those caused by aerosolized particles. Log normally distributed contamination may arise from splatters or surface-to-surface contact. Processes that are out of control may result from changes in prevalence of contamination, or increased counts when they occur.

Data are logarithmic-transform from the original concentration results:

$$y = \log_{10}(x + 0.3 d) \quad (\text{eq. F.2.})$$

where 'x' is the concentration estimated for the positive result, 'd' is the concentration corresponding to a single count result, and 'y' is the logarithmic metamer. For example, if the analytical portion is 1 ml, and there is a one decimal dilution, then a single count would result in an estimated concentration of

10 CFU/ml. Single count measurements (*i.e.*, =10 CFU/ml) would be transformed as $1.11 = \log_{10}(10+0.3 \times 10)$.

Example: Coliforms in Soft Cheese

Lots of soft cheese (*e.g.*, Brie) are sampled and tested for coliform bacteria. Specifications require each lot should not exceed 1,000 CFU/25g test portion. History is comprised of 702 samples, of which 28 were positive, for a prevalence (*p*) of 3.99%. The MTBP was 24.4 samples with a standard deviation of 27.5 samples, close to the MTBP, supporting the exponential distribution assumption.

Figure F.1. shows the g-Chart, with an UCL exception at sample #309, and a run of 9 values below the MTBP line. The exception indicates better control is possible in normal operations, and this should be explored. The run of 9 below the MTBP line, although not an exception, is suggestive of a problem with control in this range of samples.

Contamination levels of individual samples may be plotted with an UCL based on an extreme quantile of normal operations. From the historical data, nonparametric quantiles were calculated and shown in the second column of Table F.1.

Table F.1. Quantile results from Soft Cheese history

Quantile Probability	Nonparametric quantile	Adjusted Quantile Probability	Normal Quantile
99%	2.30	74.9%	2.40
99.5%	2.60	87.5%	2.77
99.9%	3.65	97.49%	3.40
99.95%	3.84	98.75%	3.62

Note: All quantiles are for $\log_{10}$ transformed positive data using eq.(F.2).

The nonparametric quantiles are derived from the EDF of the 702-sample data. As a rule, these are very imprecise for probabilities greater than $701.5 / 702 = 99.9\%$.

Parametrically, we can represent the distribution as a mixture of a binomial distribution for prevalence and a truncated normal distribution for positive observations. The hurdle threshold for positive counts is $T = \log_{10}(10 + 3) = 1.11$. The observed average and standard deviation of the positive data for *y* were 1.87 and 0.783, resp. The estimated z-score from a standard normal distribution for the threshold is 1.11, with an associated probability of only 0.03, indicating a non-truncated distribution might be useful as a rough approximation.

In Table F.1, the 'Adjusted Quantile Probability' column is the desired quantile probability 'P' (given in the first column of the table) adjusted for prevalence p. The adjusted quantile probability P* is given by

$$P^* = [P - (1 - p)] / p \quad (\text{eq. F.3.})$$

Finally the normal distribution quantiles are the quantiles associated with P*.

Note that the nonparametric and normal quantiles are in excellent agreement here, supporting the lognormal assumption. The probability of obtaining less than a single count was about 0.1%, indicating no problem with bias at the low end.

Figure F.2. shows the individuals' chart ('i-Chart') with associated UCLs for some soft cheese data. The SPC UCL line corresponds to the mean 1.87 plus 2.575 times the standard deviation of 0.783. This SPC UCL line represents the 99.5% quantile (normal vs. abnormal division) of the lognormally distributed positive result data, given that a positive result occurs. The Quantile UCL line represents the nonparametric 99.5% quantile across all results, including those which correspond to zero counts. The point at sample #12 exceeds both UCLs, indicating the point is unusual for observation as a result, and also unusual from the baseline normal distribution point of view. Sample #12 represents an unexpected shift in operations. The sample #24 result exceeds the Quantile UCL line, which means it is a rarity in sampling, but does not exceed the SPC UCL line, indicating it is not that unexpected from a positive data distribution point of view, so still represents normal operations (same distribution of positive results). This difference in interpretation between the nonparametric and parametric approaches shows another advantage (besides allowing the estimation of high probability quantiles using small samples) of the latter.

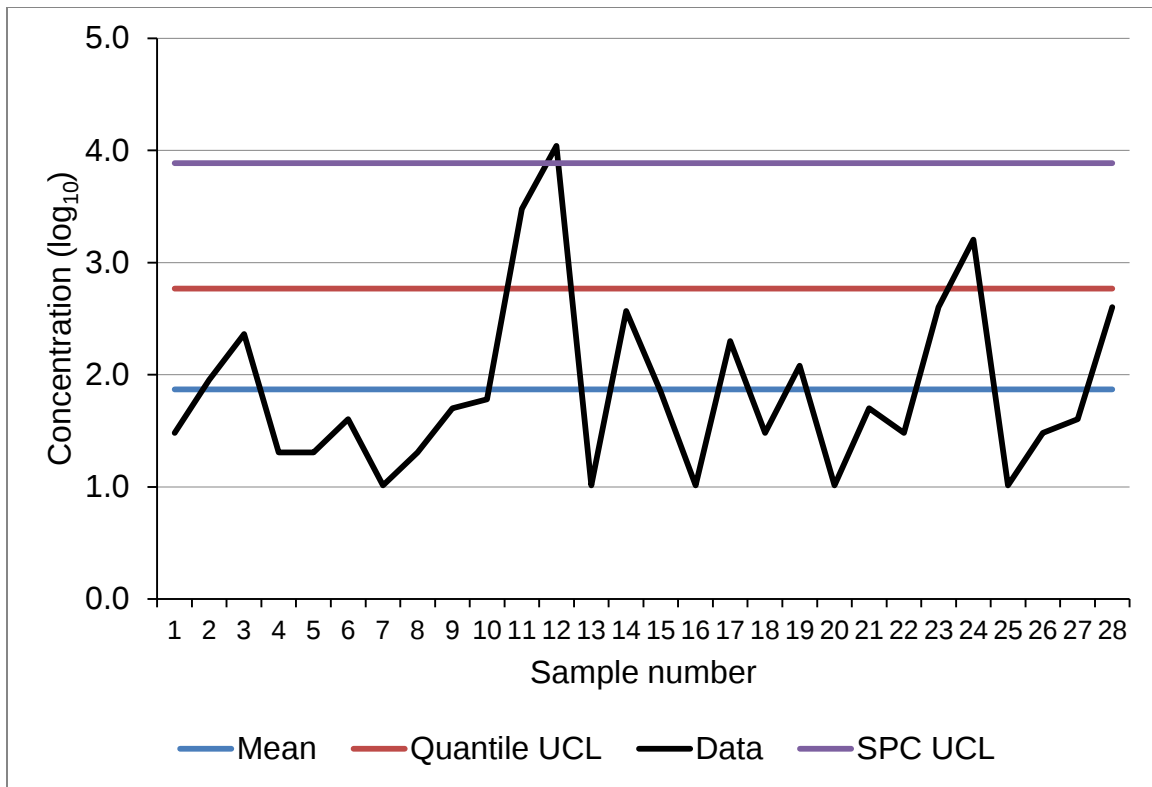


Figure F.1. g-Chart for coliforms in soft cheese. Note UCL exception around sample #309 and run of 9 values below the MTBP.

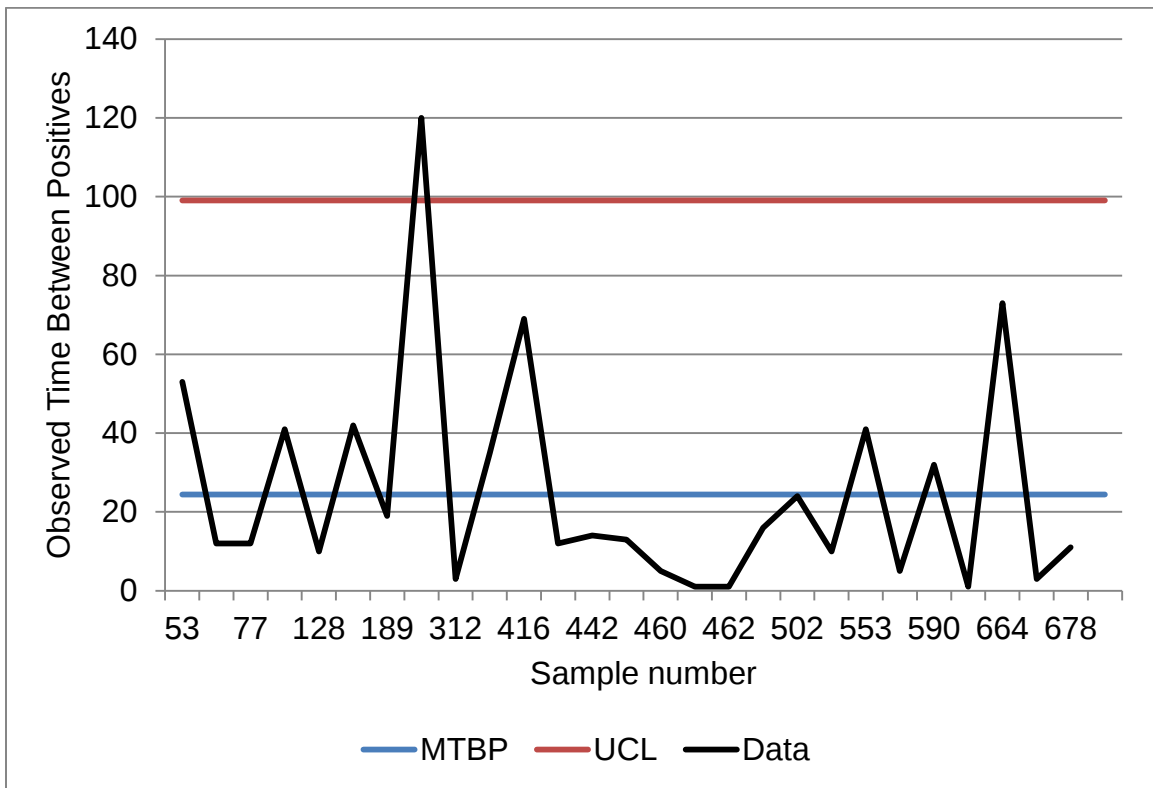


Figure F.2. Individuals chart (i-Chart) for positive samples observed for coliforms in Soft Cheese.

Appendix G. Control Chart for Moderate to High Prevalence with Quantitation

This example corresponds to prevalence in the range 10% to 95%. The sampling can be interpreted as the output of a Bernoulli process, and the prevalence estimated and controlled. It is also assumed that positive results are routinely quantitated.

Because of the moderate to high prevalence, rational subgroups (i.e., lots that represent test units belonging to a homogeneous population with the same constant population parameters) of samples may be combined to increase normality and provide better tools for prevalence SPC. If grouping is to be done by time period, equal sampling for each period is advised, and the sample size large enough to achieve an expected 5 positive results or more, and similarly for negative results. For example, if the mean prevalence is 20%, the sample size should be at least $5 / 0.2 = 25$. If the mean prevalence is 80%, the sample size would also be 25.

SPC may be carried out by a 'p-Chart', where the control limits are given by

$$UCL = \bar{p} + 3 \sqrt{\frac{\bar{p}(1-\bar{p})}{n}} \quad (\text{eq. G.1.})$$

$$LCL = \bar{p} - 3 \sqrt{\frac{\bar{p}(1-\bar{p})}{n}} \quad (\text{eq. G.2.})$$

where 'n' is the rational subgroup sample size.

The SPC of the distribution of the positive result concentrations may be performing using an individuals' chart with control limits typically as

$$UCL = \mu + 3 \sigma \quad (\text{eq. G.3.})$$

$$LCL = \mu - 3 \sigma \quad (\text{eq. G.4.})$$

The values for ρ , σ and μ need to be determined from history or a process capability study.

Although there is fixed sample size during a time period (in this case, 40 samples per quarter), the number of positive results is a random variable, so a standard X-bar chart cannot be used.

Example: Aerobic Plate Counts in Ground Beef

Lots of ground beef in cold storage are sampled and tested for aerobic plate counts (APC). Assume the microbiological guidelines require that the APC should not exceed 10,000,000 CFU/g (*i.e.*, 7.0 on a $\log_{10}(\text{CFU})$ scale).

Data consist of 455 samples, of which 393 were positive (86.4% prevalence). The positive samples had average $\log_{10}$ -transformed concentration of 5.19 with standard deviation of 1.34.

Table G.1. Quantile results from ground beef data

Quantile Probability	Nonparametric	Adjusted Quantile Probability	Normal Quantile
99%	8.00	98.84%	8.23
99.5%	8.07	99.42%	8.57
99.9%	8.28	99.88%	9.28
99.95%	8.31	99.94%	9.54

Note: All quantiles are for $\log_{10}$ -transformed positive data.

The nonparametric quantiles from the expected distribution function (EDF) are precise up to 99.5%. The agreement between nonparametric and normal-based quantiles is good, but not perfect. The probability of getting less than one count under the normal distribution model is 2.2%, a small error which should be adjusted out by using a truncated normal distribution, but which we will ignore here.

Given a prevalence of 86%, the minimum sample size required for the normal approximation to be valid is 36. Based on this, sampling was carried out by quarter of the year, with 40 samples taken randomly each quarter. For each quarter, the proportion of the 40 samples with enumerative results >0 was calculated via eq. F.1 (Appendix F).

Figure G.1. shows a p-Chart for hypothetical data. Note the exceptions at periods #7 and #22, either of which would indicate a drop in prevalence of samples with enumerative results >0 .

Figure G.2. shows an i-Chart for the samples with enumerative results >0 with control limits based on $\pm 3 \sigma$. The data are under control with no exceptions.

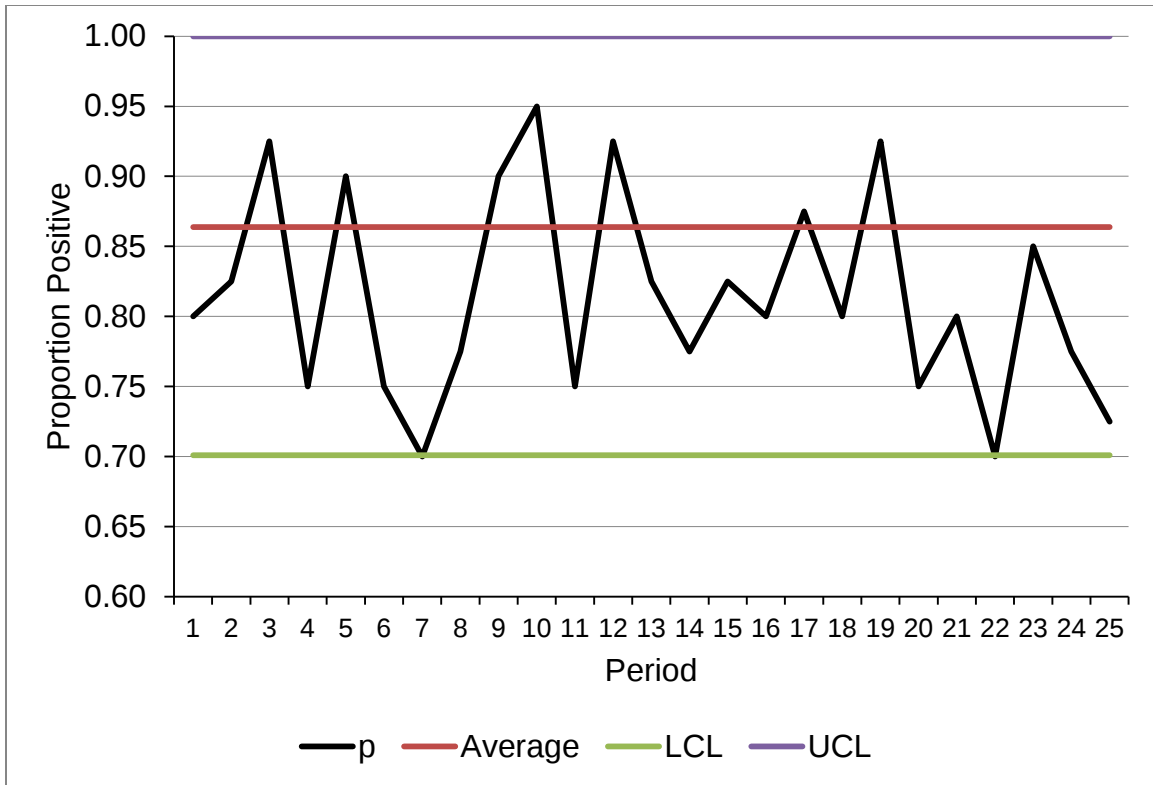


Figure G.1. p-Chart for aerobic plate count in ground beef. Note the exceptions at periods #7 and #22 which imply a drop in the proportion of samples with enumerative results >0 from that expected. There is also a general tendency to fall under the average.

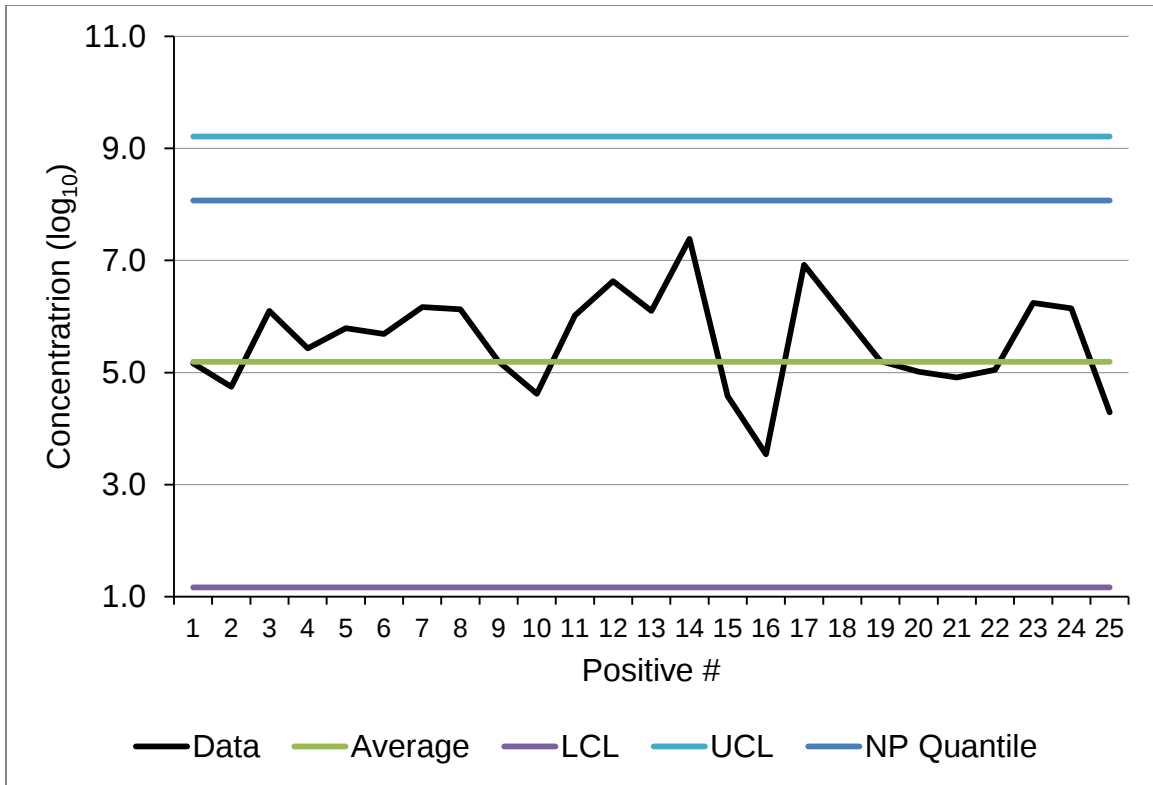


Figure G.2. Individuals' chart for aerobic plate count in ground beef.

Appendix H. Control Chart for Very High Prevalence with Quantitation

This example corresponds to prevalence in the range 95% to 100%. For this case, the number of samples with enumerative results equal to 0 is low enough to apply eq. F.2. (Appendix F).

Small rational subgroups (i.e., lots) are now possible, corresponding to shift, day, week, month or quarter, within which operations are expected to be consistent. The use of such subgroups allows control not only of the mean, but also the spread of the process.

The X-bar chart has control limits calculated as follows:

$$UCL_{ave} = \mu + A_2 R_{ave} \quad (\text{eq. H.1.})$$

$$LCL_{ave} = \mu - A_2 R_{ave} \quad (\text{eq. H.2.})$$

where the center line is μ (determined from historical data), n is the subgroup sample size, A_2 is a numerical factor available from standard control chart tables (Appendix B) and R_{ave} is the average range from the same historical dataset. The range R is the difference between the maximum and minimum values in a subgroup.

Variation is controlled by a R Chart, with control limits at

$$UCL_R = R_{ave} + D_4 R_{ave} \quad (\text{eq. H.3.})$$

$$LCL_R = 0 \text{ for } n < 6 \quad (\text{eq. H.4.})$$

where D_4 is obtained from the same table as A_2 . For most purposes, n should be between 2 and 6, with 4 or 5 preferred. The Range Chart responds to changes in *within* subgroup variation.

Table H.1. shows various scenarios for using X-bar-R- control charts for microbial testing.

Table H.1. Average-Range statistical process control chart characteristics for different sampling time frames

Average-Range Charts for Outgoing Product Sampling			
	Purpose	Verify a general good state of control at vendor output or customer receiving	
	Operation	1. Compare variation and level across periods to variation within periods. 2. Generally, start with long time-scale plans (higher numbers), with shorter time-scale plans used for tightened inspection or troubleshooting	
	Notes	1. Requires quantitative measurement result. 2. Each sampling unit may itself be a composite of specimens taken from the common time period, with a single combined test result	
	n	Sample size taken (fixed size)	
I. Sample Within Production Shift, n > 2 (best used for tightened inspection or internal control)			
	Operation	Take sample of size 'n' from each production shift. Plot average and range of each sample	
		Average chart	Range chart
	Purpose	Are the sample averages consistent across shifts and days, free of trends and disturbances?	Is the variation observed consistent within shift?
	Assignable causes	1. Personnel changes between shifts/days. 2. Introduction of new raw material lots. 3. Management changes between shifts/days. 4. Staffing and volume issues between shifts. 5. New equipment or procedures between shifts/days.	1. New employees within shift. 2. New management within shift. 3. New equipment or procedures within shift.
II. Sample Within Production Day, n > 2 (best used for tightened inspection or internal control)			
	Operation	Take sample of size 'n' from each production day, across shifts. Plot average and range of each sample	
		Average chart	Range chart
	Purpose	Are the sample averages consistent across days, free of trends and disturbances?	Is the variation observed consistent within and across different days' production?
	Assignable causes	1. Personnel changes between days. 2. Introduction of new raw material lots. 3. Management changes between days. 4. Staffing and volume issues between days. 5. New equipment or procedures between days.	1. New employees within day. 2. New management within day. 3. New equipment or procedures within day.
III. Sample Within Production Week, n = 5, 6, 7 (normal inspection)			

<i>Operation</i>	Take single unit from each production day during week, across shifts. The collection of results is the sample. Plot average and range of each sample.	
	<i>Average chart</i>	<i>Range chart</i>
<i>Purpose</i>	Average Chart PURPOSE: Are the sample averages consistent across weeks, free of trends and disturbances?	Range Chart PURPOSE: Is the variation observed consistent within and across different weeks' production?
<i>Assignable causes</i>	ASSIGNABLE CAUSES: 1. Personnel changes between weeks. 2. Introduction of new raw material lots. 3. Management changes between weeks. 4. Staffing and volume issues between weeks. 5. New equipment or procedures between days	ASSIGNABLE CAUSES: 1. New employees within week. 2. New management within week. 3. New equipment or procedures within week. 4. Day of week volume or procedure effects.
IV. Sample Within Production 'Month', n = 4 (loosened inspection)		
<i>Operation</i>	Take single unit from each production week, across days and shifts. The collection of results is the sample. Plot average and range of each sample	
	<i>Average chart</i>	<i>Range chart</i>
<i>Purpose</i>	Are the sample averages consistent across months, free of trends and disturbances?	Is the variation observed consistent within and across different months' production?
<i>Assignable causes</i>	1. Personnel changes between months. 2. Introduction of new vendors. 3. Management changes between months. 4. Staffing and volume issues between months 5. New equipment or procedures between months. 6. Seasonal changes in raw materials and production volume	1. New employees within month. 2. New management within month. 3. New equipment or procedures within month. 4. New equipment or procedures within month

Example: *Aerobic Plate Count in Bagged Salad*

Received lots of bagged salad mixed are tested for APC. Five samples are taken per lot. Based on prior test data, the long-term average APC $\log_{10}$ concentration is 5.19 with standard deviation of 1.34 and an average range of 3.12.

Figure H.1. shows the Average Chart for recent data. No abnormal behavior is apparent.

Figure H.2. shows the Range Chart for the same data. Two exceptions at the UCL are prominent.

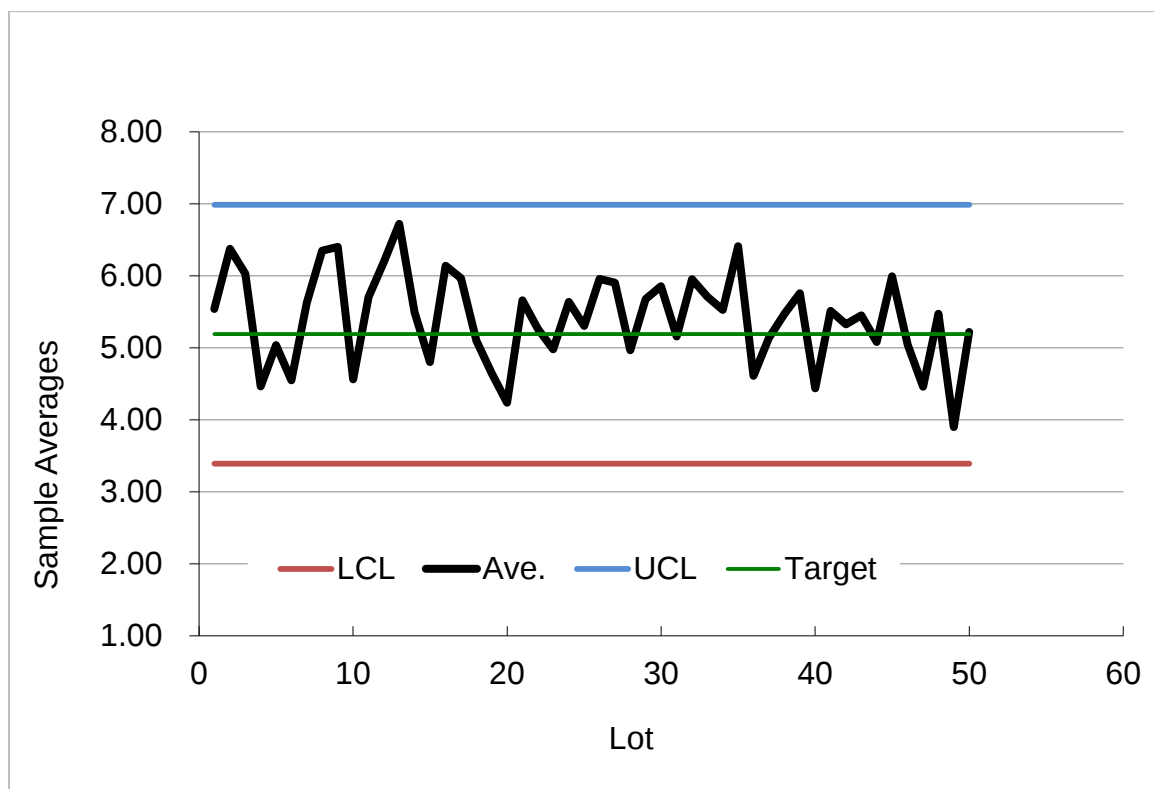


Figure H.1. Average Chart for APC in bagged salad mix.

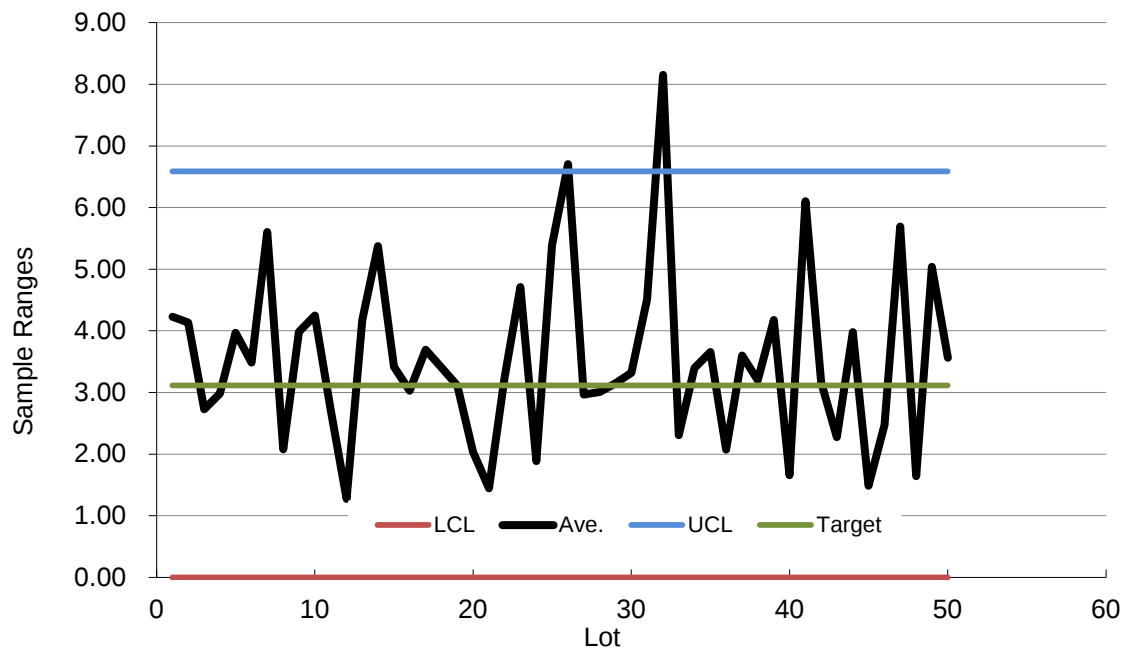


Figure H.2. Range Chart for APC in bagged salad mix. Note the two out-of-control points.

Appendix I. Number of Samples and Statistical Uncertainty about Setting Control Limits for X Bar Charts

There are no firm rules for how much data are needed to develop control charts. As the number of lots used to develop a control chart increases, the uncertainty about setting the control limits (too high or too low) decreases. However, there are diminishing returns to using more data.

For example, Figure I.1 is based on hypothetical data collected at a frequency of $n = 5$ samples per lot represented by a lognormal distribution with a geometric mean $= 3 \log_{10}$ CFU/g (1,000 CFU/g) and a standard deviation $= 1 \log_{10}$ CFU/g. The grand mean (G mean) is represented by the central solid line. The upper and lower 3σ control limits for sample means are represented by dashed lines (UCL, and LCL, respectively). The uncertainties associated with the statistics due to random sampling variability are represented by dotted lines (90% confidence limits). Assuming the process is stable over time, as the number of lots (subgroups) used to develop an average control chart increases, the uncertainty about the control limits decreases. The result of using more lots (subgroups) to develop control limits is increased confidence that the limits are not set too high or too low relative to the intended design (3 sigma). Uncertainty about the control limits decreases more slowly than the uncertainty about the mean. This is due to greater random sampling error in measures of variability.

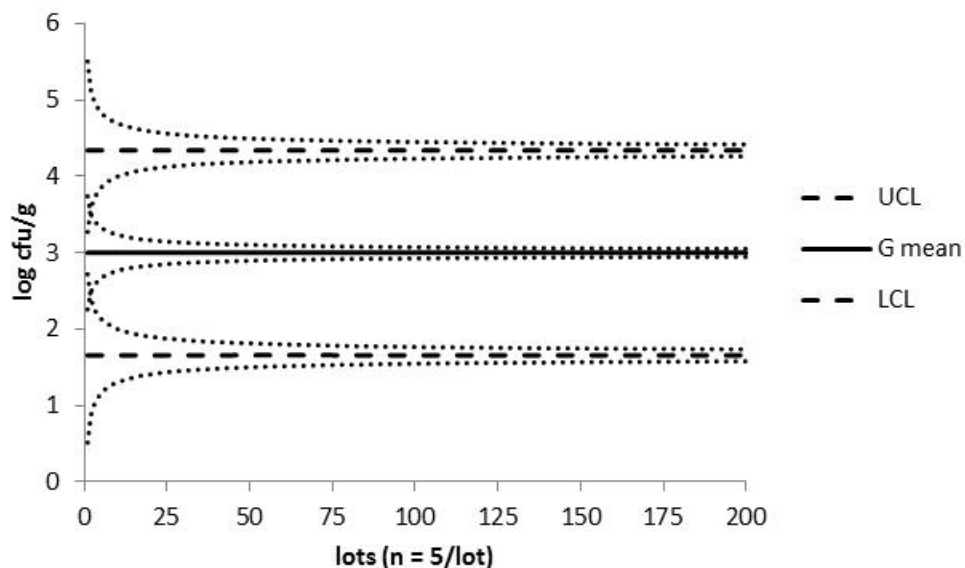


Figure I.1. Uncertainty about 3 Sigma Control Limits for Mean ($\mu = 3 \log_{10}$ CFU/g, $\sigma = 1 \log_{10}$ CFU/g)

The uncertainty about the control limits depends on the number of samples per lot (n) as well as the number of lots (subgroups) used to develop the limits. Assuming a lognormal distribution with geometric mean $= 3 \log_{10}$ CFU/g (1,000 CFU/g) and a standard deviation $= 1 \log_{10}$ CFU/g, Figure I.2 compares the

relationship between the uncertainty about average chart control limits and the number of lots used to develop the limits for $n = 5$ and $n = 3$ samples per lot (i.e., rational subgroup). Both cases show an initial rapid decrease in uncertainty in control limits followed by diminishing returns from additional lots. However, the control limit uncertainty for $n = 3$ samples per lot (subgroup) starts from a substantially higher level relative to $n = 5$ samples per lot (subgroup).

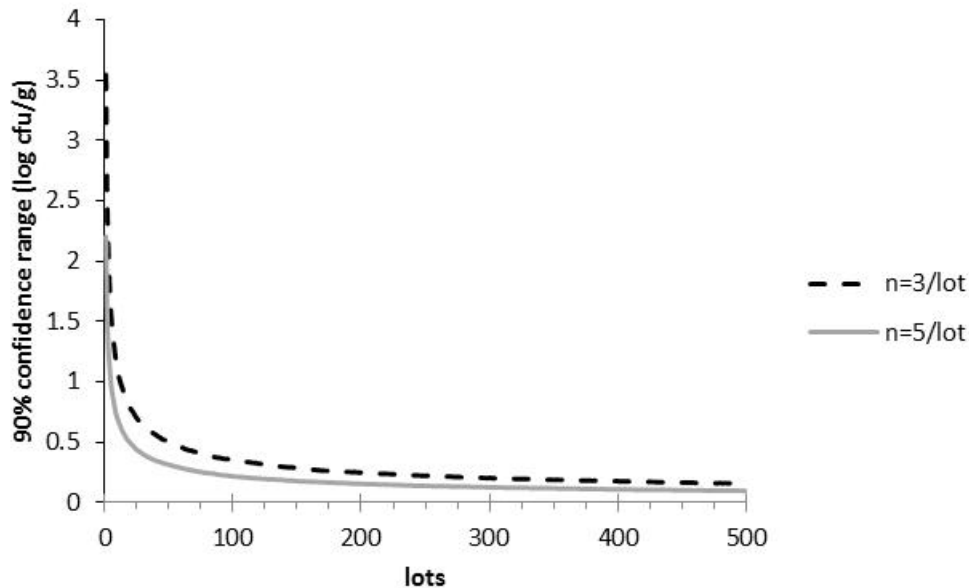


Figure I.2. 90% Confidence Range of 3 Sigma Control Limits for Mean ($\mu = 3 \log_{10} \text{CFU/g}$, $\sigma = 1 \log_{10} \text{CFU/g}$)

It should be remembered that all data included in the dataset used to compute the control limits should be consistent with a tenable assumption of a time period of unchanging conditions (e.g., the same season of raw materials, the same production process, the same equipment).

Appendix J. Microbiological and chemical limits for foods that are useful to assess process control and insanitary conditions for United States Department of Defense (DoD) use.

Introduction

The microbiological and chemical limits provided in the following tables are useful for suppliers and DOD to assess process control and sanitary conditions associated with the production of various foods. The limits and sample size or procedures described in these tables are *not* regulatory limits, although in certain cases, they may reference regulatory limits.

The food categories correspond to those listed in Appendix A where flow diagrams for manufacturing of the foods are provided. The microbiological data for the various microorganisms or classes of microorganisms can be used to develop statistical process control (SPC) charts, as well as to gain an understanding of the microbiological quality and safety associated with the various products.

The environmental monitoring program (EMP) data have less utility for development of SPC charts because of the potential high number of monitoring sites, and thus, the longer time frame required for sufficient data for SPC charts. However, the EMP data have been correlated with food-product contamination (Kornacki, 2014) and have usefulness for assessing process control, cleaning and sanitation practices, targeting supplier and DOD resources, and for trending EMP data over time to assess continuous improvement.

Each table in Appendix J includes the microorganisms that are useful for assessing process control and sanitary conditions during production of foods within the given food category. The microbiological limits for these microorganisms are provided, as well as recommended actions to be taken if the limits are exceeded. In many instances, the actions include investigating to determine a root cause, developing and implementing corrective and preventive actions, and conducting follow-up sampling and testing to determine if the corrective and preventive actions have been effective. In all tables, where applicable, these actions are identified as “Investigate” and “Implement Corrective Actions” to simplify the actions listed. The investigative and corrective action processes likely will be unique to each situation.

Samples of the food may be taken at numerous points throughout production; these are considered as in-process samples. Samples taken at the end of the production line also may be tested, with results compared against the microbiological limits. In some cases, these finished product data may be useful to assist in the development of finished product microbiological criteria; however, initially, these data should be used to assess process control and sanitary conditions, and compared against the limits provided for each criterion. When samples are taken at the end of the production line and tested, and results exceed the limits, the recommended action may be to reject the lot of food represented by the sample. This will be especially true when the microorganism detected is a pathogen and the food will not receive further processing using a validated kill step.

The number of in-process, finished product, or environmental samples to take and test may not be given for all criteria in the tables. In general, taking more samples is better; and larger numbers of samples taken for pathogens can increase the confidence of detecting pathogens present at a low prevalence. Analytical unit weights for testing should be a minimum of 25 grams; for pathogen testing, the analytical unit (usually a composite weight) in the table may specify a particular weight (e.g., 325 or 375 grams) and provide the weights for the individual samples contributing to the composite sample (e.g., 15 X 25-gram samples to result in a 375-gram analytical unit). The body of the report and Appendix I discuss how sample numbers affect the design of SPC charts.

Table J.1. Microbiological Limits for Bottled Water

Notes: The bottled water category includes bottled water described as Artesian, Mineral, Purified, Sparkling or Spring.

Criteria & EMP Target Microorganism ^a	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms	<10 in 100 ml		Reject lot. Investigate; test for generic <i>E. coli</i> . Notify local authorities if they are involved in providing water treatment.	See 21 CFR 165.110 (b)(2)(i)(A) for applicable regulatory standards
<i>E. coli</i> (generic) or thermotolerant coliforms	Negative in 100 ml		Reject lot. Investigate. Notify local authorities if they are involved in providing water treatment. If water comes in contact with food, it is recommended that the food be destroyed or reprocessed to kill vegetative cells.	See 21 CFR 165.110 (b)(2)(i)(B) for applicable regulatory standards
Enterococcus		Negative in 250 ml	Investigate. Notify local authorities if they are involved in providing water treatment. If water comes in contact with food, it is recommended that the food be destroyed or reprocessed to kill vegetative cells.	Not routinely tested; however, some countries (in the EU) test for <i>Enterococcus</i> in lieu of coliforms.
Heterotrophic plate count		<100/ml @ 22°C <20/ml @ 37°C	Investigate. Notify local authorities if they are involved in providing water treatment.	Reject or divert for further processing; investigate, implement corrective action
<i>Pseudomonas aeruginosa</i>		Negative in 250 ml	Investigate. Notify local authorities if they are involved in providing water treatment.	Not routinely tested, but may be required by individual country's regulations
Parasites & Viruses		Negative	Investigate. Notify local authorities if they are involved in providing water treatment.	Unless there is a particular concern for parasites or viruses, testing typically is not done; this will be very situational and location-dependent.

^a (Code of Federal Regulations, 2014c; European Communities, 2007; World Health Organization (WHO), 2013, 2014)

Table J. 2. Microbiological Limits for Ice, Packaged

Notes: Testing based on target microorganisms for bottled water.

Criteria & EMP Target Microorganism ^b	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms	<10 in 100ml		Investigate. Test generic <i>E. coli</i> . Notify local authorities if they are involved in providing water treatment for water becoming ice.	
<i>E. coli</i> (generic) or thermotolerant coliforms	Negative in 100 ml		Investigate. Notify local authorities if they are involved in providing water treatment for water becoming ice. If the ice is for direct consumption, it is recommended that the ice not be used. If ice comes in contact with food, it is recommended that the food be destroyed or reprocessed to kill vegetative cells.	
<i>Pseudomonas aeruginosa</i>		Negative in 250 ml	Investigate. Notify local authorities if they are involved in providing water treatment.	Not routinely tested, but may be required by individual country's regulations
Parasites & Viruses		Negative	Investigate. Notify local authorities if they are involved in providing water treatment for water becoming ice.	Unless there is a particular concern for parasites or viruses, testing typically is not done; this will be very situational and location-dependent.

^b (Code of Federal Regulations, 2014c; European Communities, 2007; World Health Organization (WHO), 2013, 2014)

Table J. 3. Microbiological and Chemical Limits for Juices and Drinks, Pasteurized and Refrigerated

Notes: Examples of these products are orange juice, carrot juice, and some tea beverages. These products are pasteurized but must be kept refrigerated to prevent spoilage. Raw citrus juices sold in the U.S. will require additional testing (Subpart B, Juice HACCP regulations). Juices with a pH>4.6 should address control of *Clostridium botulinum*.

Criteria & EMP Target Microorganism ^c	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms	<10/ml		Investigate, implement corrective action	
<i>E. coli</i> (O157:H7 or other STEC)		Negative in 10 individual 25-g samples	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	This limit is based on the FDA Juice HACCP regulation requiring a 5-log ₁₀ reduction. Processors with demonstrated control may not need to test for <i>E. coli</i> O157:H7 except for periodic verification purposes.
<i>Listeria</i> spp. (EMP) ^d	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
Patulin (in apple juice) ^e		50 µg/kg	The presence of patulin in apple juice above the limit should lead to rejection of the product. Investigate and implement corrective action.	Different countries may have different regulatory requirements. A lower limit of 10 µg/kg should be considered when apple juice products are intended for infants.
<i>Salmonella</i> (product)		Negative in 375 g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	375 g analytical unit composed of 15 X 25-g samples This limit is based on the FDA Juice HACCP regulation requiring a 5-log ₁₀ reduction. Processors with demonstrated control may not need to test for <i>Salmonella</i> except for periodic verification purposes.

^c (Code of Federal Regulations, 2014a; U. S. Department of Health and Human Services, 2004)

^d (R.B. Tompkin, 2002; R. B. Tompkin, Scott, Bernard, Sveum, & Gombas, 1999)

^e (U. S. Department of Health and Human Services, 2005e)

Table J.4. Microbiological and Chemical Limits for Shelf-stable Beverages

Notes: Examples of these products are carbonated beverages, commercial sterility/ultra-high temperature/aseptic beverages, and some juice drinks. Microbiological control is accomplished by one or more of the following: low pH, pasteurization (UHT), and carbonation

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Patulin (in apple juice) ^f		50 µg/kg	The presence of patulin in apple juice above the limit should lead to rejection of the product. Investigate and implement corrective action.	Different countries may have different regulatory requirements. A lower limit of 10 µg/kg should be considered when apple juice products are intended for infants.
Microbiological criteria (NA) ^g	NA	NA	NA	No microbiological testing recommended for this product

There are no microbiological limits set for shelf-stable beverages as these products are considered commercially-sterile (*i.e.*, stable at room temperature under normal handling and storage conditions). Suppliers should be verifying the raw materials used in the formulation of these products before the process providing commercial sterility. Shelf-stable liquid products should be examined by means other than routine microbiological testing; if inspection finds bulging containers, pH changes, odors, etc., then further investigation is warranted.

^f (U. S. Department of Health and Human Services, 2005e)

^g (Elliott & Kataoka, 2013)

Table J.5. Microbiological Limits for Dairy- Butter, margarine

Notes: Either formulated with sufficient salt or lactic acid (for unsalted butter) to prevent growth or refrigerated; products containing added seasoning/herbs/spices may have additional requirements

Criteria & EMP Target Microorganism ^h	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms ⁱ	10/g		Investigate, implement corrective action	
<i>Enterobacteriaceae</i> ⁱ		10/g	Investigate, implement corrective action	Due to certain strains being able to survive milk pasteurization, enterococci are not widely adopted as indicators of process hygiene in the dairy industry ^j
Mold/Yeast		20/g	Investigate, implement corrective action	
<i>Listeria</i> spp. (EMP) ^k	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>S. aureus</i>		100/g	Investigate, implement corrective action. if $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	

^h (National Academies of Science, 2003)

ⁱ Coliforms or *Enterobacteriaceae* are acceptable for routine testing. (Kornacki, Gurtler, & Stawick, 2013)

^j (Craven, Eyles, & Davey, 2003)

^k (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

Table J.6. Microbiological Limits for Dairy-Cheese, Hard

Notes: Ex. Parmesan, Cheddar, $a_w < 0.95$ and $pH < 5.6$. All cheeses are made with pasteurized milk.

Criteria & EMP Target Microorganism ^l	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms	<100/g		Investigate, implement corrective action	
<i>E. coli</i> (generic)		<10/g	Investigate, implement corrective action; if >100/g, reject lot	
<i>Listeria</i> spp. (EMP) ^m	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Listeria</i> spp. (product)		Negative in 25 g	Reject lot	May be in-process vat sample due to the aging process for natural cheese
<i>Salmonella</i>		Negative in 375 g	Reject lot	May be in-process vat sample due to the aging process for natural cheese.
<i>S. aureus</i>		100/g	Investigate, implement corrective action. If $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	Test for toxin if slow acid development; if positive for toxin, destroy product

^l (U.S. Department of Health and Human Services, 2010)

^m (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

Table J.7. Microbiological Limits for Dairy-Cheese, Soft, Semi-Soft, Surface-Ripened

Notes: Ex. Brie, Fresh Mozzarella, $a_w > 0.95$ and $pH > 5.4$. All cheeses are made with pasteurized milk.

Criteria & EMP Target Microorganism ⁿ	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms	<100/g		Investigate, implement corrective action	
<i>E. coli</i> (generic)		<10/g	Investigate, implement corrective action; if >100/g, reject lot	
<i>Listeria</i> spp. (EMP) ^o	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Listeria</i> spp. (product)		Negative in 125 g	Reject lot	125-g analytical unit composed of 5 x 25-g samples
<i>Salmonella</i>		Negative in 375 g	Reject lot	
<i>S. aureus</i>		100/g	Investigate, implement corrective action. if $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	Test for toxin if slow acid development; if positive for toxin, destroy product.

ⁿ (Australia New Zealand Food Authority, 2014; U.S. Department of Health and Human Services, 2010)

^o (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

Table J.8. Microbiological Limits for Dairy-Cultured, pH <4.8

Notes: Ex. Sour cream, yogurt, buttermilk; active pH control required

Criteria & EMP Target Microorganism ^p	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms	10/g		Investigate, implement corrective action; if >10/g and used for RTE foods, reject lot	
<i>Listeria</i> spp. (EMP) ^q	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
Mold/Yeast		10/g	Investigate, implement corrective action	The presence of mold and yeast may be influenced by added ingredients such as fruit purees and other inclusions. This needs to be considered in assessing mold and yeast populations as well as whether any detectable molds and yeast would grow in the product during its shelf life.
<i>S. aureus</i>		10 ³ /g	Investigate, implement corrective action. if ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	No testing recommended unless fermentation does not reach pH <4.8 in <8 h

^p (U. S. Department of Health and Human Services, 2011j; U.S. Department of Health and Human Services, 2010)

^q (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

Table J.9. Microbiological Limits for Dairy-Cultured, pH >4.8 and < 5.4

Notes: Ex. Cottage cheese, cream cheese, moisture >50%; active pH control required

Criteria & EMP Target Microorganism ^r	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms	10/g		Investigate, implement corrective action. If >10 /g and regulated under PMO, reject lot due to regulatory limit	
<i>Listeria</i> spp. (EMP) ^s	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
Mold/Yeast		10/g	Investigate, implement corrective action	The presence of mold and yeast may be influenced by added ingredients such as fruit purees and other inclusions. This needs to be considered in assessing mold and yeast populations as well as whether any detectable molds and yeast would grow in the product during its shelf life.
<i>S. aureus</i>		10 ³ /g	Investigate, implement corrective action. If ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	No testing recommended unless fermentation does not reach pH <4.8 in <8 h

^r (Bradley et al., 2013; U. S. Department of Health and Human Services, 2011j)

^s (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

Table J.10. Microbiological Limits for Dairy-Dried Products

Notes: Ex. NFDM, whey powder. This does not cover dried dairy ingredients used in infant formula; those requirements are more stringent.

Criteria & EMP Target Microorganism ^t	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC/SPC	1X10 ⁴ /g		Investigate, implement corrective action	
<i>B. cereus</i>		100/g	Investigate, implement corrective action. if $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	
Coliforms ^u	10/g		Investigate, implement corrective action	
<i>Enterobacteriaceae</i>		10/g	Investigate, implement corrective action	
<i>Listeria</i> spp. (EMP) ^v		Negative for Zone 2 or 3	Investigate, consider Zone 1 and finished product testing, implement corrective actions	
<i>S. aureus</i>		100/g	Investigate, implement corrective action. if $\geq 10^2$ /g, reject lot due to potential for enterotoxin production	
<i>Salmonella</i> (EMP) ^w	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Salmonella</i>	Negative. in 375 g		Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	As an alternative sampling option to collecting and compositing 15-25 g samples (total 375 g), an auto sampler can be used to collect small amounts of samples throughout a production run for a total of 375g ^x

^t (International Commission for the Microbiological Specifications for Foods (ICMSF), 2011; U. S. Department of Health and Human Services, 2011j; U.S. Department of Health and Human Services, 2010)

^u Coliforms or *Enterobacteriaceae* are acceptable for routine testing. (Kornacki et al., 2013)

^v (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^w (Chen et al., 2009; Grocery Manufacturers Association, 2009)

^x Recommend 1500 g per lot when high volumes of product are produced per lot (or production day).

Table J.11. Microbiological Limits for Dairy-Frozen Desserts

Notes:

Criteria & EMP Target Microorganism ^y	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		See comments	Investigate, implement corrective action.	Populations may be influenced by ingredients; product specific APC limits need to be established based on baseline testing
Coliforms	100/g		Investigate, implement corrective action.	Populations may be influenced by ingredients
<i>Salmonella</i> (product)		Negative in 250 g	Reject lot; Investigate, implement corrective action.	250 g analytical unit composed of 10 x 25-g samples
<i>Listeria</i> spp. (EMP)	Negative for Zone 2 or 3		Investigate, consider Zone 1 and product testing, implement corrective action	

^y (International Commission for the Microbiological Specifications for Foods (ICMSF), 2011)

Table J.12. Microbiological Limits for Dairy-Milk and Milk Products (Fluid)

Notes: Ex. Fluid milk, cream; Pasteurized, refrigerated; alkaline phosphatase negative (less than 2.0 micrograms phenol equivalent per g)

Criteria & EMP Target Microorganism ^z	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC/SPC	2.0 x 10 ⁴ /g		Investigate, implement corrective action	
Coliforms ^{aa}	10/g		Investigate, implement corrective action. if >10 /g and regulated under PMO, reject lot due to regulatory limit	
<i>Enterobacteriaceae</i>		10/g	Investigate, implement corrective actions	
<i>Listeria</i> spp. (EMP) ^{bb}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and product testing, implement corrective action	
<i>S. aureus</i>		100/g	Investigate, implement corrective action. if ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	No testing recommended unless temperature abuse is suspected

^z (Bradley et al., 2013; U. S. Department of Health and Human Services, 2011j)

^{aa} Coliforms or *Enterobacteriaceae* are acceptable for routine testing. (Kornacki et al., 2013)

^{bb} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

Table J.13. Microbiological Limits for Dairy- Processed Cheese

Notes: Manufactured by heating cheese with water, emulsifier and other ingredients to kill vegetative pathogens; molten cheese may then be hot-filled into loaves or blocks and chilled or cut into individual slices for use; these cheeses are intended to be stored refrigerated. Shelf-stable hot-filled cheese spreads or cheese sauces must be formulated for safety to inhibit *Clostridium botulinum*.

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC/SPC	10 ³ /g		Investigate and implement corrective action.	Test for products that are not hot-filled directly into final container. APC limit may be adjusted subject to control chart associated with Statistical Process Control. Populations are predominantly sporeformers or heat-stable spoilage microorganisms.
Coliforms		<10/g	Investigate, implement corrective action	Test for products that are not hot-filled directly into final container
<i>E. coli</i> (generic)		<10/g	Investigate and implement corrective action	Test for products that are not hot-filled directly into final container
<i>Listeria</i> spp. (EMP) ^{cc}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>S. aureus</i>		<100/g	Investigate, implement corrective action. if $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	Test for products that are not hot-filled directly into final container
<i>Salmonella</i> (EMP) ^{dd}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	Test EMP in areas where products are not hot-filled directly into final container
<i>Salmonella</i> (product)		Negative in 125 g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Test for products that are not hot-filled directly into final container

^{cc} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^{dd} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

Table J.14. Microbiological Limits for Egg Products-Pasteurized, Processed

Notes:

Criteria & EMP Target Microorganism ^{ee}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC	10 ⁴ /g		Investigate, implement corrective action	
<i>B. cereus</i>		10 ³ /g	Investigate, implement corrective action. if $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	
Coliforms	10/g		Investigate, implement corrective action	
<i>Enterobacteriaceae</i>		100/g	Investigate, implement corrective action	
<i>Listeria</i> spp. (EMP) ^{ff}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Listeria</i> spp. (product)		Negative in 100 g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	
<i>S. aureus</i>	10/g		Investigate, implement corrective action. if $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	
<i>Salmonella</i> (EMP) ^{gg}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Salmonella</i> (product)	Negative in 100 g		Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	

^{ee} (Australia New Zealand Food Authority, 2014; European Commission, 2005; International Commission for the Microbiological Specifications for Foods (ICMSF), 2011; U. S. Department of Agriculture Food Safety Inspection Service, 2014c)

^{ff} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^{gg} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

Table J.15. Microbiological Limits for Egg Products-Shell Eggs, Raw

Notes:

Criteria & EMP Target Microorganism ^{hh}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms ⁱⁱ		10 ³ /g	Investigate, implement corrective action	
<i>E. coli</i> (generic) ⁱⁱ		10 ³ /g	Investigate, implement corrective action	
<i>Enterobacteriaceae</i>		10 ⁴ /g	Investigate, implement corrective action	
<i>Salmonella</i> (EMP)	Negative for Zone 2 or 3		If environment is positive for <i>Salmonella</i> Enteritidis, conduct egg sampling per FDA Final Rule 2009	<i>Salmonella</i> Enteritidis environmental testing

^{hh} (U. S. Department of Health and Human Services, 2009)

ⁱⁱ Coliforms, *E. coli* or *Enterobacteriaceae* are acceptable for routine testing. (Kornacki et al., 2013)

Table J.16. Microbiological Limits for Grain-based products-RTE, baked items, refrigerated or TCS

Notes: Examples: focaccia, custard or cream-filled pastries, pies. Qualifying information: APC counts may be high due to containing ingredients prepared with starter culture

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
<i>B. cereus</i>		10 ³ /g	Investigate, implement corrective action. if ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	
Coliforms	100/g		Investigate process and implement corrective action	
<i>E. coli</i> (generic)		10/g	Investigate process and implement corrective action	
<i>Listeria</i> spp. (EMP) ^{jj}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	Periodic finished product testing (test/hold) for products that support growth of <i>L. monocytogenes</i>
<i>S. aureus</i>		10 ³ /g	Investigate, implement corrective action. if ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	
<i>Salmonella</i> ^{kk}		negative in 375 g	Reject lot. Investigate and implement corrective action	

^{jj} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^{kk} (Andrews, Jacobson, & Hammack, 2014)

Table J.17. Grain-based products-RTE, baked items, shelf stable, non-TCS

Notes: Examples: bread. If raw ingredients added after baking step, additional risks should be considered. ICMSF 8 does not recommend routine testing.

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Criteria & EMP Target Microorganism ^{II}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		10 ⁴ /g	Investigate process and implement corrective action	Populations predominantly sporeformers
Coliforms ^{mm}		100/g	Investigate process and implement corrective action	
<i>Enterobacteriaceae</i>		100/g	Investigate process and implement corrective action	
<i>Salmonella</i> (product)		Negative in 125 g	Reject lot; investigate process and implement corrective action	<i>Salmonella</i> testing is appropriate if raw ingredients (e.g., nuts, raw flour) are added post-baking

^{II} (International Commission for the Microbiological Specifications for Foods (ICMSF), 2011)

^{mm} Coliforms or *Enterobacteriaceae* are acceptable for routine testing. (Kornacki et al., 2013)

Table J.18. Microbiological Limits for Grain Based Products, RTE, cereals

Notes: Examples: breakfast cereals. Grain based product undergoes a lethality step; mycotoxin surveillance testing completed on incoming grains as pre-requisite program with limits based on individual country's regulations

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		5x10 ⁴ /g	Investigate process and implement corrective action	
Coliforms ⁿⁿ		100/g	Investigate process and implement corrective action	
<i>Enterobacteriaceae</i>		100/g	Investigate process and implement corrective action	
<i>Salmonella</i> (EMP) ^{oo}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	If Zone 1 positive, reject lot
<i>Salmonella</i>		Negative in 375 g	Reject lot; investigate process and implement corrective action	375-g analytical composed of 15 x 25-g samples

ⁿⁿ Coliforms or *Enterobacteriaceae* are acceptable for routine testing. (Kornacki et al., 2013)

^{oo} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

Table J.19. Microbiological Limits for Grain Based Products – RTE, cold-pressed bars

Notes; Ex. granola bars; Qualifying information: ingredients will undergo mycotoxin surveillance testing as appropriate; shelf-stable, $a_w < 0.85$

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		$5 \times 10^4/\text{g}$	Investigate process and implement corrective action	
Coliforms ^{pp}		100/g	Investigate process and implement corrective action	
<i>Enterobacteriaceae</i>		100/g	Investigate process and implement corrective action	
<i>Salmonella</i> (EMP) ^{qq}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Salmonella</i>		Negative in 375 g	Reject lot; investigate process and implement corrective action	375-g analytical unit composed of 15 x 25-g samples

^{pp} Either coliform or *Enterobacteriaceae* testing is appropriate. (Kornacki et al., 2013)

^{qq} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

Table J.20. Microbiological Limits for Grain Based Products non-RTE, dry, flour based mixes

Notes: Flour can contain pathogens occasionally and should be subjected to a lethality step prior to consumption^{rr}

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Not Applicable (NA)	NA	NA	NA	No microbiological testing recommended for this product

^{rr} (Sperber & North American Millers' Association Microbiology Working Group, 2007)

Table J.21. Microbiological Limits for Grain Based Products – Non-RTE, pasta, dried or refrigerated

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		10^6 /g		High APC counts for unheated products made with raw flour are not unexpected
<i>E. coli</i> (generic)		100/g	Investigate process and implement corrective action	Periodic testing recommended for refrigerated pasta
<i>Listeria</i> spp. (EMP) ^{ss}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	Recommended in facilities manufacturing refrigerated pasta
<i>S. aureus</i>		10^3 /g	Investigate, implement corrective action. if $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	
<i>Salmonella</i> (EMP) ^{tt}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	Recommended in facilities manufacturing dried pasta
<i>Salmonella</i>		Negative in 125 g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	

^{ss} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^{tt} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

Table J.22. Microbiological and Chemical Limits for Meals and Entrees—Non-RTE, ready-to-cook meals, includes raw ingredients

Notes: This category includes a wide variety of products and processes that will influence appropriate testing choices. Depending on their ingredients, foods that are likely to be prepared by microwave heating or those that are not labeled with validated cooking instructions may require more stringent testing (e.g. Raw poultry, beef or flour ingredients may necessitate routine, instead of non-routine, testing for *Salmonella* or *E. coli* (O157:H7 or other STEC)).

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
<i>B. cereus</i>		10 ³ /g	Investigate, implement corrective action. if ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	Test if food contains components that are high risk for <i>B. cereus</i> , such as cooked rice
Coliforms ^{uu}		10 ³ /g	Investigate, implement corrective action	
<i>E. coli</i> (O157:H7 or other STEC)		Negative in 25g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Testing if raw, non-intact beef component is present. Validated cooking instructions should be present on package.
<i>Enterobacteriaceae</i>		10 ⁴ /g	Investigate, implement corrective action	
<i>S. aureus</i>		10 ³ /g	Investigate, implement corrective action. if ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	
<i>Salmonella</i> (EMP) ^{vv}		Negative for Zone 2 or 3	Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Salmonella</i> (product)		Negative in 25 g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	
Histamine ^{ww}		50 ppm in 250 g	Reject lot; Investigate, implement corrective action	Histamine testing appropriate only when scombroid species are present; The FDA Hazards and Controls Guide lists a defect action level of 50ppm

Table J.23. Microbiological and Chemical Limits for Meals and Entrees--RTE, deli salads, sandwiches, heat-eat meals, sushi

^{uu} Coliforms or *Enterobacteriaceae* are acceptable for routine testing. (Kornacki et al., 2013)

^{vv} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

^{ww} (U. S. Department of Health and Human Services, 2011a)

Notes: Survey data indicates a wide range in microbial populations depending on specific food. Items may include ingredients that are raw.

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
<i>B. cereus</i>		10 ³ /g	Investigate, implement corrective action. If $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	Test if food contains components that are high risk for <i>B. cereus</i> , such as cooked rice
Coliforms ^{xx}		10 ⁴ /g	Investigate, implement corrective action	
<i>E. coli</i> (generic)	100/g		Investigate, implement corrective action	
<i>Enterobacteriaceae</i>		10 ⁴ /g	Investigate, implement corrective action	
<i>Listeria</i> spp. (EMP) ^{yy}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>S. aureus</i>		10 ³ /g	Investigate, implement corrective action. If $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	
<i>Salmonella</i> (EMP) ^{zz}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Salmonella</i> (product)		Negative in 375 g	Reject lot; Investigate, implement corrective action	375-g analytical unit composed of 15 x 25-g samples
Histamine ^{aaa}		50 ppm in 250 g	Reject lot; Investigate, implement corrective action	Histamine testing appropriate only when scombroid species are present; The FDA Hazards and Controls Guide lists a defect action level of 50ppm.

^{xx} Coliforms, *E. coli*, or *Enterobacteriaceae* are acceptable for routine testing. (Kornacki et al., 2013)

^{yy} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^{zz} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

^{aaa} (U. S. Department of Health and Human Services, 2011a)

Table J.24. Microbiological Limits for Meals and Entrees—RTE, sous-vide, cook and chill

Notes: These products receive a lethality treatment; presence of vegetative microbes represents post-process contamination. If not using validated *sous-vide* process for a 6-log reduction of non-proteolytic *Clostridium botulinum*, testing of vegetative microorganisms is warranted.

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		10 ³ /g	Investigate, implement corrective action	
<i>B. cereus</i>		10 ³ /g	Investigate, implement corrective action. If ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	Test if food contains components that are high risk for <i>B. cereus</i> , such as cooked rice
Coliforms ^{bbb}		100/g	Investigate, implement corrective action	
<i>E. coli</i> (generic)	10/g		Investigate, implement corrective action; reject lot or divert for recooking if appropriate	
<i>Enterobacteriaceae</i>		100/g	Investigate, implement corrective action	
<i>Clostridium perfringens</i> ^{ccc}		500/g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	If greater than 500 CFU/g, indicator of loss of process control or potential deviation from USDA cooling requirements

^{bbb} Coliforms or *Enterobacteriaceae* are acceptable for routine testing. (Kornacki et al., 2013)

^{ccc} (U. S. Department of Agriculture Food Safety Inspection Service, 1999; U.S. Department of Agriculture Food Safety Inspection Service, 1999)

Table J.25. Microbiological Limits for Meat—Beef and Pork, Non-RTE, raw (intact, non-intact)

Criteria & EMP Target Microorganism ^{ddd}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		10 ⁵ /g	Investigate, implement corrective action	
Coliforms		10 ³ /g	Investigate, implement corrective action	
<i>E.coli</i> (generic)	500/g		Investigate, implement corrective action	
<i>E. coli</i> (O157:H7 and/or other STEC) ^{eee}		Negative in 325 g	Divert for lethality step, if appropriate, or reject. Investigate and implement corrective action	Test non-intact beef (ground, tenderized, enhanced) product and intact product intended to become non-intact
<i>Enterobacteriaceae</i>		10 ⁴ /g	Investigate, implement corrective action	
<i>Salmonella</i> (product) ^{fff}		See sampling for USDA-FSIS Performance Standards	Investigate, implement corrective action	Not used an accept/reject criterion; used for process control

^{ddd} (National Advisory Committee on Microbiological Criteria for Foods, 2013; U.S. Department of Agriculture Agricultural Marketing Service (AMS), 2015)

^{eee} (U. S. Department of Agriculture, 2011)

^{fff} (U. S. Department of Agriculture Food Safety Inspection Service, 2014e)

Table J.26. Microbiological Limits for Meat—Poultry, Non-RTE, raw (intact, non-intact)

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		10 ⁶ /g	Investigate, implement corrective action	
Coliforms ^{ggg}		10 ³ /g	Investigate, implement corrective action	
<i>E. coli</i> (generic)		10 ³ /g	Investigate, implement corrective action	
<i>Enterobacteriaceae</i>		10 ⁴ /g	Investigate, implement corrective action	
<i>Salmonella</i> (product) ^{hhh}		See sampling for USDA-FSIS Performance Standards	Investigate, implement corrective action	Not used as an accept/reject criterion; used for process control

^{ggg} Coliforms, generic *E. coli*, *Enterobacteriaceae* are acceptable for testing. (Kornacki et al., 2013)

^{hhh} (U. S. Department of Agriculture Food Safety Inspection Service, 2014e); *Campylobacter* is also proposed in the USDA-FSIS Performance Standards.

Table J.27. Microbiological Limits for Meat— RTE cooked, perishable

Notes: Ex. Includes beef, pork and poultry products, deli meats, frankfurters

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		10 ³ /g	Investigate, implement corrective action	
Coliforms ⁱⁱⁱ		100/g	Investigate, implement corrective action	
<i>E. coli</i> (generic)	10/g		Investigate, implement corrective action	
<i>Enterobacteriaceae</i>		100/g	Investigate, implement corrective action	
<i>Listeria</i> spp. (EMP) ⁱⁱⁱ	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Listeria</i> spp. (product) ^{kkk}		Negative in 125 g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	125-g analytical unit composed of 5 x 25-g samples
<i>Salmonella</i>		Negative in 125 g	Investigate, implement corrective action	
<i>Clostridium perfringens</i> ^{lll}		500/g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	If greater than 500 CFU/g, indicator of loss of process control or potential deviation from USDA cooling requirements

ⁱⁱⁱ Either coliforms or *Enterobacteriaceae* are acceptable for testing. (Kornacki et al., 2013)

^{jjj} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^{kkk} (Australia New Zealand Food Authority, 2014; Canada Food Inspection Agency, 2012; U. S. Department of Agriculture Food Safety Inspection Service, 2014a; U.S. Department of Health and Human Services, 2008)

^{lll} (U. S. Department of Agriculture Food Safety Inspection Service, 1999)

Table J.28. Microbiological Limits for Meat— RTE fermented, dried

Notes: Includes beef, pork and poultry products, Jerky, dried fermented sausage, dried acidified meat sticks; e.g. $a_w < 0.85$ or $pH < 5.3$ and $a_w < 0.92$ for vacuum-packaged meat sticks. Products should be manufactured with a validated kill step for *E. coli* O157:H7 (beef) or *Salmonella* (pork, poultry) as appropriate for the given meat matrix.

Criteria & EMP Target Microorganism ^{mmm}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms ⁿⁿⁿ		100/g	Investigate, implement corrective action	
<i>E. coli</i> (generic)	10/g		Investigate, implement corrective action	
<i>E. coli</i> (O157:H7 or other STEC)		Negative in 125 g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	125-g analytical unit composed of 5 x 25-g samples
<i>Enterobacteriaceae</i>		100/g	Investigate, implement corrective action	
<i>S. aureus</i>		10^3 /g	Investigate, implement corrective action. If $\geq 10^4$ /g, reject lot due to potential for enterotoxin production	Testing of in-process (uncooked) product may be appropriate if fermentation does not meet USDA-accepted guidelines for temperature-hours ^{ooo}
<i>Salmonella</i>		Negative in 125 g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	125-g analytical unit composed of 5 x 25-g samples

^{mmm} (U. S. Department of Agriculture Food Safety Inspection Service, 2014a)

ⁿⁿⁿ Either coliforms or *Enterobacteriaceae* are acceptable for testing. (Kornacki et al., 2013)

^{ooo} (American Meat Institute Foundation, 1997)

Table J.29. Microbiological and Chemical Limits for Nuts and Nut Butters – RTE, Not processed for lethality

Notes: Ex.: include peanuts, tree nuts (e.g., walnuts, almonds, pecans, pistachios, macadamia)

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
<i>E. coli</i> (generic)		0.36 MPN/g	Investigate, implement corrective action	If 2 of 10 samples are ≥ 0.36 MPN/g, the product is violative
<i>Salmonella</i> EMP ^{ppp}	Negative for Zone 2 and 3 surfaces		Investigate root cause of positive results, conduct vector sampling and repeat sampling until confirm negative results	
<i>Salmonella</i> (product) ^{qqq}	Negative in 2 X 375-g samples		Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Two 375-g analytical units derived from 30 x 25-g samples
Toxins – Aflatoxin B1 ^{rrr}	20 ppb		Investigate, implement corrective action	This is routine testing for peanuts, pistachios & Brazil nuts, but non- routine for other nut types or situations

^{ppp} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

^{qqq} (Andrews & Hammack, 2003; U.S. Food and Drug Administration, 2015)

^{rrr} (U. S. Department of Health and Human Services, 2005a, 2005c, 2005g, 2005i)

Table J.30. Microbiological and Chemical Limits for Nuts and Nut Butters – RTE, processed for lethality

Notes: Ex.: peanut butter, almond butter, roasted nuts

Criteria & EMP Target Microorganism	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
<i>Salmonella</i> EMP ^{sss}	Negative for Zone 2 and 3 surfaces		Investigate root cause of positive results, conduct vector sampling and repeat sampling until confirm negative results	
<i>Salmonella</i> (product) ^{ttt}	Negative in 2 x 375-g samples		Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Two 375 g analytical units derived from 30 x 25-g samples
Toxins – Aflatoxin B1 ^{uuu}	20 ppb		Reject lot; investigate, implement corrective action	This is routine testing for peanuts, pistachios & Brazil nuts, but non- routine for other nut types or situations

^{sss} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

^{ttt} (Andrews & Hammack, 2003; U.S. Food and Drug Administration, 2015)

^{uuu} (U. S. Department of Health and Human Services, 2005a, 2005c, 2005g, 2005i)

Table J.31. Microbiological Limits for Produce—Fruits and Vegetables, Cut, Frozen or Refrigerated

Notes: minimally processed

Criteria & EMP Target Microorganism ^{vv}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
<i>E. coli</i> (generic)	100/g		Consider improvements in production hygiene and selection of raw materials	
<i>E. coli</i> (O157:H7 or other STEC)		Negative	Reject lot; investigate, implement corrective action	Depending on commodity, geographical location and use of GAPs
<i>Listeria</i> spp. (EMP) ^{www}	Negative zone 2 or 3		Consider zone 1 and finished product testing; implement corrective action	
<i>Listeria</i> spp. (finished product)		Negative	Reject lot; investigate, implement corrective action	Depending on commodity, geographical location and use of GAPs; Sample size may vary; e.g. 25 g
<i>Salmonella</i> (product)		Negative	Reject lot; investigate, implement corrective action	Depending on commodity, geographical location and use of GAPs; Sample size may vary; e.g. 25 g

^{vv} (European Commission, 2005; Health Canada, 2008)

^{www} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

Table J.32. Microbiological Limits for Produce—Fruits and Vegetables, Whole

Notes: Ex. products customarily consumed without cooking, tomatoes, cantaloupes, avocado, mangoes, apples, celery, carrots, berries, whole lettuce

Criteria & EMP Target Microorganism ^{xxx}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
<i>E. coli</i> (O157:H7 or other STEC)		Negative	Reject lot; investigate, implement corrective action	Depending on commodity, geographical location and use of GAPs; Sample size may vary; e.g. 25 g
<i>Listeria</i> spp. (EMP) ^{yyy}	Negative zone 2 or 3		Consider zone 1 and product testing; implement corrective action	
<i>Salmonella</i> (EMP) ^{zzz}	Negative zone 2 or 3		Consider zone 1 and finished product testing; implement corrective action	
<i>Salmonella</i> (product)		Negative	Reject lot; investigate, implement corrective action	Depending on commodity, geographical location and use of GAPs; Sample size may vary; e.g. 25 g

^{xxx} (European Commission, 2005; Health Canada, 2008; International Commission for the Microbiological Specifications for Foods (ICMSF), 2011)

^{yyy} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^{zzz} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

Table J.33. Microbiological Limits for Produce—Produce, Mushrooms

Notes: fresh, whole, sliced, not canned or marinated

Criteria & EMP Target Microorganism ^{aaaa}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
<i>E. coli</i> (generic)		10 ³ /g	Investigate, implement corrective action	
<i>E. coli</i> (O157:H7 or other STEC)		Negative for 10 individual 25-g samples	Reject or divert for further processing if appropriate; investigate, implement corrective action	No composite testing
<i>Enterobacteriaceae</i>		10 ⁴ /g	Investigate, implement corrective action	
<i>Listeria</i> spp. (EMP) ^{bbbb}	Negative zone 2 or 3		Consider zone 1 and finished product testing; implement corrective action	
<i>S. aureus</i>		10 ³ /g	Investigate, implement corrective action. If ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	
<i>Salmonella</i> (EMP) ^{cccc}		Negative zone 2 or 3	Consider zone 1 and finished product testing; implement corrective action	
<i>Salmonella</i> (product)		Negative for 2 x 375-g composite samples	Reject or divert for further processing if appropriate; investigate, implement corrective action	Two 375-g analytical units composed of 30 x 25-g samples

^{aaaa} (European Commission, 2005; Health Canada, 2008)

^{bbbb} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^{cccc} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

Table J.34. Microbiological Limits for Produce—Packaged Salads and Leafy Greens

Criteria & EMP Target Microorganism ^{dddd}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
<i>E. coli</i> (generic)	100/g		Consider improvements in production hygiene and selection of raw materials	
<i>E. coli</i> (O157:H7 or other STEC)		Negative	Reject lot; investigate, implement corrective action	Sample size may vary e.g. 25 g
<i>Listeria</i> spp. (EMP) ^{eeee}	Negative zone 2 or 3		Consider zone 1 and finished product testing; implement corrective action	
<i>Listeria</i> spp. (finished product)		Negative	Reject lot; investigate, implement corrective action	Sample size may vary; e.g. 25 g
<i>Salmonella</i> (finished product)		Negative	Reject lot; investigate, implement corrective action	Sample size may vary; e.g. 25 g

^{dddd} (International Commission for the Microbiological Specifications for Foods (ICMSF), 2011)

^{eeee} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

Table J.35. Microbiological Limits for Produce— vegetable sprouts

Criteria & EMP Target Microorganism ^{ffff}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
<i>E. coli</i> (generic)	10 ³ /g		Investigate, implement corrective action	
<i>E. coli</i> (O157:H7 or other STEC) product		Negative in 2 50-gm analytical units	Reject lot; investigate, implement corrective action	
<i>E. coli</i> (O157:H7 or other STEC) (spent irrigation water) ^{gggg}	Negative in 2 x 100-g analytical units		Reject lot; investigate, implement corrective action	
<i>Listeria</i> spp. (EMP) ^{hhhh}	Negative zone 2 or 3		Consider zone 1 and finished product testing; implement corrective action	
<i>Listeria</i> spp. (product) ⁱⁱⁱⁱ		Negative in 2 x 250-gm analytical units	Reject lot; investigate, implement corrective action	Each 250-g analytical unit composed of 5 x 50-g samples
<i>Salmonella</i> (product)		Negative in 30 x 50-gm analytical units	Reject lot; investigate, implement corrective action	
<i>Salmonella</i> (spent irrigation water) ^{cccc}	Negative in 2 x 375-gl analytical units		Reject lot; investigate, implement corrective action	

^{ffff} (Health Canada, 2006; U.S. Food and Drug Administration, 1999)

^{gggg} Sampling spent irrigation water; collect the total of 1-liter of spent irrigation water from various trays of growing sprouts. Two x 375-ml subsamples are used for *Salmonella* detection and 2 x 100-ml subsamples are used for detection of *E. coli* O157:H7.

^{hhhh} (R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

ⁱⁱⁱⁱ (Australia New Zealand Food Authority, 2014; Hitchens & Jinneman, 2013)

Table J.36. Microbiological and Chemical Limits for Seafood, Raw

Notes: Ex.: fish, shrimp, crabs. Verification testing for histamine in scombroid species only. The FDA Hazards and Controls Guide lists a defect action level of 50ppm. See Table J.37, J.38 and J. 39 if raw seafood may be used for applications without full cook such as for sushi or ceviche, additional testing may be appropriate.

Criteria & EMP Target Microorganism ^{jjjj}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
Coliforms		10 ³ /g	Investigate, implement corrective action	Routine testing is not recommended for a raw product that is not intended to be consumed raw.
<i>Salmonella</i>		Negative	Divert for reprocessing if appropriate or reject; investigate, implement corrective action	Consider sampling in facilities or countries where growing, harvesting or handling conditions result in significant prevalence of <i>Salmonella</i> in the product. Treat raw seafood as RTE food if it may be used for applications without full cook such as sushi or ceviche (See Table J.37 and J.38); Sample size may vary; e.g. 25 g. ^{kkkk}
Histamine		50 ppm	Reject lot; investigate, implement corrective action	Scombroid species only; see sampling details ^{llll}

^{jjjj} (Ahmed & Institute of Medicine (U.S.). Committee on Evaluation of the Safety of Fishery Products., 1991; Australia New Zealand Food Authority, 2001, 2014; International Commission for the Microbiological Specifications for Foods (ICMSF), 2011; U. S. Department of Health and Human Services, 2011a)

^{kkkk} Sample size may vary depending on intended usage, e.g. for sushi or ceviche without full cook (Andrews & Hammack, 2003)

^{llll} (U. S. Department of Health and Human Services, 2011a)

Table J.37. Microbiological and Chemical Limits for Seafood--RTE, Fish, Cold Smoked

Notes: Verification testing for histamine in scombroid species only. The FDA Hazards and Controls Guide lists a defect action level of 50ppm

Criteria & EMP Target Microorganism ^{mmmm}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC (EMP)	10/cm ²		Investigate, implement corrective action	Routine testing for general status of cleaning and disinfection can be done by swab sampling and determining the aerobic plate count. Product contact surfaces should contain less than 10 CFU/cm ²
<i>Listeria</i> spp. (EMP) ⁿⁿⁿⁿ	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Listeria monocytogenes</i> (product)		Negative in 5 individual 25-g analytical units	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	
<i>Salmonella</i>		Negative	Divert for reprocessing if appropriate or reject; investigate, implement corrective action	Sample size may vary; e.g. 25 g
Histamine		50 ppm	Reject lot; investigate, implement corrective action	Scombroid species only; see sampling details ^{oooo}

^{mmmm} (International Commission for the Microbiological Specifications for Foods (ICMSF), 2011; U. S. Department of Health and Human Services, 2011a)

ⁿⁿⁿⁿ (Scott et al., 2005; R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^{oooo} (U. S. Department of Health and Human Services, 2011a)

Table J.38. Microbiological and Chemical Limits for Seafood-- RTE, cooked or hot smoked

Notes: Ex.: includes cooked and hot smoked products, cooked crabmeat, lobster meat, shrimp, crayfish, surimi, seafood salads, hot-smoked fish.

Histamine testing recommended for scombroid species only with Defect Action Level 50ppm

Criteria & EMP Target Microorganism ^{pppp}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		10 ⁵ /g	Investigate Source of Post Cook Handling and Storage Contamination	
Coliforms ^{qqqq}		100/g	Investigate; implement corrective action	For cooked seafood other than shrimp and crabmeat
Coliforms ⁿⁿⁿⁿ		5x10 ³ /g	Investigate; implement corrective action	For cooked crabmeat- fresh (Handled after final cook)
Coliforms ⁿⁿⁿⁿ		10 ³ /g	Investigate; implement corrective action	For cooked shrimp (Handled after final cook)
<i>Listeria</i> spp. (EMP) ^{rrrr}	Negative for Zone 2 or 3		Investigate, consider Zone 1 and finished product testing, implement corrective action	
<i>Listeria monocytogenes</i> (product)		Negative in 5 individual 25-g analytical units	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	
<i>S. aureus</i>	10 ³ /g		Investigate, implement corrective action. If ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	Routine testing is recommended especially for products that are handled after the final cook (kill) step
<i>Salmonella</i>		Negative	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Validated cooking will destroy <i>Salmonella</i> ; if HACCP plans are in place to control cooking and recontamination after cooking, there should be no need for routine testing. Testing could be done as a verification or investigation. Sample size may vary; e.g. 25 g
Histamine		50 ppm	Reject lot; investigate, implement corrective action	Scombroid species only; see sampling details ^{ssss}

^{pppp} (Australia New Zealand Food Authority, 2014; International Commission for the Microbiological Specifications for Foods (ICMSF), 1986; U. S. Department of Health and Human Services, 2011a)

^{qqqq} (Buchanan, 1991)

^{rrrr} (Scott et al., 2005; R.B. Tompkin, 2002; R. B. Tompkin et al., 1999)

^{ssss} (U. S. Department of Health and Human Services, 2011a)

Table J.39. Microbiological Limits for Seafood--RTE, Raw Molluscan Shellfish

Notes: Ex.: molluscan shellfish such as oysters, clams, mussels, scallops intended to be eaten without a full cook. Shellfish must be from approved harvest waters from countries with MOU (*i.e.*, New Zealand, Mexico, Korea, Canada) with the United States. Shellfish from any other source should not be accepted by DOD. Investigational testing for aquatic toxins. While *V. vulnificus* and *parahaemolyticus* may be a concern in RTE, raw molluscan shellfish, no limits can be recommended at this time.

Criteria & EMP Target Microorganism ^{tttt}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC		1.5x10 ⁶ /g	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	
Coliforms (fecal)		3.3x10 ² /100g	Investigate; implement corrective action; enumerate generic <i>E. coli</i>	
<i>E. coli</i> (generic)		3.3x10 ² /100g	Investigate; implement corrective action	

^{tttt} (National Advisory Committee on Microbiological Criteria for Foods, 1992; U. S. Department of Health and Human Services, 2011a)
Each analytical unit is comprised of 10-12 individual shellfish composited into one unit. See text FDA Fish and Fishery Products Hazards and Controls Guidance for sampling procedure.

Table J.40. Microbiological Limits for Spices, Herbs, Coffee and Tea

Notes: defined as ready-to-eat

Criteria & EMP Target Microorganism ^{uuuu}	Microbiological Limit		Recommended Action if Limit is Exceeded	Comments
	Routine	Non-Routine		
APC	10 ⁵ /g		Investigate	
<i>B. cereus</i>		10 ⁴ /g	Investigate, implement corrective action. if ≥10 ⁴ /g, reject lot due to potential for enterotoxin production	
Coliforms		10 ⁴ /g	Investigate, implement corrective action	
<i>E. coli</i> (generic)		10/g	Investigate, implement corrective action	
<i>E. coli</i> (O157:H7 or other STEC)		Negative	Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Number and size of samples will vary depending on product
Mold/Yeast		10 ⁴ /g	Investigate, implement corrective action	
<i>Salmonella</i> (EMP) ^{vvv}	Negative zone 2 or 3		Consider zone 1 and finished product testing; implement corrective action	
<i>Salmonella</i>	Negative		Divert for reprocessing, if appropriate, or reject. Investigate and implement corrective action	Number and size of samples will vary depending on product; routine testing of <i>Salmonella</i> in roasted coffee may not be applicable
Mesophilic sporeformer		10 ⁵ /g	Investigate, implement corrective action	

^{uuuu} (Health Canada, 2008; Sagoo et al., 2009)

^{vvv} (Chen et al., 2009; Grocery Manufacturers Association, 2009)

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Appendix K. Sampling for Lot Acceptance or Rejection

In lot acceptance sampling, a sample of n units is drawn from a lot, and a characteristic of interest (e.g., presence of a pathogen or concentration of an indicator organism) of the sample units is analyzed. The test portion or analytical unit may represent an entire sample unit (e.g., enrichment of a 25-g sample), a composite of multiple sample units (e.g., enrichment of sixty 6.25-g sample units into an approximate 375 g composite test portion), or a portion or aliquot of a sample unit (e.g., enumeration of 1 ml of diluted homogenate prepared from a 25-g sample unit). Based on the sample results and the lot acceptance criteria, a lot disposition decision is made to either accept or reject the lot.

For lot acceptance sampling to have direct impact, lots must vary with respect to the analyte of interest. If the analyte is homogeneous among lots, sampling will accept some lots and reject others by chance alone, and the accepted lots are no better than the rejected lots. Lot acceptance sampling schemes may involve normal, tightened, or reduced inspection levels. Lot acceptance sampling may be applied lot-by-lot, or skip-lot sampling may be applied to less than 100% of lots. Conventional rules for switching between inspection levels depend on the history of supplier conformance to specifications and are available as guidelines for frequency and intensity of lot acceptance sampling schemes (e.g., MIL-STD-1916 [available at [http://guidebook.dcm.mil/34/milstd1916\(15\).pdf](http://guidebook.dcm.mil/34/milstd1916(15).pdf)], ISO 2859-1, ISO 2859-3, /ASQ Z1.4 [available at www.asq.org]).

Lot Acceptance Sampling for Attributes

Current microbiological lot acceptance sampling schemes are based on lot acceptance sampling for attributes. Both microbial presence/absence data obtained from enriched samples and quantitative concentration data divided into numerical ranges are classified as attributes. Two-class sampling plans are applicable where product quality is divided into two attribute classes. For sampling based on detection methods, the classes are presence or absence. For sampling based on enumeration methods, the classes are $x \leq m$ or $x > m$, where x is a measure of concentration (e.g., CFU/g, CFU/ml, CFU/cm², as appropriate), and m is the microbiological limit separating acceptable from unacceptable concentrations. Three-class sampling plans are applicable where product quality is measured by enumeration methods and results are divided into three attribute classes: $x \leq m$, $m < x \leq M$, or $x > M$; where m is the limit for a marginally acceptable concentration (note the change in meaning from the 2-class plan), and M is the limit for an unacceptable concentration (i.e., similar in meaning to m in the 2-class plan). Two- and three-class sampling plans also may be used for process control (3), but in the context of lot acceptance sampling, a consequence of non-conformance with a microbiological criterion is rejection of the lot represented by the samples.

Performance Characteristics of the Sampling Plans

The performance characteristics of microbiological lot acceptance sampling plans are generally described in terms of the probability of acceptance (p_a) for a given lot composition under specified microbiological limits. The operating characteristic (OC) curve plots the probability of accepting the lot versus a measure of its quality (e.g., the proportion of analytical units exceeding a given limit). In practice, the probability of lot acceptance also depends on the sampling procedures and analytical methods specified under the microbiological criteria (e.g., test sensitivity and specificity, percent recovery in enumeration, compositing). The statistical performance characteristics of a lot-acceptance sampling plan reflect the different probabilities of rejecting lots of different qualities. In addition to this direct, or curative, effect of a sampling plan, for a continuing series of lots presented for inspection, lot acceptance sampling also may have an indirect, or preventative, effect by exerting economic pressures on suppliers to prevent or limit the frequency and severity of non-conformance through process control measures (2, 6, 8) .

Two-Class Plans

For two-class sampling plans based on a maximum limit (m), the probability of lot acceptance (p_a) can be calculated directly from the proportion of non-conforming analytical units (p). For presence/absence sampling plans, p refers to the proportion of test-positive analytical units. For concentration-based sampling plans, p refers to the proportion of analytical units with CFU/g $\geq m$. A two-class sampling plan is defined by the sample size n and the maximum number of non-conforming analytical units allowed (acceptance number) c . In general, the probability of lot acceptance (p_a) is the probability that the number of non-conforming independent sample units in the sample of n is less than or equal to c :

$$p_a = \sum_{i=0}^{i=c} C_i^n (p)^i (1-p)^{n-i} \quad (\text{eq. K.1.})$$

where $C_i^n = \frac{n!}{i!(n-i)!}$ (the binomial coefficient, or combination of n things taken i at a time), p is the proportion of non-conforming analytical units, n is the number of samples drawn from the lot, and c is the acceptance number.

Most two-class sampling plans for pathogens specify $c = 0$. In this case, p_a simplifies to:

$$p_a = (1-p)^n \quad (\text{eq. K.2.})$$

For presence/absence sampling plans, it is important to note that the proportion of test positives depends on the size of the analytical unit (test portion). If analytical units of different sizes are examined from the same food lot, the proportion of test positives will be lower for smaller analytical units. For example, for a given concentration, a 25-g sample is less likely to contain at least one microorganism than a 100-g sample. Therefore, prevalence needs to be referenced to the size of the analytical unit (e.g.,

prevalence in 25 grams). If the detection method is less than 100% sensitive, the apparent prevalence (proportion of test positives) is less than the true prevalence (actual proportion of positives). For concentration-based sampling plans, equations K.1. and K.2. assume that measurement error is negligible (~100% recovery) and that false positive results are very unlikely (~ 100% specificity). If measurement error is substantial, the probability of lot acceptance may be affected by the microbial distribution (not just the proportion of non-conforming sample units) because measurement error can result in misclassification of sample units above or below the limit (m).

Figure K.1. presents the operating characteristic curves for $c = 0$ two-class sampling plans over a range of sample sizes (n).

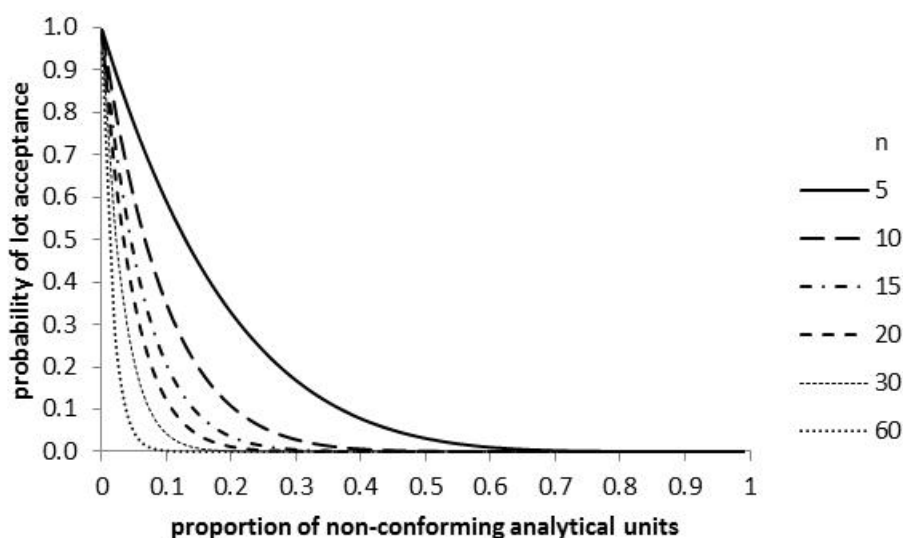


Figure K.1. Operating Characteristic Curves for Two-Class Sampling Plans with $c = 0$.

Table K.1. summarizes the performance of an $n = 5$, $c = 0$ two-class sampling plan with $m = \text{absence in 25 g}$ with probability of acceptance (p_a) = 0.95, 0.5, and 0.05.

Table K.1. Summary Performance of $n = 5$, $c = 0$ Two-Class Sampling Plan

n	5
c	0
m	absence in 25 g
p_a	p
0.95	0.0102
0.50	0.1294
0.05	0.4507

Alternatively, the probability of acceptance for two-class sampling plans can be calculated based on an assumed statistical distribution of the microbial concentration in

a lot. Assuming that the average concentration is log normally distributed and that the number of CFU in an analytical unit varies randomly according to the Poisson distribution (a Poisson-Lognormal distribution), Table K.2. summarizes the performance of an $n = 5$, $c = 0$ two-class sampling plan with $m =$ absence in 25 g. The values (probability of acceptance) shown in Table K.2. assume a perfect detection method (100% sensitivity and 100% specificity). The calculations can be performed using on-line resources (http://www.icmsf.org/publications/sampling_plans.html; <http://www.fstools.org/sampling>). For further details about the Poisson-Lognormal used by these on-line calculators, see (7) .

Table K.2. Performance of $n = 5$, $c = 0$, $m =$ absence in 25-g Two-Class Sampling Plan

N	c	M	Probability of lot acceptance	stdev* = 0.25** (log ₁₀ CFU/g)	stdev = 0.50 (log ₁₀ CFU/g)	stdev = 0.80 (log ₁₀ CFU/g)	stdev = 1.2 (log ₁₀ CFU/g)
				Geometric mean concentration (log ₁₀ cfu/g)***			
5	0	absence in 25 g	0.95	-3.46	-3.67	-4.08	-4.81
			0.50	-2.32	-2.48	-2.74	-3.14
			0.05	-1.64	-1.69	-1.74	-1.79

Note: Probability of acceptance (p_a) values assume negligible measurement error.

*stdev = standard deviation

**In many applications, measurement error alone exceeds 0.25 log₁₀ CFU/g. We include this standard deviation value to help the reader understand the derivation of values for sampling plans commonly presented in the literature (e.g. (3, 4))

***Technically, the geometric mean is the exponentiated mean of the logarithms of individual concentrations. For example, if the mean of the log-transformed values = -2 log₁₀ CFU/g, the geometric mean = 0.01 CFU/g. However, because -2 log₁₀ and 0.01 are equivalent, we adopt the common usage in the food microbiology literature of "geometric mean" to refer to the mean of the log-transformed values to differentiate it from the arithmetic mean on the original scale (CFU/g). For the lognormal distribution, the geometric mean represents the median because the normal distribution is symmetric.

It should be noted that in terms of consumer health risk, the arithmetic mean (the average, or expected value) concentration is more relevant than the geometric mean (median) concentration (5) . Table K.3. presents the arithmetic mean concentration for the 5 percent probability of acceptance distributions in Table K.2.

Table K.3. Arithmetic Mean for Lognormal Distributions

geometric mean (log ₁₀ CFU/g)	standard deviation (log ₁₀ CFU/g)	arithmetic mean (CFU/g)
-1.64	0.25	0.0270
-1.69	0.50	0.0396
-1.74	0.80	0.0993
-1.79	1.20	0.7377

As an example interpretation of Table K.3, if the arithmetic mean concentration is 0.0396 CFU/g, a 25-g serving would contain an average of 1 CFU. Note that there is no direct correspondence between the probability of lot acceptance and the level of risk indicated by the arithmetic mean concentration. This illustrates that evaluating the food safety impact of sampling plans is not as straightforward as calculating their statistical operating characteristics.

Three-Class Plans

Three-class sampling plans are based on a marginal limit (m) and a maximum limit (M). A three-class sampling plan is defined by the sample size n and c the maximum number of marginal analytical units allowed, or acceptance number. (The acceptance number for analytical units exceeding M is zero.) For three-class sampling plans, the probability of lot acceptance (p_a) can be calculated directly from the proportion of marginally acceptable (p_m) and unacceptable (p_d) analytical units (1):

$$p_a = \sum_{i=0}^c C_i^n (p_m)^i (1 - p_d - p_m)^{n-i} \quad (\text{eq. K.3})$$

where $C_i^n = \frac{n!}{i!(n-i)!}$ (the binomial coefficient), p_m is the proportion of marginally acceptable analytical units (with $m < x \leq M$), p_d is the proportion of analytical units with $x > M$, n is the number of samples drawn from the lot, and c is the acceptable number of marginal units.

Figure K.2 summarizes the operating characteristics of an $n = 5$, $c = 2$ three-class sampling plan for probability of acceptance (p_a) = 0.95, 0.5, and 0.05.

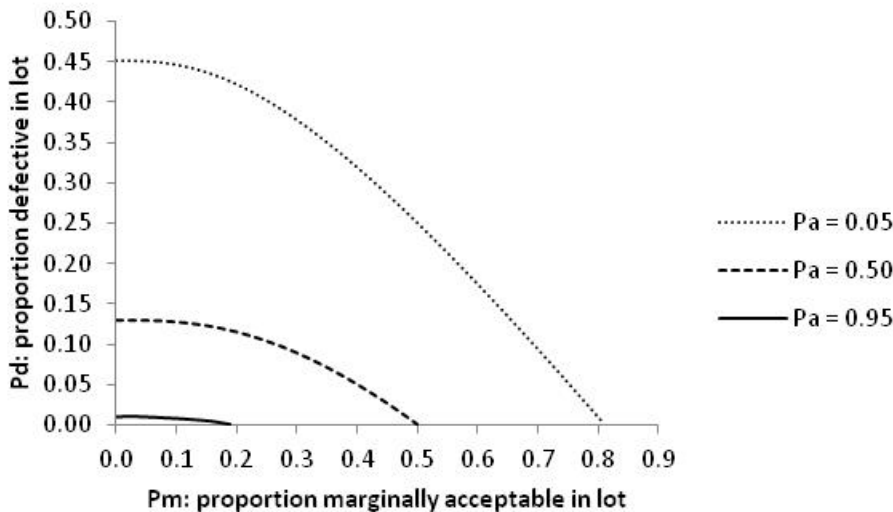


Figure K.2. Operating Characteristic Contours for Three-Class Plan: $n = 5$, $c = 2$

Table K.4 presents selected performance characteristics for an $n = 5$, $c = 2$ three-class sampling plan.

Table K.4. Performance Characteristics for $n = 5$, $c = 2$ Three-Class Sampling Plan: Probabilities of Acceptance (p_a) for Lots Containing Indicated Proportions p_d and p_m

Proportion unacceptable (p_d)	Proportion Marginal (p_m)								
	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
0.90	<*	0.0000							
0.85	<	<	0.0000						
0.80	<	<	<	0.0000					
0.75	<	<	<	<	0.0000				
0.70	<	<	<	<	<	0.0000			
0.65	0.0051	<	<	<	<	<	0.0000		
0.60	0.0101	0.0092	0.0074	0.0051	<	<	<	0.0000	
0.55	0.0182	0.0170	0.0146	0.0111	0.0073	<	<	<	0.0000
0.50	0.0310	0.0294	0.0262	0.0213	0.0156	0.0099	0.0051	<	<
0.45	0.0500	0.0481	0.0438	0.0374	0.0294	0.0209	0.0129	0.0065	<
0.40	0.0774	0.0750	0.0697	0.0614	0.0508	0.0389	0.0270	0.0163	0.0080
0.35	0.1156	0.1127	0.1063	0.0959	0.0822	0.0663	0.0497	0.0338	0.0201
0.30	0.1675	0.1642	0.1564	0.1438	0.1267	0.1062	0.0840	0.0618	0.0414
0.25	0.2367	0.2327	0.2236	0.2084	0.1875	0.1620	0.1334	0.1039	0.0753
0.20	0.3270	0.3224	0.3117	0.2938	0.2687	0.2375	0.2018	0.1638	0.1258
0.15	0.4429	0.4377	0.4253	0.4044	0.3748	0.3373	0.2938	0.2463	0.1974
0.10	0.5896	0.5837	0.5695	0.5454	0.5108	0.4666	0.4143	0.3563	0.2952
0.05	0.7727	0.7661	0.7501	0.7225	0.6826	0.6310	0.5692	0.4995	0.4250
0.00	0.9988	0.9914	0.9734	0.9421	0.8965	0.8369	0.7648	0.6826	0.5931

p_d	p_m								
	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90
0.50	0.0000								
0.45	<	0.0000							
0.40	<	<	0.0000						
0.35	0.0098	<	<	0.0000					
0.30	0.0243	0.0117	<	<	0.0000				
0.25	0.0498	0.0289	0.0137	<	<	0.0000			
0.20	0.0902	0.0590	0.0339	0.0160	0.0053	<	0.0000		
0.15	0.1500	0.1064	0.0689	0.0393	0.0184	0.0060	<	0.0000	
0.10	0.2342	0.1762	0.1239	0.0797	0.0451	0.0210	0.0068	<	0.0000
0.05	0.3488	0.2742	0.2046	0.1428	0.0912	0.0513	0.0237	0.0077	<
0.00	0.5000	0.4069	0.3174	0.2352	0.1631	0.1035	0.0579	0.0266	0.0086

Note: Probability of acceptance (p_a) values calculated assuming negligible measurement error.

* $p_a < 0.005$.

Note that some combinations of p_m and p_d presented in Figure K.2. and Table K.4. may not be plausible for certain applications. For example, consider a three-class sampling

plan for mesophilic aerobic bacteria in dried milk with $m = 10^4$ CFU/g, and $M = 10^6$ CFU/g. Based on Table K.3, $p_a = 0.05$ for the combination $p_m = 0.05$ and $p_d = 0.45$. However, if the distribution is lognormal, this combination of p_m and p_d values would imply a lot of dried milk with a geometric mean aerobic plate count of $4 \log_{10}$ CFU/g (10,000 CFU/g), a standard deviation of $16 \log_{10}$ CFU/g, and a maximum concentration in excess of $40 \log_{10}$ CFU/g.

Alternatively, the probability of acceptance for three-class sampling plans can be calculated based on an assumed statistical distribution of the microbial concentration in a lot. In this case, eq. K.3. above is still used to calculate the probability of lot acceptance, but the marginal and unacceptable proportions (p_m and p_d) are derived from the assumed statistical distribution. Table K.5. summarizes the performance of $n = 5$, $c = 2$ three-class sampling plans based on assuming a lognormal distribution.

Table K.5. Performance of $n = 5$, $c = 2$ Three-Class Sampling Plans

n	c	m	M	Probability of lot acceptance	stdev* = 0.25 ($\log_{10}$ CFU /g)	stdev = 0.50 ($\log_{10}$ CFU /g)	stdev = 0.80 ($\log_{10}$ CFU /g)	stdev = 1.2 ($\log_{10}$ CFU /g)
					Geometric mean concentration ($\log_{10}$ CFU /g)			
5	2	10^4 CFU /g	10^6 CFU /g	0.95	3.78	3.56	3.29	2.82
				0.50	4.00	4.00	3.99	3.89
		$4 \log_{10}$ CFU /g	$6 \log_{10}$ CFU /g	0.05	4.22	4.44	4.68	4.90
5	2	<3MPN/g	9.8 MPN/g	0.95	0.25	-0.19	-0.87	-1.79
				0.50	0.47	0.33	0.05	-0.38
				0.05	0.68	0.76	0.79	0.78

*stdev = standard deviation

As an example of Table K.5. calculations, consider the lognormal distribution with geometric mean of $4.68 \log_{10}$ CFU /g (47,863 CFU /g) and standard deviation of $0.8 \log_{10}$ CFU /g (denoted by shaded cell of Table K.5.) For the first sampling plan, the marginal limit (m) of $4 \log_{10}$ CFU /g is the 20th percentile of the distribution. The maximum limit (M) of $6 \log_{10}$ CFU /g is the 95th percentile of the distribution. As a result, $p_m = 0.95 - 0.20 = 0.75$ and $p_d = 1 - 0.95 = 0.05$. Looking up these values in Table 3 (or inserting the values into eq. 3) results in probability of lot acceptance (p_a) = 0.05. The calculations can be performed using on-line resources (http://www.icmsf.org/publications/sampling_plans.html; <http://www.fstools.org/sampling>).

Impact of Lot Acceptance Sampling Plans

As noted above, the impact of lot acceptance sampling plans depends on variability among lots. The Food and Agriculture Organization/World Health Organization jointly

supported development of a web-based analytical tool that can analyze the direct impact of lot acceptance sampling plans based on user-specified distributions of contamination between and within lots (<http://www.fstools.org/sampling>).

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Appendix L. Design of 2-class and 3-class Sampling Plans

Two- and three-class sampling plans are attribute sampling plans where quantitative microbiological concentration data are divided into two or three classes, respectively. The key parameters of these plans are: 'n', the sample size; 'c_m', the acceptance number for sample units which exceed m in concentration; and 'c_M', the acceptance number for sample units which exceed M in concentration. The sample of n units is presumed to be a 'rational subgroup', such as units chosen from the same lot or time period of production.

Some additional notation that will be useful in the discussion of 2- and 3-class sampling plans are:

P_m: The percentile rank of m in *normal* production.

$$Q_m = 1 - P_m$$

P_M: The percentile rank of M in *normal* production.

$$Q_M = 1 - P_M$$

y_m = log₁₀(m + 0.3 d), where 'd' is the dilution factor from counts to concentration

y_M = log₁₀(M + 0.3 d), where 'd' is the dilution factor from counts to concentration

2-Class Sampling Plans

In a '2-Class' sampling plan, only the M quantile is used.

There are several approaches to designing a 2-class sampling plan.

Approach1: Given M and associated error fraction target, find n and c_M

This approach is used primarily in designing sampling plans *for acceptance testing*, and the focus is on the *false negative fraction* ('consumer's risk' or 'type II error' or 'β').

Here 'M' denotes a median concentration that, if exceeded, represents 'unacceptable' product which should be rejected with high reliability by the plan.

Subject matter expertise or specifications provides a value for M that should be rejected with high reliability ('Power' = 1 – β), say 95% or 99% or 99.9%. The parameters n and c_M are chosen to achieve the power required. For this purpose, M is presumed to be the median of the distribution of concentration for an unacceptably contaminated lot of product.

The probability of rejecting this type of unacceptable lot is

$$1 - \beta = P[(\# > M) > c_M] \quad (\text{L.1.})$$

calculated from a binomial distribution with $p = P[X \leq M] = 0.50$.

Some possible plans are:

Table L.1. 2-Class plans, given M as median of 'unacceptable' lots

n	c	Power Target	Power Actual
4	0	95.00%	93.75%
5	0	95.00%	96.88%
6	0	95.00%	98.44%
10	1	95.00%	98.93%
20	5	95.00%	97.93%
10	1	99.00%	98.93%
20	4	99.00%	99.41%
10	0	99.90%	99.90%
20	3	99.90%	99.87%

Note that this type of plan is based on specifications, so is not generally useful for SPC.

Approach 2: Given P_M and associated error fraction target, find n and c_M

This approach is used in designing sampling plans *for process control*, and the focus is on the *false positive fraction* ('producer's risk' or 'type I error' or ' α ' or 'FAR').

M here represents a specified high quantile of the concentration of 'normal' production. Its value is determined from its percentile rank P_M either nonparametrically or parametrically based on historical data. The parameters n and c_M are chosen to achieve the maximum false positive fraction α allowed, typically 1% or 0.1%, depending upon sampling rate. Note that M is presumed to be a high quantile of the distribution of concentration during 'normal' production under statistical process control.

The probability of a randomly chosen lot from normal production failing is

$$\alpha = P[(\# > M) > c_M] \quad (\text{eq. L.1.})$$

calculated from a binomial distribution with n trials and $p = Q_M$.

Some possible plans are:

Table L.2. 2-Class plans, given M and α

n	c	α Target	Prob. $\leq M$	α Actual
1	0	5.00%	95.00%	5.00%
2	1	5.00%	95.00%	0.25%
3	1	5.00%	95.00%	0.72%
5	1	5.00%	95.00%	2.26%
10	2	5.00%	95.00%	1.15%
1	0	1.00%	99.00%	1.00%
2	1	1.00%	99.00%	0.01%
3	1	1.00%	99.00%	0.03%
5	1	1.00%	99.00%	0.10%
10	1	1.00%	99.00%	0.43%
1	0	0.10%	99.90%	0.10%
2	1	0.10%	99.90%	0.00%
3	1	0.10%	99.90%	0.00%
5	1	0.10%	99.90%	0.00%
10	1	0.10%	99.90%	0.00%

Approach 3: Given n, c_M and error fraction target, find P_M

This approach results in achievement of an exact false positive fraction α , and again is used in designing sampling plans *for process control* with the focus on the *false positive fraction* ('producer's risk' or 'type I error' or ' α ' or 'FAR').

M represents a high quantile of 'normal' production whose value is determined from its percentile rank P_M which solves the following equation

$$\alpha = P[(\# > M) > c_M] \quad (\text{eq. L.2.})$$

calculated from a binomial distribution with n trials and $p = Q_M$.

Some possible plans are:

Table L.3. 2-Class plans, given n , c and α

n	c	α Target	Prob. $\leq M$	α Actual
1	0	5.00%	95.00%	5.00%
2	1	5.00%	77.65%	5.00%
3	1	5.00%	86.46%	5.00%
5	1	5.00%	92.36%	5.00%
10	2	5.00%	91.28%	5.00%
1	0	1.00%	99.00%	1.00%
2	1	1.00%	90.00%	1.00%
3	1	1.00%	94.10%	1.00%
5	1	1.00%	96.73%	1.00%
10	1	1.00%	98.45%	1.00%
1	0	0.10%	99.90%	0.10%
2	1	0.10%	96.80%	0.10%
3	1	0.10%	98.20%	0.10%
5	1	0.10%	99.00%	0.10%
10	1	0.10%	99.52%	0.10%

3-Class Sampling Plans

In a '3-Class' sampling plan, both the m and M quantiles are used.

We will discuss again the same three approaches to designing a 3-class sampling plan.

Approach 1: Given m , M and error fraction target, find n , c_m and c_M

This approach once again is used *for acceptance testing*, and the focus is on the *false negative fraction* ('consumer's risk' or 'type II error' or ' β ').

Here ' M ' again denotes the median concentration that, if exceeded, represents 'unacceptable' product that should be rejected with high reliability by the plan. The quantile ' m ' denotes the median concentration that separates 'acceptable' from 'marginal' product. Presumably 'acceptable' product should be rejected infrequently.

Subject matter expertise or specifications provide a value for M that should be rejected with high reliability ('Power' = $1 - \beta$), say 95% or 99% or 99.9%. Such expertise or specifications also provides the value for m that should be rejected 50% of the time (*i.e.*, the 'indifference' level). The parameters n , c_m and c_M are chosen to achieve the statistical power required.

The probability of rejecting unacceptable lots with median concentration M is determined from eq.(L.1.) and the probability of rejecting indifferent lots with median concentration m is

$$0.5 \sim P[(\# > m) > c_m] \quad (\text{eq. L.3.})$$

calculated from a binomial distribution with $p = P[X \leq m] = 0.50$.

Some possible plans are:

Table L.4. 3-Class plans, given m, M as median concentrations

n	m c	M c	M Power Target	M Power Actual	m Power Target	m Power Actual
4	1	0	95.00%	93.75%	50.00%	68.75%
5	2	0	95.00%	96.88%	50.00%	50.00%
6	2	0	95.00%	98.44%	50.00%	65.63%
7	3	1	95.00%	93.75%	50.00%	50.00%
10	4	1	95.00%	98.93%	50.00%	62.30%
7	3	0	99.00%	99.22%	50.00%	50.00%
10	4	1	99.00%	98.93%	50.00%	62.30%
10	4	0	99.90%	99.90%	50.00%	62.30%

Approach 2: Given P_m , P_M and error fraction target, find n , c_m and c_M

This approach is used primarily in designing sampling plans *for process control*, and the focus is on the *false positive fraction* ('producer's risk' or 'type I error' or ' α ' or 'FAR').

M and m represent specified quantiles of the concentration of 'normal' production under statistical control, with $m < M$. Their values are determined from their percentile ranks P_m and P_M either nonparametrically or parametrically based on historical data. The parameters n , c_m and c_M are chosen to achieve the maximum false positive fraction α allowed, typically 1% or 0.1%, depending upon sampling rate.

The probability of a randomly chosen lot from normal production failing from both rejection rules for the special case that $c_M = 0$ is

$$1 - \alpha = \sum_{k=0}^c \binom{n}{k} u^k p^{n-k} \quad (\text{eq. L.4.})$$

where $p = P_m$ and $u = P_M - P_m$.

An additional design rule for 3-class plans is to make the rejection rules for m and M roughly of equal impact in the determination of the total false positive fraction. This can be achieved by determining P_M for the 2-class plan with sample size n and acceptance number $c_M = 0$ and half the false positive fraction α , and then choosing the parameters related to m. We assume therefore that P_M has been determined from

$$\alpha / 2 = P[(\# > M) > 0] \quad (\text{eq. L.5.})$$

calculated from a binomial distribution with n trials and $p = Q_M$.

Some possible plans are:

Table L.5. 3-Class sampling plan given m and M probabilities

n	c	α Target	Probability $\leq m$	Probability $\leq M$	Exact α $m + M$
5	1	0.50%	98.50%	99.95%	0.454%
5	1	1.00%	97.50%	99.90%	1.047%
5	1	5.00%	94.50%	99.49%	4.720%
5	1	10.00%	91.50%	98.98%	9.650%
5	2	0.50%	93.50%	99.95%	0.493%
5	2	1.00%	91.50%	99.90%	1.019%
5	2	5.00%	85.00%	99.49%	4.894%
5	2	10.00%	80.00%	98.98%	9.919%
5	3	0.50%	85.00%	99.95%	0.470%
5	3	1.00%	81.00%	99.90%	1.041%
5	3	5.00%	71.00%	99.49%	5.028%
5	3	10.00%	64.50%	98.98%	10.045%

Approach 3: Given n , c_m , c_M and error fraction target, find P_m and P_M

This approach results in achievement of an *exact* false positive fraction α , and again is used in designing sampling plans *for process control* with the focus on the *false positive fraction* ('producer's risk' or 'type I error' or ' α ' or 'FAR').

M and m again represent specified quantiles of the concentration of 'normal' production under statistical control, with $m < M$. Their values are determined from their percentile ranks P_m and P_M either nonparametrically or parametrically based on historical data. The parameters n , c_m and c_M are specified, and P_m and P_M chosen to achieve the maximum false positive fraction α allowed, typically 1% or 0.1%, depending upon sampling rate.

As in the section titled ‘Given P_m , P_M and error fraction target, find n , c_m and c_M ’, we assume $c_M = 0$ to simplify the equations, and choose P_M to satisfy eq.(L.5.). The implicit eq.(L.4.) is then solved for P_m .

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Some possible plans are:

Table L.6. Find m for 3-class plan from given α , M and σ

N	c	FAR Target	Probability $\leq M$	Probability $\leq m$
5	1	0.200%	99.980%	98.966%
5	1	0.200%	99.980%	98.966%
5	1	0.200%	99.980%	98.966%
5	1	0.500%	99.950%	98.342%
5	1	0.500%	99.950%	98.342%
5	1	0.500%	99.950%	98.342%
5	1	1.000%	99.900%	97.608%
5	1	1.000%	99.900%	97.608%
5	1	1.000%	99.900%	97.608%
5	1	5.000%	99.495%	94.176%
5	1	5.000%	99.495%	94.176%
5	1	5.000%	99.495%	94.176%
5	2	0.200%	99.980%	95.237%
5	2	0.200%	99.980%	95.237%
5	2	0.200%	99.980%	95.237%
5	2	0.500%	99.950%	93.436%
5	2	0.500%	99.950%	93.436%
5	2	0.500%	99.950%	93.436%
5	2	1.000%	99.900%	91.610%
5	2	1.000%	99.900%	91.610%
5	2	1.000%	99.900%	91.610%
5	2	5.000%	99.495%	84.770%
5	2	5.000%	99.495%	84.770%
5	2	5.000%	99.495%	84.770%
5	3	0.200%	99.980%	87.778%
5	3	0.200%	99.980%	87.778%
5	3	0.200%	99.980%	87.778%
5	3	0.500%	99.950%	84.500%
5	3	0.500%	99.950%	84.500%
5	3	0.500%	99.950%	84.500%
5	3	1.000%	99.900%	81.392%
5	3	1.000%	99.900%	81.392%
5	3	1.000%	99.900%	81.392%
5	3	5.000%	99.495%	71.088%
5	3	5.000%	99.495%	71.088%
5	3	5.000%	99.495%	71.088%

Approach 4: Finding m and M from P_m and P_M

The concentrations m and M may be determined nonparametrically as quantiles of an observed empirical cumulative distribution function (ECDF) at probabilities P_m and P_M . Acceptable accuracy requires the number of underlying observations $N \geq 2 / Q_M$. E.g., for $P_M = 99.9\%$, $Q_M = 1 - P_M = 0.001$ and the requirement is that $N \geq 2000$. Lack of available data typically limits this approach to $P_M = 99\%$ or less.

Extension to higher quantiles is possible by fitting available data to a parametric model, such as a normal distribution for the $\log_{10}$ -transformed concentrations. Suppose this has been done, and the prevalence of zero results is P_0 , and the mean and standard deviation of the $\log_{10}$ -transformed concentrations are estimated at ' m ' and ' s '.

If the entire mixture ECDF (including zero results) is the desired basis for quantiles, compute adjusted quantiles for P_m and P_M as

$$P_m' = 1 - (1 - P_m) / (1 - P_0) \quad (\text{eq. L.6.})$$

$$P_M' = 1 - (1 - P_M) / (1 - P_0) \quad (\text{eq. L.7.})$$

Find y_m and y_M as the quantiles of the normal distribution with mean m and standard deviation s corresponding to P_m' and P_M' . Then compute m and M as the antilogs of y_m and y_M . Note that observed zero results are still used in applying the rejection rules.

Appendix M. Implied False Alarm Rate in a 3-class Sampling Plan Given m , M , n , c , and σ .

In addition to designing 3-class sampling plans to achieve a desired false alarm rate (FAR), we can also investigate the FAR implied by existing plans. We begin by assuming that the unacceptable concentration limit M is chosen according to some criterion and then evaluate the impact of different levels of process variability (σ) on the FAR under existing 3 class sampling plans.

M percentile criterion: First, assume that M is chosen to be an extreme percentile in the right hand tail of the distribution, such as the 99.5th percentile. This means that M is chosen such that $p_d = 0.5\%$ of individual samples have an unacceptable concentration. For a given sampling plan, the FAR attributable to M (FAR_M) = $1-(1-p_d)^n$. This is simply the complement of eq. K.2. (Appendix K). For an $n = 5$ sampling plan, $FAR_M = 2.5\%$. For a given sampling plan, the overall FAR is given by the complement of eq. K.3. (Appendix K). The overall FAR can be partitioned into portions attributable to M and m : $FAR = FAR_M + FAR_m$. Assuming a lognormal distribution, given p_d and $\sigma_{\log10}$ values, we can calculate p_m from existing sampling plans based on the ratio of the limits (M/m). Given a fixed M percentile, the implied $\mu_{\log10}$ and percentile of the specified marginal limit value (m) will vary depending on the process variability (σ). This has important consequences for the FAR. Table M.1 presents the FARs implied by existing $n = 5$, $c = 2$ three-class sampling plans over a range of $\sigma_{\log10}$ values.

Table M.1. False alarm rates implied by existing 3-class sampling plans (M percentile)

n	c	$\log(M/m)$	$\sigma_{\log10}$	M percentile	FAR(%)	$FAR_M(\%)$	$FAR_m(\%)$
5	2	1	0.25	7.7	99.6	2.5	97.1
			0.50	71.8	15.8	2.5	13.3
			0.80	90.8	3.1	2.5	0.6
			1.20	95.9	2.5	2.5	0.0
5	2	2	0.25	0.0	100.0	2.5	97.5
			0.50	7.7	99.6	2.5	97.1
			0.80	53.0	45.2	2.5	42.8
			1.20	81.8	6.6	2.5	4.1

FAR_M criterion: Second, assume that M is chosen to achieve a desired FAR_M for a given sampling plan, such as the conventional 0.3%. For a given sampling plan, $p_d = 1-(1-FAR_M)^{1/n}$. For an $n = 5$ sampling plan, $p_d = 0.05\%$ ($M = 99.95^{\text{th}}$ percentile). Based on the same approach used to generate Table M.1, Table M.2 presents implied FAR.

Table M.2. False alarm rates implied by existing 3-class sampling plans (FAR_M)

n	c	$\log(M/m)$	$\sigma_{\log 10}$	m percentile	FAR(%)	$FAR_M(\%)$	$FAR_m(\%)$
5	2	1	0.25	23.2%	91.4%	0.3%	91.2%
			0.50	89.8%	1.2%	0.3%	0.9%
			0.80	97.8%	0.3%	0.3%	0.0%
			1.20	99.3%	0.3%	0.3%	0.0%
5	2	2	0.25	0.0%	100.0%	0.3%	99.7%
			0.50	23.2%	91.4%	0.3%	91.2%
			0.80	77.9%	7.8%	0.3%	7.5%
			1.20	94.5%	0.4%	0.3%	0.1%

The take away message from Tables M.1 and M.2 is that designing 3-class sampling plans based on n , c , m , and M without considering process variability (σ) can result in highly inconsistent false alarm rates. On the other hand, the analysis in this section assumes that limits are based on reliable data from an ideal process that is under statistical control and capable of meeting microbiological specifications. If the m and M limit values are chosen instead on the basis of strong observed associations with certain concentrations being exceeded (e.g., measurements exceeding M are associated with observed pasteurization process failures, measurements exceeding m and associated with significantly reduced shelf life), then frequent occurrences where the microbiological criteria were exceeded may indicate unsatisfactory process quality. Under such circumstances, the events would be mischaracterized as false alarms because the exceedances indicate a lack of process capability to meet microbiological specifications.