Memorandum

Date May 28, 2025

From Ashley Nazario Toole (Innovative Foods Staff (IFS))

Through

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Subject Cell Culture Consultation (CCC) 000005, Cultured *Oncorhynchus kisutch* cell material

To Administrative File, CCC 000005

Submission Received Date: June 27, 2022, Disclosable Safety Narrative; June 3, 2022, Supplemental, Confidential Material

Amendments Received Date: January 17, 2023; May 3, 2023; July 28, 2023; January 24, 2024; August 30, 2024; December 11, 2024; April 2, 2025; April 11, 2025

Sponsor: Wildtype (Wildtype, the firm)

Summary

- The Food and Drug Administration (FDA, we) evaluated the food that is the subject of CCC 000005 submitted by Wildtype.
- This food is defined as the cell material at harvest, comprised of cultured coho salmon (*Oncorhynchus kisutch*) cells of the mesenchymal lineage, as produced by the method of manufacture described in CCC 000005.

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Human Foods Program
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- The cells used to establish the cell lines are originally isolated from muscle and connective tissue of salmon at the fry stage of development. The isolated cell lines are determined to be of mesenchymal lineage through standard methods validated for their intended purpose, including microscopy and RNA-sequencing. Species identity of master cell banks was verified by *cytochrome c oxidase subunit I* (*COI*) mitochondrial gene sequencing (DNA barcoding).
- The cell lines are established by the selection of mesenchymal progenitors with demonstrated proliferative and differentiation capacity that are then adapted to suspension culture. Cells are proliferated through a seed train to successively increase the volume of the suspension culture.[1]
- The cells are harvested using bowl centrifugation, washed three times with a water and sugar solution, rapidly cooled using blast chillers, and stored frozen with continuous temperature monitoring.
- The harvested cell material, following washing, is described as mesenchymal tissue of coho salmon (*Oncorhynchus kisutch*).[2] Microbial and toxic heavy metal specifications for the harvested cell material are provided.
- We evaluated information about the cell lines, the production process (including cell bank establishment), substances used in the production process, and properties of the harvested cell material, including information available in both the disclosable safety narrative as well as supporting, corroborative information in the supplemental, confidential material.
- Based on the data and information presented in CCC 000005, we have no questions at this time about Wildtype's conclusion that foods comprising or containing cultured coho salmon cell material resulting from the production process defined in CCC 000005 are as safe as comparable foods[3] produced by other methods. Furthermore, at this time we have not identified any information indicating that the production process as described in CCC 000005 would be expected to result in food that bears or contains any substance or microorganism that would adulterate the food.[4]

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[1] During the cell line establishment process, Wildtype selects for pluripotent cells of the mesenchymal lineage. The firm characterizes the capacity of the primary isolated cells to differentiate into various cell types, including muscle (myocytes), fat (adipocytes), and connective tissue cells (fibroblasts). The firm creates cell banks using undifferentiated cells that exhibit differentiation potential. The banked, undifferentiated cells are used as inputs into the production process. Wildtype does not induce differentiation (i.e., introduce media components to stimulate changes in gene expression profiles, leading to the development of distinct cell types) prior to cell harvest.

[2] Wildtype reports analytical data on the composition of the harvested cell material from several production runs as one element in characterizing the identity of its product to demonstrate the capability to meet or exceed specifications for food contaminants, and to demonstrate the consistency of its production process. Some variations in fatty acids, amino acids, and minerals were observed in the data from these batch analyses of the harvested cell material, relative to a conventional salmon product. In all cases levels were consistent with those found in commonly consumed foods. Wildtype's conclusions regarding the safety of its product are not based on the establishment of exact equivalence of all nutrients.

[3] Wildtype identifies "conventional salmon" as a comparator. In the firm's submission, wild coho salmon (*Oncorhynchus kisutch*) is utilized as a conventional comparator.

[4] Our review did not address other provisions of the Federal Food, Drug and Cosmetic Act.

Production Method

Wildtype describes an overall production process involving the establishment of a cell bank that provides a standardized source of cells for food production, and a production process including proliferation of the cells in a seed train followed by harvest of the cell material for subsequent conventional food processing.[5]

Wildtype states that a food safety and quality system is in use during production, and provides information about the following programs and measures that will be used in its production facilities, including:

- A Hazard Analysis and Critical Control Point (HACCP) plan, developed in accordance with 21 CFR 123.6, which addresses hazards specific to seafood products and applicable to Wildtype's production process;
- A current good manufacturing practice (cGMP) program that includes all the items enumerated in 21 CFR 117 subpart B;
- Validated sanitation processes, an environmental monitoring program, and hygienic zoning;
- A supplier approval program;
- An allergen control program;
- Controls for prevention of biological, chemical, and physical hazards;
- Document and records control including material and product specifications;
- A product release system involving quality assurance review for incoming raw materials, intermediate products, and finished food products;
- Batch record review; and
- Traceability of raw materials and finished food products.

Wildtype also notes the use of internal quality assurance and auditing programs, as well as annual cell culture and sanitation employee re-training programs.

An overview of the production process, potential hazards or quality issues at each process step, and management strategy is provided in Table 1 based on the information provided by Wildtype. A more detailed version of this table is provided in the Appendix of this memorandum.

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[5] Wildtype's June 27, 2022, disclosable safety narrative described a maturation step occurring on a plant-based scaffold made of materials commonly used in food production. In the amendment dated July 28, 2023, the firm subsequently changed its process to remove this maturation step. In the firm's current production process, cells are combined with food ingredients post-harvest, when they are no longer viable. We consider the post-harvest steps of the production process to be outside the scope of the CCC evaluation, which, per the March 2019 Formal Agreement considers cell isolation through the time of harvest. However, we note that Wildtype performs a post-harvest thermal processing step of the finished food product consisting of the harvested cell material and food ingredients, which is identified as a mitigation and control strategy for some adventitious agents identified by the firm as potential hazards during the production process prior to harvest.

Table 1: Overview of potential identity, quality, and safety issues

Process Step	**Potential Issues**	**Management Strategy**
Cell Isolation	Cell identity; contaminants from source, reagents, or environment	Antibiotics and antifungal application, aseptic procedures, cGMP, hygienic condition, supplier documentation, sterile filtration, sterile rinsing, testing, thermal step, visual observation
Establishment of Cell Lines	Cell identity; contaminants from materials or environment; appropriate adaptation to culture	Allergen controls, aseptic procedures, documentation, environmental monitoring, hygienic condition, supply-chain controls, sterile filtration, sterilization, testing, thermal step, visual observation
Establishment of Master Cell Bank and Working Cell Bank	Cell identity; contaminants from materials, equipment, or environment; media components	Allergen controls, aseptic procedures, cGMP, documentation, hygienic condition, environmental monitoring, food safety assessment[6], sterilization, supply-chain controls, testing program, thermal step, visual observation
Proliferation Phase	Contaminants from materials, equipment, or environment; media components	Allergen controls, aseptic procedures, cGMP, documentation, environmental monitoring, food safety assessment, sterilization, sanitation controls, supply-chain controls, testing program, thermal step, visual observation, X-ray
Harvest of Cell Material	Contaminants from materials, equipment, or environment; media components	Allergen controls, aseptic procedures, cGMP, compositional analysis, environmental monitoring, food safety assessment, specifications, sterilization, supply-chain controls, testing program, thermal step, visual observation, washing step, X-ray

[6] "Food safety assessment" indicates evaluation of the use of substances or materials based on commonly established paradigms for evaluating chemical, biochemical, and toxicological data in conjunction with estimates of exposure for their intended use to assess whether such use is consistent with applicable safety standards.

Cell Banking

Wildtype provides information about the establishment of cell banks used in the subsequent production process. The firm describes cell banking as a three-step, non-recurrent research and development process that occurs upstream of the production process. The cell banks described in the firm's manufacturing process are a collection of cryopreserved cells derived from a single tissue source from a single animal. Wildtype uses a two-tiered strategy in which there is both a primary cell bank (the master cell bank; MCB) and secondary cell banks (the working cell bank; WCB) all derived from a single cell line expansion. The steps involved include:

- Cell isolation
- Establishment of cell lines
- Establishment of MCBs
- Establishment of WCBs

Cell Isolation

The cells used to establish the cell banks are mesenchymal cells isolated from skeletal muscle and connective tissue of fry stage coho salmon acquired from a Washington state hatchery.[7] Hereafter, the source is referred to as the "source animal" or "animal source." Reagents used at this stage may include cell culture media, media components, and antibiotics and antifungals.

Potential hazards and quality issues identified by Wildtype at this stage include:

- Source animal health prior to tissue procurement resulting in cells contaminated by adventitious agents such as aquatic microflora[8], pathogenic bacteria[9], viruses[10],

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[7] Wildtype's June 27, 2022, disclosable safety narrative also described use of a cell line originating from a Canadian institute. In the amendment dated January 17, 2023, the firm noted that its current production process only uses a cell line isolated from the fry stage of development, originating from a Washington state hatchery.

[8] Microorganisms identified by Wildtype as microflora associated with living fish include *Psychrobacter* spp., *Moraxella* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Shewanella* spp., *Flavobacterium* spp., *Cytophaga* spp., *Aeromonas* spp., *Corynebacterium* spp., and *Micrococcus* spp. In the June 27, 2022, disclosable safety narrative, Wildtype states that these potential hazards are mitigated in the firm's production process by isolating source cells under sterile conditions and monitoring cell cultures for microbial contamination.

[9] Potential bacteria identified by Wildtype as being associated with fish include *Clostridium botulinum*, *C. perfringens*, various *Vibrio* species, *Plesiomonas shigelloides, Aeromonas hydrophila*, *Listeria monocytogenes, Campylobacter jejuni*, *Yersinia enterocolitica*, *Escherichia coli*, *Shigella* spp., and *Salmonella* serovars. In the June 27, 2022, disclosable safety narrative, Wildtype describes its mitigation and control strategies, including performing cell isolation and cell line establishment under aseptic conditions and use of a thermal processing step following harvest.

[10] Potential viruses of concern identified by Wildtype include norovirus and hepatitis A. The firm states that these viruses are predominantly found in shellfish and arise from contaminated water or human handling. In the June 27, 2022, disclosable safety narrative, Wildtype describes its mitigation and control strategies, including performing cell isolation and cell line establishment under aseptic conditions, establishment of employee hygiene practices throughout production, and the use of a thermal processing step following harvest. In the amendment

parasites[11], prions, or toxic heavy metals (such as arsenic, mercury, cadmium, or lead); and

- Introduction of adventitious agents from human handling or the local environment, including viruses (norovirus, hepatitis A) and bacteria such as *Escherichia coli,* fecal coliforms, *Listeria monocytogenes*, *Salmonella* serovars*,* and *Staphylococcus aureus*.

Wildtype provides health documentation from the State of Washington Department of Fish and Wildlife for the animal source. Donor animal health is assessed prior to cell isolation from skeletal muscle and connective tissue, which is performed under aseptic conditions to mitigate microbial, viral, and parasitic contamination. Additionally, the firm states that tissue (i.e., muscle biopsy) specimens are treated with hydrogen peroxide and ethanol prior to cell isolation to control for bacterial contaminants.

Wildtype states that potential adventitious agents (e.g., norovirus, hepatitis A) can enter the production process via contaminated water and human handling. However, the risks associated with these pathogens are mitigated by using EPA-regulated municipal water filtered through 0.2 μm filters, as well as the use of aseptic and cGMP techniques, including the use of laminar flow hoods, gloves, and gowning. The risk of prion transmission is mitigated by isolating cells from skeletal muscle tissue, where prions are not commonly found.

Wildtype screens the isolated cells to select for cells that have desired characteristics, including attachment affinity for uncoated cell culture vessel surfaces and the ability to thrive in various nutrient formulations. Selected cells are characterized with respect to general shape (cellular morphology), proliferative capacity, genetic stability using exome sequencing (i.e., DNA sequencing focusing on the protein-coding regions of the genome) and RNA sequencing over the course of multiple generations, and gene expression patterns. RNA sequencing and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) to evaluate gene expression patterns of target genes [12] is used to confirm cells are of mesenchymal lineage, which can

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dated January 17, 2023, Wildtype performed one-time testing on representative batches of harvested cell material and confirmed norovirus and hepatitis A were not detected.

[11] Potential parasites identified by Wildtype as being associated with a variety of marine fish, including salmon, include tapeworms (*Diphyllobothrium* spp.), roundworms (*Anisakis* spp. and *Pseudoterranova* spp.), liver flukes (*Opisthorchis* spp. and *Clonorchis* spp.), lung flukes (*Paragonimus* spp.), intestinal flukes (*Heterophyidae* and *Echinostomatidae*), and protozoa *(Cryptosporidium parvum*, *Cyclospora* spp., and *Giardia* spp.). In the June 27, 2022, disclosable safety narrative, Wildtype describes its mitigation and control strategies, including performing cell isolation and cell line establishment under aseptic conditions, and use of a thermal processing step following harvest.

[12] In the amendment dated April 2, 2025, Wildtype provides details regarding the genetic stability and gene expression profile of its production cell line. The firm performed baseline exome sequencing and gene expression analyses on the production cell line in 2020, which serves as the control for re-sequencing and gene expression analyses performed over multiple passages of cells collected over the course of four years. The firm reports that exome sequencing results over the course of multiple passages revealed no changes in the DNA sequence of the protein-coding region of the genome. FDA notes that exome sequencing does not fully explore the non-coding regions of the genome and may not detect larger structural variations (e.g., deletions, duplications, inversions) that can impact genetic stability. To further assess genetic stability, the firm monitors the phenotypic stability of the cultured cells (i.e., cell growth, viability, metabolism (e.g., cell culture pH)), as well as gene expression levels of the cultured cells to detect statistically significant changes in gene expression levels which may indicate a loss of

acquire characteristics of muscle (myocytes), fat (adipocytes), and connective tissue cells (fibroblasts) through differentiation. Reagents used at this stage may be cell culture media, media components, and antibiotics and antifungals.

Wildtype describes methods used for originating tissue isolation, including the cell culture media. Cell culture media inputs used during the cell banking and production stages are sourced from suppliers with tightly controlled quality standards. Antibiotics and antifungals are used to support establishment of sterile culture conditions during the development of the cell bank.

Establishment of Cell Lines

Wildtype propagates and screens the isolated cells to assess proliferation rate, cell viability, differentiation potential, and the ability to grow in suspension within the cell culture media without the need for attachment to surfaces.

Potential hazards and quality issues identified by Wildtype at this stage include:

- Incorrect source-animal identity resulting in isolated cells of incorrect origin;
- Introduction of adventitious agents from human handling or the local environment, including bacterial (*Mycoplasma* spp., *L. monocytogenes*, *Salmonella* serovars*, S. aureus,* and *Fusobacterium* spp.) and fungal contamination (such as *Aspergillus* spp.); and
- Carry-over of heavy metal contaminants from isolated cells.

Wildtype confirms the species identity of cell lines using DNA barcoding of the *COI* mitochondrial gene, specifically, where *COI* PCR products are Sanger sequenced and aligned to the Barcode of Life database to confirm the cell line species identity as coho salmon (*Oncorhynchus kisutch*).

Wildtype screens for the presence of *Mycoplasma* spp. during cell line establishment using in-house tests, including fluorescent staining, microscopy, and PCR. The firm also screens for additional adventitious agents and for sterility for purposes of quality and safety. The firm discusses details of testing, which is conducted using validated methods by a third-party laboratory using either observation of potential microbial growth under permissive conditions (for aerobic plate count (*S. aureus*), anerobic plate count (*Fusobacterium* spp.), and fungal growth (*Aspergillus* spp.)) or real-time PCR analysis (for *L. monocytogenes* and *Salmonella* serovars). Third-party toxicological testing using validated methods is conducted to measure the levels of toxic heavy metals, including arsenic, mercury, cadmium, and lead in the harvested cell material at the end of the proliferation stage. Wildtype compares the results of

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genetic stability. Wildtype's gene expression analyses of the production cell line (*via* RNA sequencing and RT-qPCR over multiple passages) revealed no changes in gene expression levels, providing additional support for the firm's conclusion that the production cell line is genetically stable. The gene expression analyses also support Wildtype's conclusions regarding the cell type identity of its production cell line. Specifically, the firm notes that the expression of myogenic (e.g., *MyoD*, *Myf5*, *MyoG*, *Pax7*) and adipogenic (e.g., *C/EBPβ*) genes in its production cell line is consistent with cells of mesenchymal lineage.

the third-party microbiological and toxicological tests to the analytical testing results for wild coho salmon (*Oncorhynchus kisutch*).

Establishment of MCB and WCB

Wildtype describes its MCB and WCB as a collection of cryopreserved cells derived from muscle and connective tissue sourced from a single salmon at the fry stage of development. Cell lines from a single source are seeded, expanded, washed, and centrifuged to remove residual media. The cell pellet is resuspended in media containing a cryoprotectant and aliquoted to create three lots designated as MCB and two lots designated as WCB.

Potential hazards and quality issues identified by Wildtype at this stage include:

- Use of an unintended cell line due to documentation or handling errors;
- Presence of residual reagents (i.e., cryoprotectants) used during cell line establishment in the harvested cell material;
- Introduction of adventitious agents from the local environment or from animal-derived reagents (e.g., porcine trypsin, fetal bovine serum (FBS), bovine serum albumin (BSA)) used in the establishment of the MCB and WCB.

As described above in the "Establishment of Cell Lines" section, Wildtype verifies the genetic identity of cell lines as coho salmon (*Oncorhynchus kisutch*) using *COI* barcoding prior to cell banking. Records for each cell line are securely maintained and include the cell line name, operator name, and date. Vials of MCBs and WCBs are clearly labeled and color coded and a separate liquid nitrogen storage tank is maintained for each seafood species under development at Wildtype.

Wildtype states that it periodically tests for the presence of the cryoprotectant, dimethyl sulfoxide (DMSO), in the harvested cell material. The firm provides analytical results for a single batch of the harvested cell material, demonstrating that DMSO was below the limit of detection (LOD) of <50 ppm.

Food safety and quality management systems are in place to account for the potential risks associated with the use of animal-derived substances during cell line establishment, including a Supplier Approval Program and vendor verification that animal-derived components test negative for relevant adventitious agents. All bovine-derived substances are verified to be sourced from bovine spongiform encephalopathy (BSE)-free/risk-negligible herds and compliant with 21 CFR 189.5, prohibited cattle materials. The firm notes that the controls in place are adequate to manage contamination risk from sera and any other animal-derived substances that could be used in production.[13]

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[13] Wildtype provides a description of the process used to verify that animal-derived components used during cell line establishment are free of adventitious agents in the amendment dated April 2, 2025. The firm provides certificates of analysis (COAs) for porcine trypsin and FBS (in the amendment dated April 2, 2025), as well as BSA (in the amendment dated April 11, 2025), demonstrating that the animal-derived components are tested and

For quality purposes, one vial of every lot that is submitted to Wildtype's MCB is tested by a third-party accredited laboratory for absence of aerobic and anaerobic bacterial growth and fungal growth using sterility testing and anerobic culture conditions. Lots are also screened in-house to detect intracellular *Mycoplasma* spp. contamination prior to submitting new MCB and WCB vials.[14] *Mycoplasma* spp. screens are conducted using fluorescence microscopy and PCR, which are standard and validated methods to detect for the presence of *Mycoplasma* spp. contamination in cell culture.

Production Process

Wildtype provides information about its production process, including:

- The proliferation phase using suspension culture; and
- Cell harvest

Wildtype states that the firm's food safety and quality systems are based on the requirements prescribed in 21 CFR 123.6 with a HACCP plan and in 21 CFR part 117 subpart B, cGMP. The food safety and quality systems are supplemented by the firm's high standards of cleanliness, education, training, and supervision needed by cell culture operations. Wildtype applies allergen controls, including separating MCB and WCBs by species and a label review program to prevent cross-species contamination. Batch records are maintained to provide traceability of every input used during the commercial production process, track corrective actions, and enable quality assurance personnel to verify all quality and safety standards are met prior to release of the finished food product into commerce. Wildtype also states that all incoming raw materials, such as powdered cell culture media, are obtained through a supplier approval program which includes hazard assessments of materials, review of supplier qualifications, incoming materials inspections, supplier performance monitoring (including annual inspections), notice of supplier non-conformance and corrective actions (as applicable), supplier approval checklist, and record keeping. Upon receipt by Wildtype, raw materials are inspected, validated (content and quality attributes), and stored under appropriate conditions. Liquid media is sterilized using an appropriate filter and stored at 2-8 °C. Wildtype states that the firm uses food contact materials that are permitted for their intended use throughout the production process.[15]

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certified to not contain relevant adventitious agents (e.g., bovine-associated viruses tested per 9 CFR 113.53 (c), porcine-associated viruses tested per 9 CFR § 113.53 (d)), including the prion causative agent of bovine spongiform encephalopathy (BSE).

[14] Sterility of MCB and WCB vials is evaluated by testing for adventitious agents including *Staphylococcus* spp., *Fusobacterium* spp., *Aspergillus* spp., and *Mycoplasma* spp. using methods validated for their intended use.

[15] The production conditions described by the firm would be consistent with food type 1 (nonacid, aqueous products; may contain salt or sugar or both (pH above 5.0)) and conditions of use type D (hot filled or pasteurized below 66 °C). The various food types and conditions of use are described in Appendix V of FDA's "Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances (Chemistry Recommendations)."

Wildtype's production process utilizes aseptic techniques or hygienic controls at different steps of the process. The production process is described as consisting of the: (1) proliferation phase using suspension fed-batch culture, and (2) harvest of cell material from bioreactors using bowl centrifugation followed by washing with a water and sugar solution.

Wildtype states that it maintains sterile technique throughout the proliferation and harvest phases. The proliferation phase involves handling cells in small-scale culture vessels or in closed, stainless steel bioreactor systems. Wildtype states that, for smaller-scale culture vessels, any cell passages or sampling are conducted in high-efficiency particulate air-filtered biological safety cabinets by operators trained on appropriate aseptic practices. Cultures in larger suspension bioreactors use aseptic procedures to reduce the risk of introducing contaminants, and stainless-steel culture vessels are sterilized using a combination of validated standard operating procedures (SOPs) using cleaning agents and high temperature steam. Wildtype notes that the presence of potential adventitious agents of concern (*Salmonella* serovars*, L. monocytogenes*, and *S. aureus*) in culture during the proliferation phase would negatively affect the production capacity of the cell culture system, trigger quality control checks, and are inherently self-limiting. Nevertheless, Wildtype uses real-time pH monitoring of cultures in smaller-scale culture vessels and bioreactors to detect the presence of microbial contamination. Cultures that experience a pH above 7.8 or below 7.1 are determined to be at risk for contamination and subjected to further screening including microscopy. If an adventitious agent is detected, the cell culture is immediately sterilized and discarded.

After the proliferation phase is complete, cells are harvested under sterile conditions and stored at low temperatures (frozen at ≤-20 °C). Wildtype also states that an environmental monitoring program is in place to assess the effectiveness of overall hygienic practices in the manufacturing facility.

Proliferation Phase Using Suspension Culture

One or more vials of frozen cells from the WCB are thawed and placed in sterile culture medium in a small vessel under sterile conditions. Following multiple rounds of cell division and growth (proliferation), the cells are transferred to larger vessels (passaging). This process, under continued sterile conditions, is repeated with increasingly larger vessels to accumulate the desired quantity of cells (i.e., a seed train). The seed train cultures are agitated so that the cells are suspended in a homogeneous mixture within the liquid culture medium inside the vessel. Wildtype states that all vessels are either pre-sterilized single-use, food grade plastic, or reusable vessels that can be cleaned and sterilized between uses.

Wildtype identifies potential hazards associated with this production stage including:

- Use of an unintended cell line from another species due to documentation or handling errors;
- Introduction of adventitious agents via contaminated culture media components or inadequate sterilization of bioreactors;
- Introduction of adventitious agents present in the local environment of the production facility during passaging from one vessel to another;
- Introduction of metal fragments produced by metal-to-metal contact in bioreactor; and

- Introduction of media components that could persist in the cell material through proliferation and washing at harvest, thus being present as residues in the harvested cell material.

Wildtype manages the risk associated with the first hazard by utilizing a two-tiered preventive control plan. First, cell lines derived from different species are maintained in separate liquid nitrogen storage tanks sufficiently labeled as a precaution to prevent potential errors during the cryopreservation step. Second, when removing cryovials for thaw, two individuals independently certify that the storage tank and cryovial species match and are the correct species for production. Seed train operators review the two written certifications and sign off that the species thawed matches the production target species before moving cell lines into production.

Wildtype manages the risks associated with the next two hazards, introduction of adventitious agents (bullets two and three above), through the sterile procedures and monitoring programs discussed above in the "Production Process" section and through third-party testing of the harvested cell material and the finished food product (e.g., the harvested cell material mixed with food ingredients). Preventive controls associated with the introduction of metal fragments and other foreign material include visual inspection and X-ray screening of the finished food product. Safety considerations associated with the introduction of media components that could persist in the harvested cell material are discussed below in the section, "Substances Used in the Production Process."

Cell Harvest

Wildtype states that at the end of proliferation, cells are collected from the bioreactor via bowl centrifugation and washed three times with a water and sugar solution. After washing, cells are frozen and stored in a ≤-20 ˚C freezer ahead of post-harvest processing steps.

The potential hazards identified by the firm associated with this production stage include:

- Introduction of adventitious agents during the cell culture process; and
- Media components that could be present as residues after washing.

Wildtype manages risks associated with the introduction of adventitious agents (e.g., *Salmonella* serovars*, Listeria* spp.*, S. aureus*) by practicing aseptic technique, employing cGMPs, and through the adventitious agent tests and specifications discussed in the "Characterization of the Harvested Cell Material" section. Furthermore, Wildtype performs a post-harvest thermal processing step on the finished food product to inactivate potential adventitious agents, mitigating the risks of their introduction at prior steps in the production process. Wildtype provides data and information demonstrating the effectiveness of the post-harvest thermal processing step which is an important process control identified in the firm's HACCP plan.

Safety considerations associated with the use of media components that could be present as residues after washing are discussed in the subsequent section, "Substances Used in the Production Process."

Substances Used in the Production Process

Wildtype provides information about the substances used during its production process in the form of cell culture media and other components, including:

- nutrients used to support primary cell metabolism;
- substances to manage properties of the culture medium;
- substances intended to support cell proliferation during culture; and
- substances used to harvest the cell material.

For each substance, Wildtype provides information about the identity and the basis for its safety conclusion, and in certain cases information about estimated consumer exposure.[16]

The firm's cell culture medium is described as containing substances required for growth, including amino acids, nucleotides, energy substrates (e.g., sugars), fatty acids, vitamins, minerals, trace elements, and salts. Additional substances identified by Wildtype during the production phase (i.e., proliferation and harvest) include emulsifiers and surfactants, antioxidants, a growth factor, and a wash solution (i.e., a water and sugar solution).[17] The firm explains that most of these substances are already widely consumed by humans as part of the U.S. food supply and notes that many are present in commonly consumed, commercially available conventional salmon. The firm states that the non-nutrient substances listed above are largely removed from the harvested cell material by washing[18], and that residual levels in the product do not present concerns given the available safety information and existing use or presence in the U.S. food supply. No antibiotic or antifungals are used during the proliferation and harvest stages of the production process.

Wildtype describes the firm's general framework for evaluating substances intended for use during the proliferation and harvest stages of production, including whether substances used during proliferation and harvest are currently authorized by FDA for use in human food as a result of a food additive regulation or effective food contact notification, or FDA evaluation of a generally recognized as safe (GRAS) notice. The firm also considered prior use or natural presence in conventional food and anticipated dietary exposure. In particular, Wildtype discusses the firm's intended use of media supplements to support proliferation and metabolism of cells in culture, specifically, a recombinant salmon growth factor and a non-

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[16] A complete list of these substances was provided to FDA as supporting, corroborative information in the supplemental, confidential material.

[17] Wildtype informed FDA of updates to substances used during proliferation and harvest in the amendment dated August 30, 2024. At FDA's request, the firm provided a complete list of all substances used during the production process to FDA in a December 11, 2024, amendment to the supplemental, confidential material.

[18] Wildtype provides exposure estimates (i.e., estimated daily intakes (EDIs)) for substances used in the production process as supporting, corroborative information in the supplemental, confidential material. In the August 30, 2024, amendment Wildtype provided updated theoretical EDIs based the conservative assumption that the concentration of substances in the harvested cell material is the same as the level of the substance in the cell culture medium. Analytical EDIs are based on the residual levels of substances in three, non-consecutive batches of harvested cell material.

proteinogenic amino acid, respectively. The firm also publicly discloses data and information regarding a substance, methyl-β-cyclodextrin, for which the intended uses are not addressed by an existing, authorizing regulation, effective food contact notification, FDA evaluation of a GRAS notice, or another authorization, including the identity, toxicological studies or other relevant safety data, and estimates of consumer exposure informed by batch analysis of its harvested cell material. In addition to its discussion on the relevant safety-related information of these components of the cell culture medium, the firm also considered the estimated intake level derived from its analytical data from the harvested cell material for each component with reference to levels present in one or more currently consumed comparator foods. This information provided by Wildtype is described in more detail below.

Recombinant growth factors

In the amendment dated August 30, 2024, Wildtype states that Fibroblast Growth Factor 2 (FGF2), also known as basic fibroblast growth factor, is the only growth factor used in the firm's current production process. Other functional proteins described in Wildtype's June 27, 2022, submission (i.e., insulin, insulin-like growth factor (IGF), albumin, and transferrin) are no longer used in the production process.

Wildtype states that the use of FGF2 in production is necessary to replace biological sources that would ordinarily be available to animal cells *in vivo,* that the gene sequence is derived from salmon, and that this protein is highly conserved in humans and in animals that are consumed by humans. Wildtype's assessment of the firm's intended uses considered several factors, including:

- published information on the levels of FGF2 in serum from healthy men and women;
- published information on the stability of growth factors;
- analytical data on the presence of FGF2 in Wildtype's cultured cell material after harvest and post-harvest processing;
- a published non-oral study of human recombinant FGF2; and
- the physiological ranges of this protein in conventional seafood.

As part of the firm's material input safety assessment, Wildtype notes that its assessment of the available scientific literature indicates that growth factors are generally recognized as having low stability and degrade quickly with temperature fluctuations, both heating and freezing, indicating that Wildtype's post-harvest thermal processing and blast chilling steps will denature and deactivate residual FGF2. The firm discusses a modification to FGF2 which enhances thermostability of the protein at temperatures used for cell growth, but references published studies indicating that the modified, thermostable FGF2 would be inactivated by both proteases in the cell culture medium and at higher temperatures in the range Wildtype uses for its post-harvest thermal processing step. Moreover, Wildtype provides data supporting

that FGF2 is not detected in its finished food product (i.e., a ready-to-eat coho salmon product).[19]

Wildtype referenced a non-oral one-month study conducted in dogs that reported a no observed adverse effect level (NOAEL) of >480 mg/kg body weight (bw)/d for recombinant human FGF2, which shares homology with, but is not identical to, the recombinant salmon FGF2 used by the firm. No recombinant proteins derived from the human genome are used in the firm's production process.

The firm cites data reporting that the peak concentration of FGF2 in rainbow trout is 10-100 ng/mL and <4.0 ng/L and <10.8 ng/L in men and women, respectively. Wildtype further states that, given the firm's production process and the naturally occurring levels, FGF2:

- exposure from the harvested cell material is infinitesimally small compared to total circulating FGF2 levels in humans (<11 and <29.7 ng, in men and women, respectively);
- is present in the harvested cell material at lower levels to those found in foods derived from fish muscle tissue, such as chinook salmon or rainbow trout;
- would be present at very low or undetectable levels in the finished food product after conventional food processing; and
- would likewise be broken down by both heat (e.g., cooking) and gastric fluids.

Substances to manage properties of the culture media

Methyl-β-cyclodextrin is a cyclic oligosaccharide with a hydrophobic cavity that is used in cell culture to increase the solubility of non-polar substances such as fatty acids and cholesterol. Methyl-β-cyclodextrin is not the subject of an existing U.S. food ingredient authorization or GRAS notice evaluated by FDA, nor does it have publicly available safety data. However, the firm notes that methyl-β-cyclodextrin shares high structural similarity to β-cyclodextrin, a compound for which there is extensive published safety data that may be used to read-across to methyl-β-cyclodextrin. Wildtype notes that β-cyclodextrin is used in various foods, is the subject of GRAS notice 000074, and had safety data establishing an acceptable daily intake (ADI) of 5 mg/kg bw/d. Wildtype analyzed the concentration of methyl-β-cyclodextrin in its harvested cell material and reported an EDI of <0.22 mg/kg bw/d based on analytical data. FDA asked the firm to consider whether its use of methyl-β-cyclodextrin, combined with levels of β-cyclodextrin found in the diet, would exceed the ADI for this substance. The firm noted that the EDI for β-cyclodextrin at the 90th percentile is 0.15 mg/kg bw/d, and that addition of methyl-β-cyclodextrin from Wildtype's harvested cell material would result in a cumulative EDI of <0.37 mg/kg bw/d, which is substantially lower than the ADI of 5 mg/kg bw/d for β-cyclodextrin.

[19] Wildtype conducted testing on its finished food product (i.e., a ready-to-eat coho salmon product) to validate its conclusion regarding the anticipated residual level of the thermostable FGF2 protein used in the firm's production process. All results were consistent with the conclusion that this protein would be present at low to undetectable levels in the firm's finished food product.

Nutrients used to support primary cell metabolism

Wildtype considered relevant data and information on substances used to support primary cell metabolism, including available toxicological data, presence in foods, and presence in the firm's harvested cell material. The firm reports that these substances are present in the harvested cell material at levels comparable to those found in conventional salmon, or at levels found in other commonly consumed foods while also being well below reference exposure values identified by various regulatory bodies that assess the safety of food, or both.

Characterization of Harvested Cell Material

Identity

As described above, during cell line establishment Wildtype selects for and banks pluripotent, mesenchymal cells and uses *COI* mitochondrial gene sequencing to verify the species identity of the MCB as coho salmon (*Oncorhynchus kisutch*). The firm also implements cell bank inventory controls (i.e., separating MCB vials for different species in different liquid nitrogen dewars, WCB vial inventory controls, and two-person checks and sign-off) to ensure that the correct WCB vials are used as inputs in the production process. The firm verifies the species identity of the harvested cell material as coho salmon (*Oncorhynchus kisutch*) using *COI* mitochondrial gene sequencing. Wildtype examines cultured cells during the proliferation phase of the production process to ensure that cell growth rate, size, and morphology conform to expected, well-characterize phenotypic parameters (i.e., growth rate ±35% or less than the average expected growth rate; cell size ±25% deviation from expected diameter of 11-18 μm; expected cell shape (uniform, spherical, and without membrane irregularities)). The firm examines the harvested cell material to ensure the cells demonstrate the expected phenotypic characteristics.

Wildtype does not routinely verify the cell type identity of the harvested cell material using gene expression or cell marker analyses (e.g., RT-qPCR, immunofluorescence); however, the firm verifies that its production cell line is comprised of cells that express mesenchymal lineage genes, as described in the previous section "Establishment of Cell Lines." The firm acknowledges that it is theoretically possible for cells to undergo spontaneous differentiation during the proliferation stage of the production process. Wildtype states that, should spontaneous differentiation occur during the production process, the presence of spontaneously differentiated cells in the harvested cell material would not pose a safety concern, considering that mesenchymal lineage cell types (i.e., fat, muscle, connective cells) are regularly found in conventional salmon. To support its conclusions regarding the cell type identity of the harvested cell material, the firm states that, to date, it has not observed spontaneously differentiated cells in the harvested cell material. Further, the firm explains that spontaneous differentiation, while theoretically possible, is unlikely to occur during the production of the harvested cell material for several reasons: first, given the fact that terminal differentiation leads to proliferative arrest (i.e., the end of cell division), spontaneous differentiation would slow the growth rate and would be readily detected; second, as noted above, the firm has yet to observe spontaneously differentiated cells in its harvested cell material.

Adventitious Agents and Contaminants

Wildtype describes compositional analysis and characterization of the harvested cell material. The compositional analyses of the harvested cell material include major nutrients (proteins and amino acids, fats, carbohydrates, minerals, and vitamins), and some residues of media components. Wildtype provides information and specifications for adventitious agent and toxic heavy metals that could potentially be present in the harvested cell material and presents results from three independent batches demonstrating conformance with the stated specifications.[20] Data was concurrently generated using wild-caught coho salmon as a comparator.

Wildtype provides microbial specifications for each batch of harvested cell material, including:

- Aerobic plate count (<100 colony-forming units (CFU/g)
- Yeast/mold (<20 CFU/g)
- *Enterobacteriaceae* (<20 CFU/g)
- Total coliforms (<100 CFU/g)
- *Escherichia coli* (<20 CFU/g)
- *Escherichia coli* O157:H7 (not detected in 25 g)
- *Campylobacter* spp. (not detected in 25 g)
- *Salmonella* serovars (not detected in 25 g)
- *Listeria* spp. (not detected in 25 g)
- *Staphylococcus aureus* (<20 CFU/g)[21]
- *Bacillus cereus* (<100 CFU/g)
- *Clostridium perfringens* (<10 CFU/g)
- *Clostridium botulinum* (not detected in 8 g)

Microbial testing was performed using methods validated for their intended purposes.

Wildtype provides specifications for toxic heavy metals that are commonly considered in conventional food manufacturing and could potentially be present as contaminants in the harvested cell material.[22] Toxic heavy metal specifications for each batch of harvested cell material include:

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[20] In the amendment dated April 2, 2025, Wildtype commits to testing each batch of harvested cell material for adventitious agents and toxic heavy metals and states that it will notify FDA in a supplement to CCC 000005 should it make changes to its testing regime.

[21] Wildtype addresses the potential for production of *S. aureus* and *C. botulinum* toxins in its production process. In the amendment dated July 28, 2023, the firm performed one-time testing on representative batches of harvested cell material and confirmed these toxins were not detected. The firm states that testing for the presence of *S. aureus* and *C. botulinum* were selected because the organisms are a necessary precursor to the associated toxins. Wildtype also notes that it performs routine pH monitoring and microscopy during the early stages of the production process and further mitigates the potential for contamination via cGMP during the production process.

[22] Wildtype provides the same toxic heavy metal specifications for the finished food product.

- Arsenic (<50 ppb)[23]
- Lead (<20 ppb)
- Mercury (<20 ppb)
- Cadmium (<20 ppb)

Composition

Wildtype conducted a compositional analysis of three independent batches of the harvested cell material, including proximates, amino acids, vitamins, and minerals.[24] Proximates include moisture, protein, fat, ash, and carbohydrate content, and the firm provided specifications ranges for proximates in the harvested cell material. As a point of reference, the firm provides nutrition data from a U.S. Department of Agriculture (USDA) database on conventional salmon products.[25] The harvested cell material was washed with a water and sugar solution, resulting in moisture content that was somewhat higher in the firm's independent batches relative to the conventional coho salmon reference data. The total fat content was lower in the harvested cell material (1.13%-1.75%) versus the conventional coho salmon comparator (5.93%). The total amino acid content and individual amino acids were lower in the harvested cell material relative to the conventional coho salmon comparator, which the firm notes is likely due to the absence of protein-rich extracellular structures that are found in conventional salmon products. The relative levels of minerals were similar between the harvested cell material and conventional coho salmon, with modest decreases in the levels of iron, magnesium, potassium, and selenium in the harvested cell material.

Wildtype provides compositional analyses for its finished food product (i.e., a ready-to-eat coho salmon product) for reference.

FDA's Evaluation

FDA evaluated the information provided by Wildtype with respect to the established cell lines, cell banks, substances used in the production process, and properties of the harvested cell material that collectively are the subject of CCC 000005. The primary focus of FDA's evaluation is the information on which the firm relies to conclude that the harvested cell material is safe for use as food and does not contain substances or microorganisms that would adulterate the food.

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[23] In the amendment dated December 11, 2024, Wildtype reduced its specification for arsenic to <50 ppb.

[24] As noted in the previous section, "Substances Used in the Production Process," in the amendment dated August 30, 2024, Wildtype notified FDA of updates to its production process, including the use of animal-component free medium supplemented with a recombinant salmon FGF2. The firm provides analytical data for proximates (moisture, total fat, protein, ash, carbohydrates), vitamins, minerals, toxic heavy metals, trace metals, and fatty acids for three non-consecutive batches of harvested cell material in the amendment dated August 30, 2024.

[25] Wildtype provides comparator data for conventional salmon products from the USDA FoodData Central in the amendment dated December 11, 2024. Notably, comparator data from the USDA FoodData Central entry for "Fish, salmon, coho, farmed, raw" (FDC ID 173715) is available for moisture, protein, ash, carbohydrates, minerals, and some fatty acids. *Trans* fat comparator data from data from "Fish, salmon, coho, farmed, raw" (FDC ID 173715) was not available, and, as such, the firm provides *trans* fat comparator data for "Fish, salmon, sockeye, raw" (FDC ID 173691) and "Fish, salmon, pink, raw" (FDC ID 175138).

Wildtype provides information on the establishment of the cell lines used to produce the food that is the subject of CCC 000005. FDA considered the information on the source and lineages of the cell lines, the culture adaptation process, the processes used to immortalize the cell lines (selection of cells based on attachment affinities and nutritional conditions), and the harvested cell material. We also considered the information provided by Wildtype with respect to the observed behavior of the cell lines in culture, the genetic capacity of animal cells to produce toxins or other potentially harmful substances, and the viability of cells following harvest.

The information reported was consistent with salmon cells that displayed enhanced replicative capacity under *in vitro* conditions. However, once removed from the protected and controlled environment of the bioreactor the cells die quickly, removing any replicative capacity. Subsequent food processing (such as cooking) would further break down cellular structures and contents. Digestion after consuming food made from the harvested cell material would also break down any residual cellular structure. No information presented by the firm or otherwise available to us indicated any mechanism by which the harvested cell material, once rendered non-living, heated, consumed, and digested, would retain any replicative capacity or the ability to induce replicative capacity in living cells exposed to this material.

Wildtype notes the harvested cell material will present the same allergenicity concerns to consumers who may be allergic to conventional salmon. The firm states that it will address concerns regarding allergenicity of the harvested cell material through product labeling.

In summary, we did not identify any properties of the cells as described that would render them different from other animal cells with respect to safety for food use.

Regarding the production process, FDA considered the data and information pertaining to the firm's hazard analysis for each phase in the production process and its rationales for risk-based preventive controls, including Wildtype's assessment of potential sources for introduction of adventitious agents, and the corresponding mitigation and control strategies for each hazard identified. We also considered Wildtype's use of a HACCP plan developed in accordance with 21 CFR 123.6, cGMPs, and supporting programs such as environmental monitoring, sanitation control, supply-chain controls, and other controls. No data and information presented by the firm or otherwise available to us indicated that the selected test strategies would be inadequate to control for the presence of biological, chemical, or physical hazards or to maintain product quality. We note the self-limiting nature of quality failures related to adventitious agent control in the production process. In summary, we did not identify elements of the production process, as described in CCC 000005, that indicate an unaddressed food safety risk resulting from microbial, viral, or other contaminants.

FDA considered the general framework that Wildtype used for evaluating the safety of each substance for its intended use as well as the complete list of substances provided as supporting, corroborative information in the supplemental, confidential material, including the identity, intended use, anticipated dietary exposure, and relevant data on safety and existing authorizations or evaluations in the U.S. We also considered the data and information presented by the firm regarding its assessment of the use of a thermostable, recombinant FGF2 derived from the salmon genome, including the intended use level, anticipated digestive fate, and corroborative analytical data regarding residual presence of the recombinant salmon FGF2 used in the production process in its finished food product. Furthermore, FDA considered that

the analytical data-based EDI of <0.22 mg/kg bw/d for methyl-β-cyclodextrin is well below the ADI of 5 mg/kg bw/d for the structurally similar, well-studied β-cyclodextrin, supporting the safety of methyl-β-cyclodextrin, a substance new to the U.S. human food supply. We note that the substances described by Wildtype have no intended technical or functional effect in the harvested cell material and if present are expected to be at minimal levels.

We did not identify any substance uses that would lead us to question Wildtype's conclusion regarding the safety of its food given available information, existing uses or authorizations in food, and anticipated exposure. We noted moderately lower levels of several nutritional components relative to conventional salmon (discussed above in the "Characterization of the Harvested Cell Material" section); however, the harvested cell material, which is the subject of CCC 000005, is expected to be mixed with food ingredients to produce the finished food product. Therefore, the nutritional composition of the finished food product containing the harvested cell material will depend on the type and amount of other ingredients in the product. Regarding the use of any food contact materials throughout the production process, we note that the production conditions described by the firm during culture for food production and immediately subsequent to harvest are consistent with food type 1 (nonacid, aqueous products; may contain salt or sugar or both [pH above 5.0]) and conditions of use type D (hot filled or pasteurized below 66 °C) save for post-harvest storage (conditions F or G for refrigeration or frozen storage, respectively). Thus, any food contact materials authorized for these conditions would be appropriate.[26]

FDA reviewed the data and information that was provided on the identity and composition of the harvested cell material, including batch test data for constituents and contaminants, and specifications. We considered the analytical data provided by Wildtype on the composition of the harvested cell material from several production runs as one element in characterizing the identity of its product, as evidence of the firm's ability to conform to its stated specifications for food contaminants, and as relevant information in evaluating the relationship between the production process described in CCC 000005 and the properties of the harvested cell material produced through that process. We evaluated the firm's specifications for toxic heavy metals to ensure they were as low as reasonably possible and were consistent with levels that are considered safe in food.

We also considered information relating to compositional analysis. We did not consider the establishment of exact equivalence of all nutrients and components relative to a particular conventional comparator as a necessary component of Wildtype's safety conclusion, nor did we interpret the analytical data provided by the firm as definitive nutritional information regarding either harvested cell material produced through the process defined in CCC 000005 or food products that contain this material.

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[26] As noted earlier, the various food types and conditions of use are described in Appendix V of FDA's "Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances (Chemistry Recommendations)."

Conclusions

Based on our evaluation of the data and information that Wildtype provides in CCC 000005, as well as other information available to FDA, we did not identify a basis for concluding that the production process as described would be expected to result in food that bears or contains any substance or microorganism that would adulterate the food. We have no questions at this time about Wildtype's conclusion that foods comprised of, or containing, the harvested cell material resulting from the production process defined in CCC 000005 are as safe as comparable foods produced by other methods.

ASHLEY E. NAZARIO-TOOLE -S

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Ashley Nazario Toole

Appendix: Summary of potential identity, quality, and safety issues

Process Step	Potential Issues	Management Strategy
Cell Isolation	Cell line identification, cells from different line or species inadvertently used	cGMP, supplier documentation
Cell Isolation	Carryover of adventitious agents such as bacteria, fungi, viruses, parasites, and prions from source animal	Animal health documentation, antibiotics and antifungal application, cGMP, sterile filtration, testing, thermal step
Cell Isolation	Introduction of adventitious agents during isolation	Antibiotic solution, aseptic procedures, hygienic condition, sterile rinsing, testing, thermal step, visual observation via gross inspection and microscopy
Cell Isolation	Introduction of contaminants in laboratory reagents	Sterilization, supply-chain controls, thermal step, visual monitoring (SOPs)
Cell Isolation	Facility environment contamination	Aseptic procedures, hygienic condition, thermal step
Establishment of Cell Lines	Cell line identification, cells from different line or species inadvertently used	Color-coded label, PCR testing
Establishment of Cell Lines	Cells do not display expected growth profile; genetic instability	Exome sequencing, gene expression analyses, testing, monitoring doubling time
Establishment of Cell Lines	Contamination with adventitious agents	Aseptic procedures, cGMP, hygienic conditions, sterilization, testing, use of antimicrobials, thermal step, visual observation via pH check and microscopy
Establishment of Cell Lines	Introduction of adventitious agents from media components	Sterile filtration, supply-chain controls, testing, thermal step, visual monitoring
Establishment of Cell Lines	Introduction of microbial toxins (e.g., histamine)	Testing
Establishment of Cell Lines	Introduction of chemical hazards	Allergen controls, cGMP, document control and training, sanitation controls, supply-chain controls
Establishment of Cell Lines	Introduction of physical hazards	cGMP, sanitation controls, sterile filter, supply-chain controls, visual monitoring, X-ray
Establishment of Cell Lines	Facility environment contamination	Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls
Establishment of MCB and WCB	Cells from different line or species inadvertently used	PCR testing, color-coded label
Establishment of MCB and WCB	Genetic instability	Exome sequencing, gene expression analyses, monitoring doubling time

Establishment of MCB and WCB	Introduction of adventitious agents	Aseptic procedures, cGMP, environmental monitoring, sterilization, testing, visual observation via pH check and microscopy
Establishment of MCB and WCB	Contamination with adventitious agents from original animal source	Testing
Establishment of MCB and WCB	Contamination with adventitious agents from culture media components	Aseptic procedures, cGMP, environmental monitoring, sanitation controls, sterile filter, supply-chain controls, testing
Establishment of MCB and WCB	Introduction of chemical hazards	Allergen controls, cGMP, document control and training, sanitation controls, supply-chain controls, testing
Establishment of MCB and WCB	Introduction of physical hazards	cGMP, sanitation controls, sterile filter, supply-chain controls, visual monitoring, X-ray
Establishment of MCB and WCB	Facility environment contamination	Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls
Proliferation Phase (Cell Thaw, Seed Train, and Proliferation)	Inadvertent introduction of cryoprotectant	Food safety assessment, testing
Proliferation Phase	Cells from different line or species inadvertently used	Dedicated storage tanks for cell lines of different species, sign-off procedure, supply-chain controls, testing
Proliferation Phase	Phenotypic stability	Monitoring cell density and cell metabolism
Proliferation Phase	Introduction of adventitious agents during proliferation phase	Aseptic procedures, cGMP, environmental monitoring, sanitation controls, testing, thermal step, visual monitoring
Proliferation Phase	Contamination with adventitious agents from media components	Supply-chain controls, testing, thermal step
Proliferation Phase	Contamination with adventitious agents through inadequate sterilization of vessels and transferring between vessels	cGMP, environmental monitoring, sanitation controls, testing, thermal step
Proliferation Phase	Introduction of media components that could persist as residues in harvested cells	Food safety assessment
Proliferation Phase	Introduction of media components that could accumulate in the cells before harvest	Compositional analysis at harvest, food safety assessment
Proliferation Phase	Introduction of chemical hazards	Allergen controls, cGMP, sanitation controls, supply-chain controls

Proliferation Phase	Introduction of physical hazards	cGMP, sanitation controls, supply-chain controls, sterile filter, visual monitoring, X-ray
Proliferation Phase	Facility environment contamination	Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls
Harvest of Cell Material	Presence of adventitious agents from culture process	Culture monitoring, specifications, testing, thermal step
Harvest of Cell Material	Migration of contaminants from food contact materials	Use of authorized food contact materials
Harvest of Cell Material	Presence of residual media components after harvest	Analytical testing, food safety assessment, wash steps
Harvest of Cell Material	Presence of elemental contaminants (metals) after harvest	Specifications, testing
Harvest of Cell Material	Introduction of chemical hazards	Allergen controls, cGMP, document control and training, sanitation controls, supply-chain controls
Harvest of Cell Material	Introduction of physical hazards	cGMP, sanitation controls, supply-chain controls, sterile filter, visual monitoring, X-ray
Harvest of Cell Material	Facility environment contamination	Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls