U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Memorandum

Date July 24, 2025

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Through

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Subject Cell Culture Consultation (CCC) 000039, Cultured *Gallus gallus domesticus* cell material

To Administrative File, CCC 000039

Submission Received Date: May 21, 2024

Amendments Received Date: October 4, 2024; November 5, 2024; January 15, 2025; March 7, 2025; March 20, 2025; May 28, 2025; June 9, 2025; June 25, 2025[1]

Sponsor: Believer Meats (Believer, the firm)

Summary

The Food and Drug Administration (FDA, we) evaluated the food that is the subject of CCC 000039 submitted by Believer.

[1] Believer submitted a pre-filing amendment as supporting, corroborative information in the supplemental, confidential material on April 12, 2024, regarding discontinuing the use of one of the substances used in the production process as well as another substance from the wash solution.

- This food is defined as the cell material at harvest, comprised of cultured chicken (*Gallus gallus domesticus*) fibroblasts, as produced by the method of manufacture described in CCC 000039.
- The cell lines are originally isolated from fertilized chicken eggs and determined to be fibroblasts through standard methods validated for their intended purpose, including microscopy, RNA sequencing, and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). Species identity of the primary master cell bank was determined using PCR amplification of *MT-CYB*, a mitochondrial gene which encodes cytochrome B. Species identity of the secondary master cell bank and the manufacturer's working cell bank were confirmed using a quantitative PCR (qPCR) chicken ID kit.
- The cell lines are established using spontaneous immortalization, in which cells are propagated until small populations spontaneously break through the replicative limits and demonstrate stable population doubling times. Cell lines are then adapted to growth in suspension culture. Cells are proliferated through a seed train until an adequate number of cells are available to seed a bioreactor system.
- The cells are cultured to increase cell volume in a suspension culture proliferation phase.
- The cells are harvested from the bioreactors and washed in a sodium chloride solution within a continuous closed system.
- The harvested cell material, following washing, is described as cultured chicken (*Gallus gallus domesticus*)[2] fibroblasts, similar in composition and nutritional characteristics to conventional chicken breast products. 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 000039, we have no questions at this time about Believer's conclusion that foods comprised of or containing cultured chicken cell material resulting from the production process defined in CCC 000039 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 000039 would be expected to result in food that bears or contain any substance or microorganism that would adulterate the food.[4]

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[2] Believer 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 moisture, protein and amino acids, fat and fatty acids, vitamins, and minerals were observed in the data from these batch analyses of the harvested cell material, relative to a conventional chicken product. In all cases levels were consistent with those found in commonly consumed foods. Believer's conclusions regarding the safety of its product are not based on the establishment of exact equivalence of all nutrients.

[3] Believer identifies "conventional chicken" as a comparator. In the firm's submission, chicken breast (*Gallus gallus domesticus*) is utilized as a conventional comparator.

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

Production Method

Believer 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 seed cell expansion, suspension culture proliferation in a bioreactor, followed by harvest of the cell material for subsequent conventional food processing.

Believer 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 current good manufacturing practice (cGMP) program that includes all the items enumerated in 21 CFR part 117 subpart B;
- Development of a hazard analysis and risk-based preventive controls (HARPC) food safety plan, including preventive measures and corrective actions for prevention and mitigation of biological, chemical, and physical hazards;
- Validated sanitation processes and an environmental monitoring program;
- A supplier approval program;
- An allergen control program;
- In-process checks and controls of key process parameters;
- 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 products;
- Batch record review; and
- Traceability of raw materials and finished products.

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 Believer. A more detailed version of this table is provided in an Appendix of this memorandum.

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

Process Step	**Potential Issues**	**Management Strategy**
Cell Isolation	Cell identity; contaminants from source, reagents, or environment	Aseptic procedures, hygienic condition, labeling, sterile filtration, supply-chain controls, testing, visual observation
Establishment of Cell Lines	Cell identity; contaminants from materials or environment; appropriate adaptation to culture	Allergen controls, aseptic procedures, cGMP, document control and training, environmental monitoring, hygienic condition, supply-chain controls, sterile filtration, sterilization, testing program, visual observation
Establishment of Primary and Secondary Master Cell Banks and Manufacturer's Working Cell Bank	Cell identity; contaminants from materials, equipment, or environment; media components	Allergen controls, aseptic procedures, cGMP, document control and training, environmental monitoring, food safety assessment[5], hygienic condition, sterile filtration, supply-chain controls, testing program, visual observation
Proliferation Phase	Contaminants from materials, equipment, or environment; media components	Allergen controls, aseptic procedures, cGMP, document control and training, environmental monitoring, food safety assessment, sterile filtration, sterilization, sanitation controls, supply-chain controls, testing program, visual observation
Harvest of Cell Material	Contaminants from materials, equipment, or environment; media components	Allergen controls, aseptic procedures, cGMP, compositional analysis, document control and training, environmental monitoring, food safety assessment, specifications, sterilization, supply-chain controls, testing program, visual observation, washing step; for the finished food product, use of a metal detector[6]

[5] "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.

[6] 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 Believer utilizes a metal detector post-harvest on the finished food product consisting of the harvested

Cell Banking

Believer provides information about the establishment of cell banks used in the subsequent production process. Believer defines a cell bank in the firm's manufacturing process as a collection of cryopreserved cells derived from a single tissue source from a single fertilized egg. The firm describes cell banking as a multi-step process that occurs upstream of the production process. Specifically, Believer uses a system in which there is a primary master cell bank (primary MCB), a secondary master cell bank (secondary MCB) derived from a subset of cells stored in the primary MCB, and the manufacturer's working cell bank (MWCB) derived from a subset of cells stored in the secondary MCB. The steps involved include:

- Cell isolation
- Establishment of cell lines
- Establishment of primary MCBs
- Establishment of secondary MCBs
- Establishment of MWCBs

Unless otherwise noted, reagents used at these stages may include materials of bovine origin (i.e., fetal bovine serum, bovine catalase, bovine serum albumin), antibiotics (penicillin and streptomycin), and other cell culture media inputs. All cell culture media inputs used during the cell banking and production stages are sourced from suppliers that meet Believer's food safety and quality standards.

Cell Isolation

The cells used to establish the cell banks are fibroblasts isolated from fertilized eggs (i.e., embryos) of the Israeli Baladi chicken. Hereafter, the source is referred to as the "source animal" or "animal source."

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cell material and food ingredients, which is identified as a mitigation and control strategy for physical hazards identified by the firm as potential hazards during the production process prior to harvest.

- Potential hazards and quality issues identified by Believer at this stage include: Source animal health prior to tissue procurement resulting in cells contaminated by adventitious agents such as pathogenic bacteria[7], viruses[8], fungi[9], or parasites[10];
- Incorrect source animal or tissue identity resulting in isolated cells of incorrect origin;
- Introduction of adventitious agents from contaminated non-animal sourced reagents, the local environment, water, or human handling including viruses (norovirus) and bacteria, including *Mycoplasma* spp., *Salmonella* serovars, *Campylobacter* spp., *Escherichia coli*, *Listeria* spp., *Bacillus cereus*, and *Staphylococcus aureus*; and
- Introduction of adventitious agents from animal-derived reagents (i.e., fetal bovine serum, bovine catalase, bovine serum albumin).

Believer states that the health of the source animal is confirmed through microbiological and viral testing of embryonic tissue cells and isolated primary cells. In addition to an aerobic plate count being performed, embryonic tissue from the source animal and isolated primary cells are also tested for specific infectious agents known to infect poultry, including viruses (Newcastle disease virus, avian influenza A), bacteria (*E. coli, S. aureus, Pseudomonas aeruginosa, Salmonella* serovars), and fungi (*Candida* spp., yeast, mold).

Believer visually observes cell morphology and uses a clear labeling system to avoid inadvertently using the incorrect cell line during the production process. Furthermore, genetic species validation of the secondary MCB and MWCB is performed by PCR analyses, as described in more detail in the below sections, "Establishment of Cell Lines," "Establishment of the Primary MCB," and "Establishment of the MWCB."

Believer screens tissue samples from the donor animal for the presence of *Mycoplasma* spp. during cell line development using an in-house PCR method, and for the presence of viruses

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[7] Potential bacteria identified by Believer as being associated with poultry include *Campylobacter* spp., *Salmonella* serovars, *E. coli*, *Chlamydia psittaci, Erysipelothrix rhusiopathiae*, *Yersinia* spp., *Mycobacterium avium*, and *Listeria* spp. Believer describes its mitigation and control strategies, including performing cell isolation and cell line establishment under aseptic conditions, performing sterility testing of the MWCB, and performing species-specific testing on the primary and secondary MCBs for *Campylobacter* spp., *Salmonella* serovars, and *E. coli*.

[8]Potential viruses identified by Believer as being associated with poultry include Newcastle disease virus (avian paramyxovirus 1), avian influenza A, West Nile virus, and Eastern, Western, and Venezuelan equine encephalitis virus alphaviruses. Of these, Believer notes that only Newcastle disease virus and avian influenza virus A are prevalent in chickens. Believer states that although Newcastle disease virus and avian influenza virus A do not post food safety concerns, the firm still tested isolated cells and all cell banks for these viruses using validated PCR-based methods.

[9] Potential fungi identified by Believer as being associated with poultry include *Histoplasma capsulatum* and *Cryptococcus neoformans*, as well as yeasts and molds, generally. Believer describes its mitigation and control strategies, including performing cell isolation and cell line establishment under aseptic conditions and performing sterility testing of the MWCB.

[10] Potential parasites identified by Believer as being associated with poultry include *Giardia duodenalis, Giardia intestinalis, Giardia lamblia,* and *Toxoplasma gondii*. Believer notes that parasites exhibit a complex life cycle which requires animal hosts that are absent in Believer's production process.

prevalent in chickens (Newcastle disease virus, avian influenza A) using qPCR. Believer also screens for additional adventitious agents and for sterility for purposes of quality and safety.

Believer states that potential adventitious agents of human concern may enter the production process via contaminated water, reagents, and human handling. However, the risks associated with these pathogens are mitigated using sanitation controls, such as using annually-tested municipal water treated with a reverse-osmosis system that filters water to <0.001 μm, and further filtering reagents through 0.2 μm filters; the small size of these filters excludes bacterial adventitious agents of concern. Believer mitigates risks of contamination through human handling by employing personnel hygiene practices, handwashing, aseptic procedures, and cGMP techniques. Specifically, cells are handled aseptically in biosafety cabinets, which are cleaned and sanitized before and after every use and monitored through ATP swabbing. To mitigate the risks of contamination from human handling, Believer employs personnel hygiene practices, enforces handwashing, and uses cGMP techniques implemented in the facility.

To mitigate the risk of adventitious agents arising from animal-derived reagents, Believer screens the cell line for bovine and porcine viruses[11] using an RNA sequencing-based method described in the below section, "Establishment of Cell Lines." All bovine-derived substances used during cell isolation, cell line establishment, as well as during establishment of the primary and secondary MCBs are verified to be sourced from bovine spongiform encephalopathy (BSE)-free/risk-negligible herds and compliant with 21 CFR 189.5, prohibited cattle materials.

Establishment of Cell Lines

Believer screens the isolated cells to select for individual cells that have desired characteristics, including the ability to exhibit a stable phenotype with repeated, linear growth (cell immortalization), characteristics of connective tissue cells (fibroblasts), the potential to acquire characteristics of fat cells (adipocytes) through differentiation, and the ability to grow in suspension within the cell culture media without the need for attachment to surfaces (suspension adaptation).

The cell line exhibits cell immortalization due to spontaneous immortalization through selection in culture. Suspension adaptation is achieved using standard methods of media support and selection.

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

- Inadvertent use of a different cell line or species;
- Unintended effects of adaptation to culture (e.g., genetic instability);

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[11] In the May 21, 2024, disclosable safety narrative, Believer noted that *in silico* analysis of this RNA sequencing data screens for viruses of both bovine and porcine origin. The firm listed this assay as a risk mitigation strategy for adventitious agents arising from animal-derived reagents, which included porcine trypsin used during cell line development. In the amendment dated May 28, 2025, the firm clarified that porcine trypsin was not used during the production process, including during cell line initiation, and that instead the firm used a trypsin-like enzyme produced by a non-pathogenic and non-toxigenic strain of *Pichia pastoris*.

- Introduction of adventitious agents from contaminated non-animal sourced reagents, the local environment, water, or human handling including viruses (norovirus) and bacteria, including *Mycoplasma* spp., *Salmonella* serovars, *Campylobacter* spp., *E. coli, Listeria* spp., *B. cereus*, and *S. aureus*; and
- Introduction of adventitious agents from animal-derived reagents (i.e., fetal bovine serum, bovine catalase, bovine serum albumin).

Believer confirms the cell line species identity using PCR analysis of *MT-CYB*, the mitochondrial gene that encodes cytochrome B. Gel electrophoresis is performed on PCR products alongside positive and negative controls to confirm the genetic identity of the cell line as chicken (*Gallus gallus domesticus*), and to also confirm that the cell line is not contaminated with DNA from any other species maintained at the facility (i.e., ovine, porcine, bovine). Believer performs additional testing to confirm species identity of the secondary MCB and the MWCB, as discussed in the below sections, "Establishment of the Secondary MCB" and "Establishment of the MWCB."

Believer confirms the fibroblast identity of the cell line by using RNA sequencing analysis on the primary cell isolates, immortalized adherent cell line, and suspension-adapted cell line to demonstrate that the cell line has expression patterns that are characteristic of fibroblast cells.

Believer demonstrated genotypic and phenotypic stability of its cell line through karyotyping and single nucleotide variation (SNV) analysis of the *TP53* gene, tracking culture doubling time, and testing cells for differentiation potential. Using karyotyping, Believer demonstrated that the cell line retains normal chromosome distribution over 500 population doublings. SNV analysis was used to compare the sequence of the *TP53* gene, which encodes the tumor suppressor protein p53, between the suspension-adapted cell line and the primary chicken fibroblasts. Believer stated that no mutations occurred in the *TP53* gene of the suspension-adapted cell line, indicating that the cell line retains the ability to carry out DNA repair. Believer also stated that the cell line maintained a stable doubling time over 800 days. Furthermore, Believer tested the differentiation potential of its cultured fibroblasts after 576 population doublings (i.e., 1.6 years of continuous growth) and found that the cells retained the potential to undergo adipogenesis when treated with a mixture of lecithin and fatty acids.

To mitigate the risk of the introduction of adventitious agents from non-animal sourced reagents, the local environment, water, or human handling, Believer employs the sanitation controls described in the above section, "Cell Isolation."

Food safety and quality management systems are in place to account for the potential risks associated with the use of animal-derived reagents during cell line establishment, including a Supplier Approval Program and vendor verification that animal-derived reagents test negative for relevant adventitious agents. The firm notes that the controls in place are adequate to manage contamination risk from sera and any other animal-derived reagents that could be used in production.

To mitigate the risk of adventitious agents arising from animal-derived (i.e., bovine) reagents, Believer compared RNA sequencing data collected from its cell line to a database the firm generated with sequences of common bovine viruses. The firm did not detect any viral

sequences in its cell line and concluded that there are no bovine viruses present in its cell line that pose food safety concerns.

Establishment of Primary MCB

Believer states that individual cell lines displaying desired properties described in "Establishment of Cell Lines" (i.e., immortalization, suspension-adaptation) are prepared for storage in a primary MCB, which is defined by Believer as a collection of cryopreserved cells derived from a single tissue source from a single animal. The primary MCB consists of frozen ampules of cells stored in liquid nitrogen at two independent facilities.

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

- Inadvertent use of a different cell line or species;
- Introduction of adventitious agents from contaminated non-animal sourced reagents, the local environment, water, or human handling including viruses (norovirus) and bacteria, including *Mycoplasma* spp., *Salmonella* serovars, *Campylobacter* spp., *E. coli, Listeria* spp., *B. cereus*, and *S. aureus*; and
- Introduction of adventitious agents from animal-derived reagents (i.e., fetal bovine serum, bovine catalase, bovine serum albumin).

To ensure that the appropriate cell line is selected for the production process, cell lines derived from a different species or that are used for research purposes are stored on separate racks within liquid nitrogen storage dewars from cell lines used for production of the harvested cell material. Vials are labeled with the lot number and species identity, while accompanying records capture information about each time cell lines are thawed and expanded.

To mitigate the risk of the introduction of adventitious agents from non-animal sourced reagents, the local environment, water, or human handling, Believer employs the sanitation controls described in the above section, "Cell Isolation."

Believer mitigates the risk of adventitious agents introduced from animal-derived reagents as described in the above section, "Establishment of Cell Lines." Briefly, Believer states that the cell line tested negative for polyadenylated viruses of bovine origin using an RNA-sequencing based method.

For quality purposes, Believer also sends samples of the primary MCB for testing by a third-party accredited laboratory. Testing is performed using validated methods for either observation of potential growth under permissive conditions (aerobic plate count, yeast and mold, *E. coli*, *Salmonella* serovars, *Listeria* spp.), enzyme-linked fluorescent immunoassay (*Campylobacter* spp.), PCR *(Mycoplasma* spp., Newcastle disease virus, avian influenza A), and RNA sequencing (polyadenylated bovine viruses of human food safety concern).

Believer tests the primary MCB for two species of *Mycoplasma* which commonly infect avian species, *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. Additional testing for other species of *Mycoplasma* is performed on the secondary MCB and the MWCB, as described in the below sections, "Establishment of the Secondary MCB" and "Establishment of the MWCB."

Establishment of Secondary MCB

Believer expands a subset of cells from the primary MCB and adapts this culture to growth in serum-free medium, in which fetal bovine serum is not used. These cells are cryopreserved and stored to form the secondary MCB.

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

- Inadvertent use of a different cell line or species;
- Introduction of adventitious agents from contaminated non-animal sourced reagents, the local environment, water, or human handling including viruses (norovirus) and bacteria, including *Mycoplasma* spp., *Salmonella* serovars, *Campylobacter* spp., *E. coli, Listeria* spp., *B. cereus*, and *S. aureus*; and
- Introduction of adventitious agents from animal-derived reagents (i.e., bovine catalase, bovine serum albumin).

To ensure that the appropriate cell line is selected for the production process, Believer uses a validated qPCR-based method to confirm the genetic identity of the cell line in the secondary MCB as chicken (*Gallus gallus domesticus*), and to verify that the secondary MCB is not contaminated with ovine, porcine, or bovine DNA.

To mitigate the risk of the introduction of adventitious agents from non-animal sourced reagents, the local environment, water, or human handling, Believer employs the sanitation controls described in the above section, “Cell Isolation.”

Further, Believer sends samples of the secondary MCB for testing by a third-party accredited laboratory. Testing is performed using validated methods. The secondary MCB was tested for aerobic plate count, yeast and mold, and specific adventitious agents (i.e., *E. coli*, *Salmonella* serovars, and *Campylobacter* spp.), and for viruses of bovine origin.

Believer also uses an in-house nested-PCR method to test the secondary MCB for over 100 different species of *Mycoplasma*, and states that *Mycoplasma* spp. were not detected.

Believer mitigates the risk of adventitious agents introduced from animal-derived reagents as described in the above section, “Establishment of Cell Lines.”

Believer states that two recombinant proteins derived from the human genome (rHP), insulin and fibroblast growth factor 2 (rH FGF-2), are used in its serum-free medium to stabilize gene expression in the cells. Both of these recombinant proteins are produced by non-pathogenic and non-toxigenic strains of *E. coli*. The firm states that rHPs were (1) only used to establish the secondary MCB; (2) were subsequently removed during establishment of the MWCB; (3) are not used in the production process (i.e., cell proliferation and harvest); and, (4) because of the high dilution factors of up to 10^{-22} in the production process, the use of rH FGF-2 and insulin in establishing the secondary MCB has no impact on the identity or safety of the harvested cell material produced using Believer’s manufacturing process described in CCC 000039. The firm provided FDA with a certificate of analysis for rH FGF-2 demonstrating a purity of ≥95%, an endotoxin level below 0.1 ng/μg of protein, and the sequence of the protein

to support the safe use of rH FGF-2. In addition, the firm provided a certificate of analysis for rH insulin demonstrating that impurities do not exceed 1.0% and that bacterial endotoxins are below 10 IU/mg. The firm states that the sequences of rH insulin and rH FGF-2 are highly similar to those found in agriculturally relevant species.

Establishment of MWCB

Believer expands a population of cells from the secondary MCB and adapts the culture to growth in animal component-free media. This includes removal of the remaining animal-derived reagents, i.e., bovine catalase and bovine serum albumin, from the media formulation. Then, Believer adapts these cells to growth in media that is free of rHPs (i.e., rH insulin and rH FGF-2). These cells are then cryopreserved and stored to form the MWCB.

Antibiotics are not used during or after the establishment of the MWCB and are not present in the harvested cell material based on analytical testing.

Believer states that rHPs used during establishment of the secondary MCB are catabolized by cells and subjected to high dilution factors (up to 10^{-22}) in the production process. Further information is discussed in the above section, "Establishment of the Secondary MCB."

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

- Inadvertent use of a different cell line or species;
- Unexpected growth profile of cell line;
- Carryover of adventitious agents from previous cell line establishment steps; and
- Introduction of adventitious agents from contaminated non-animal sourced reagents, the local environment, water, or human handling including viruses (norovirus) and bacteria, including *Mycoplasma* spp., *Salmonella* serovars, *Campylobacter* spp., *E. coli, Listeria* spp., *B. cereus*, and *S. aureus*.

To ensure that the appropriate cell line is selected for the production process, Believer uses a validated qPCR-based method to verify the species of the cells in the MWCB as chicken (*Gallus gallus domesticus*), and to verify that the MWCB is not contaminated with ovine, porcine, or bovine DNA.

Believer monitors freezer temperatures to ensure that cells are properly stored in a manner that prevents contamination by adventitious agents or deterioration of the MWCB. To ensure the cells retain expected phenotypes, Believer tests the MWCB for expression of tropomyosin, a marker for fibroblasts, using an in-house qPCR method. Believer also monitors the growth rate of cells and discards any cultures that do not meet the established specifications for the population doubling time set by the firm.

In establishing the MWCB, Believer adapts the culture to growth in animal component-free media. As such, Believer mitigates the risk of adventitious agents introduced from animal-derived reagents by limiting the use of all animal-derived reagents to the cell isolation through establishment of the primary and secondary MCB phases of the production process. As

mentioned, the cell line is adapted to growth in animal component-free media before being deposited into the MWCB.

To mitigate the risk of the introduction of adventitious agents from non-animal sourced reagents, the local environment, water, or human handling, Believer employs the sanitation controls described in the above section, "Cell Isolation." Further, Believer sends samples of the MWCB for testing by a third-party accredited laboratory for testing of general sterility, *Mycoplasma* spp. using nested-PCR, as well as Newcastle disease virus and avian influenza A using PCR. Testing is performed using validated methods.

Believer sets specifications for the MWCB to ensure that cell lines employed in the production process conform to the firm's identity, quality, and safety standards.

Believer provides the following specifications related to the genotype and phenotype of the MWCB:

- Species determination (*Gallus gallus domesticus*)
- Tropomyosin expression (Cq < 26)
- Doubling time (dT < 30 hours)
- Morphology (normal)[12]

Believer provides microbial specifications for each MWCB, including:

- Sterility (negative)
- *Mycoplasma* spp. (not detected)
- Newcastle disease virus (not detected)
- Avian influenza A (not detected)

Production Process

Believer provides information about its production process, including:

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

Believer states that the firm's food safety and quality systems are based on the requirements of 21 CFR part 117 subpart B, including the requirements of (cGMP and HARPC). The food safety and quality systems are supplemented by the firm's high standards of cleanliness, education, training, and supervision needed during the production process. Believer's production process utilizes aseptic techniques or hygienic controls at different steps of the process. The production process is described by the firm as consisting of: (1) proliferation phase using suspension

[12] Believer uses confocal microscopy to monitor the morphology of the cells and describes the morphology of fibroblasts as spindle-shaped.

culture, (2) harvest of cell material from bioreactors using filtration followed by washing with a saline (sodium chloride) solution.

Believer reviews allergen declarations on all raw materials, which are sourced from approved suppliers, verified upon receipt, and documented to ensure traceability. Believer states that it utilizes soy-derived protein during production of the harvested cell material. The firm states that it will address concerns regarding allergenicity of the harvested cell material through product labeling. As a precaution, Believer has tested its harvested cell material for the presence of egg proteins (e.g., ovomucoid, ovalbumin, ovotransferrin) to ensure that egg allergens, which are encoded in the chicken genome, but are unlikely to be expressed in the firm's fibroblast cell line, are not present. Believer noted that egg allergens are absent from the harvested cell material at a limit of quantitation of 2.5 ppm.

Bovine serum albumin, also present in fetal bovine serum, is one of the allergens present in cow's milk along with other milk proteins such as β-lactoglobulin, α-lactalbumin, and casein. Among cow's milk proteins, α-lactalbumin, β-lactoglobulin, and casein are recognized as major allergens, whereas bovine serum albumin is considered a minor allergen. Based on use levels of bovine serum albumin and fetal bovine serum during cell banking and a dilution factor of 10^{-22}, the combined estimated daily intake (EDI) of albumin was calculated as 9.34^{-20} mg/d by the firm. For milk allergic patients, the median MEDs (minimal eliciting dose) for allergenicity were 396 mg (range: 3.3-7920 mg), 534 mg (range: 9.9-6600 mg), and 396 mg (range: 9.8-9000 mg) protein respectively, for mild, moderate, or severe reactions (Zhu et al., 2015).[13] The ED10 doses (the eliciting dose predicted to provoke a reaction in 10% of individuals with a specific food allergy) for milk were 15.1, 51.4, and 21.4 mg protein for mild, moderate, and severe reactions, respectively, with a combined value of 25.2 mg. Based on these values, the EDI of albumin (9.34^{-20} mg/d) is negligible compared to the lowest dose (3.3 mg) that elicited an allergic reaction to milk proteins. Believer notes that animal-derived reagents are not used following establishment of the secondary MCB.

Believer describes a supplier qualification plan and incoming material controls that comply with 21 CFR part 117 subpart G, Supply-Chain Program, noting that the plan is applicable for all raw materials, packaging materials, consumables, food contact materials, and equipment used in the production process. [14] Believer states that the firm uses food contact materials that are permitted for their intended use throughout the production process.

Proliferation Phase Using Suspension Culture

Frozen cells from the MWCB are thawed and placed in sterile culture medium in a small vessel (i.e., shaker flasks) under sterile conditions. Following multiple rounds of cell division and

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[13] Zhu, J., Pouillot, R., Kwegyir-Afful, E. K., Luccioli, S., & Gendel, S. M. (2015). A retrospective analysis of allergic reaction severities and minimal eliciting doses for peanut, milk, egg, and soy oral food challenges. Food and Chemical Toxicology, 80, 92-100.

[14] 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)."

growth (proliferation), the cells are transferred to larger vessels (passaging). This process, under continued sterile conditions, is repeated with increasingly larger vessels[15] to accumulate the desired quantity of cells (i.e., a seed train). Once there is a sufficient amount of cells, batches of cells grown in the seed-train bioreactor are seeded into a closed-system large-scale bioreactor to continue proliferation, where waste materials are exchanged for fresh[16] media to achieve higher densities of cell growth. The cell material is then removed from the large-scale bioreactor and washed in a closed-system centrifuge.

Believer identifies potential hazards associated with this production stage, including:

- Introduction of adventitious agents from media components; and
- Introduction of adventitious agents from the environment or food contact surfaces.

Believer manages the risk associated with the introduction of adventitious agents during the production process by filtration of air and water sources, as well as filter sterilization of media components. Any inputs to the bioreactors (i.e., growth media, washing agents) are sterile filtered through a 0.2 μm membrane to avoid the introduction of adventitious agents through these sources. Believer monitors dissolved oxygen (DO) concentrations within the bioreactors, using a rapid drop in DO as an indicator of contamination. Believer reiterates that animal-derived reagents are not used following establishment of the secondary MCB, minimizing the risk of adventitious agents arising from media components derived from animal sources.

Believer states that the production process is conducted in an aseptic, closed system. The proliferation phase begins in a biosafety cabinet, which is sterilized before the production of each lot using a steam-in-place procedure. The starter culture is evaluated microscopically for visual signs of contamination before being used to seed a closed-system bioreactor used for growing cells in successively larger volumes (i.e., seed train). Personnel are subject to hygiene

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[15] Believer discussed its intention to scale up production at its production facility in North Carolina, using bioreactors that support larger culture volumes than those used at the Rehovot, Israel facility. In an amendment dated January 15, 2025, the firm describes the ways in which the control and mitigation strategies for the identified hazards apply to the large-scale production process. Further, in the amendment dated May 28, 2025, the firm states that is does not expect an increase in the scale of the production process to affect the safety, identity, or regulatory status of the harvested cell material. The firm states that it plans to generate additional data to confirm that the harvested cell material produced using large-scale bioreactors meet the safety criteria outlined in CCC 000039, including the same nutritional, chemical, and microbiological specification parameters outlined in CCC 000039. The firm commits to notifying FDA of any changes to the manufacturing process that impact the safety, identity, or regulatory status of the harvested cell material, including whether it identifies any new hazards that are not adequately covered by its mitigation and control strategies, and, if necessary, submitting a supplement to the completed consultation describing and addressing those changes.

[16] In the May 21, 2024, disclosable safety narrative, Believer discusses a rejuvenation step in which spent media undergoes a filtration process to remove waste materials (e.g., ammonia, lactate), such that the media can be recycled for use in the bioreactor. In the amendment dated January 15, 2025, Believer notes that the rejuvenation step is an optional process that was not used to produce Believer's harvested cell material to date. The firm noted that it would revisit the step with FDA before implementing it in its production process. As such, FDA notes that we did not consider the rejuvenation step during our evaluation of CCC 000039.

management programs, including wearing protective gowns. Steam-in-place sterilization is conducted on the bioreactor and its supply lines/tanks.

Cell Harvest

Believer states that at the end of proliferation, cells are collected from the bioreactor via filtration and washed with a saline (sodium chloride) solution using a closed system centrifuge. After exiting the bioreactor, cells are kept under sanitary, but not sterile, conditions.

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

- Presence of residual media components without an existing U.S. human food authorization;
- Presence of residual cleaning agents used during the firm's clean-in-place processes for sterilizing food contact surfaces;
- Adventitious agents arising from the environment; and
- Introduction of adventitious agents in the wash buffer.

Safety considerations associated with the use of media components that may be present as residues in the harvested cell material following washing are discussed in the subsequent section, "Substances Used in the Production Process."

Believer states that sterilization of food contact surfaces, an environmental monitoring program, and testing of the harvested cell material for the presence of adventitious agents all mitigate the risks of contamination occurring during cell harvest.

Specifically, Believer mitigates the risk of contaminating the harvested cell material with residual cleaning agents by rinsing food contact surfaces with water following the firm's clean-in-place procedures and testing the rinse water to ensure that cleaning agents are effectively removed. Believer states that a verified and validated sanitation process completely removes cleaning agents employed in the production process. Further, Believer manages risks associated with growth of adventitious agents by practicing aseptic technique and employing cGMPs to minimize the potential for introduction of adventitious agents in the production process. Believer filters all water sources and sterilizes all food contact surfaces.

Based on the firm's hazard analysis, Believer performs both periodic environmental monitoring and, for each lot of the harvested cell material, adventitious agent testing to monitor for adventitious agents introduced either at the cell proliferation or harvest phases of the production process.

Substances Used in the Production Process

Believer 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 media;

- substances intended to support cell proliferation during culture; and
- substances used to harvest the cell material.

For each substance, Believer provides information about the identity and the basis for its safety conclusion, and in certain cases information about either the theoretical estimated consumer exposure, or in other cases, provides EDIs based on analytical measurements in the harvested cell material.[17]

Believer describes the firm's proprietary cell culture medium as containing substances required for growth, including amino acids, fatty acids, sugars, minerals, trace elements, vitamins, and salts. Additional substances identified by Believer during the production phase (i.e., proliferation and harvest) include emulsifiers and surfactants, a soy-derived enzyme, and a saline wash buffer. The firm explains that most of these substances are already widely consumed by humans as part of the U.S. food supply. The firm states that the water-soluble non-nutrient substances 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. Believer also states that no antibiotics or antifungals are used during the proliferation and harvest stages of production.

Believer 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. Additionally, the firm considered prior use or natural presence in conventional food and anticipated dietary exposure. The firm also publicly discloses data and information regarding a substance, hydroxypropyl-β-cyclodextrin HPβCD, 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 this component of the cell culture medium, the firm also considered the estimated intake level derived from its analytical data from the harvested cell material for the component with reference to levels present in one or more currently consumed comparator foods. The firm states that residual levels of the substance in the harvested cell material are undetectable. This information provided by Believer is described in more detail below.

Substances to manage properties of the culture media

HPβCD 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. HPβCD is

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

[18] Believer provides exposure estimates (i.e., EDIs) for substances used in the production process as supporting, corroborative information in the supplemental, confidential material.

not the subject of an existing U.S. food ingredient authorization or GRAS notice evaluated by FDA, nor is it naturally present in the U.S. human food supply. Believer cited a 12-month feeding study in rats which described a no observable adverse effect level (NOAEL) of 500 mg/kg body weight bw)/d for HPβCD. Believer provided analytical data demonstrating that HPβCD is not present above the limit of quantitation (< 0.009% w/w in the harvested cell material. The firm calculated an EDI of 14.31 mg/d (or 0.24 mg/kg bw/d in a 60 kg adult) based on a conservative assumption that HPβCD is present at the limit of quantitation. The firm noted that the EDI of HPβCD from the harvested cell material is 2,000-fold below the NOAEL of 500 mg/kg bw/d. The firm also notes that HPβCD 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 HPβCD, further supporting its safe use.

Nutrients used to support primary cell metabolism

Believer considered relevant data and information for 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 chicken, or at levels found in other commonly consumed foods while also being well below reference exposure values identified by various authoritative bodies that assess the safety of food, or both.

Characterization of Harvested Cell Material

Identity

As described above, during cell line establishment Believer selects for and banks pluripotent, fibroblast cells and uses PCR amplification of the *MT-CYB* mitochondrial gene to verify the species identity of the primary MCB as chicken (*Gallus gallus domesticus*), and a qPCR method to validate the species identity of the secondary MCB and MWCB. Believer characterizes its cell line as fibroblasts by using microscopy to examine the spindle-shaped morphology of the cells and RT-qPCR to measure gene expression levels. Believer demonstrated that expression levels of the fibroblast markers Tropomyosin 1 (*TPM1*), fibroblast specific protein 1 (*FSP1*), integrin B3 (*ITGB3*), and collagen 8A1 (*COL8A1*) are stable over 19 days of culture.

Believer implements cell bank inventory controls (i.e., separating MCB and MWCB vials for different species on different racks of a liquid nitrogen dewar) to ensure that the correct MWCB vials are used as inputs in the production process. The firm examines cultured cells during the proliferation phase of the production process to ensure that cell growth rate, morphology, and gene expression conform to expected, well-characterized phenotypic parameters (i.e., doubling time <30 hours, normal morphology, Cq <26 for tropomyosin expression). The firm verifies the cell type identity of the harvested cell material as fibroblasts by using RT-qPCR to measure the expression levels of tropomyosin in the harvested cell material.

Specifications for cell line stability include:

- Expression levels of a fibroblast marker, tropomyosin (<26 Cq)

Adventitious Agents and Contaminants

Believer describes compositional analysis and characterization of the harvested cell material. The compositional analyses of the harvested cell material includes major nutrients (protein and amino acids, fats, carbohydrates, minerals, and vitamins), and some residues of media components. Believer provides information and specifications for adventitious agents 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. Data was concurrently generated using store-bought ground chicken and store-bought chicken breast as comparators.

Believer provides specifications for each batch of the harvested cell material, including:

- Total plate count (<5,000 colony-forming units (CFU)/g)[19]
- Yeast (<100 CFU/g)
- Mold (<200 CFU/g)
- *E. coli* (<10 CFU/g)
- *Salmonella* serovars (not detected in 25 g)
- *Enterobacteriaceae* (≤50 CFU/g)

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

In addition to the specifications above, Believer provides results from analytical testing of potential adventitious agents including: *E. coli*, yeast, mold, *Salmonella* serovars, *Enterobacteriaceae*, coliforms, coagulase-positive *Staphylococcus aureus*, mesophilic sulphite-reducing clostridia, *L. monocytogenes*, and *Pseudomonas* spp.; as well as toxic heavy metals (lead, arsenic, cadmium, and mercury), based on the analysis of three independent batches. Believer states that all microbial tests were negative at the limit of detection, and that toxic heavy metals are below levels that would result in safety concerns. Believer states that all analytical methods are validated for their intended purpose.

Believer 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. Toxic heavy metal specifications for each batch of harvested cell material include:

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[19] In the May 21, 2024, disclosable safety narrative, the firm reported the results of the analysis of total plate count as <10 CFU/g in three non-consecutive batches of harvested cell material. FDA asked the firm to consider lowering its specification as low as reasonably possible, in line with the results of the batch data; however, in an amendment dated May 28, 2025, the firm stated that the specification of <5,000 CFU/g is based on commercial food safety standards. The firm further noted that the specification is for the harvested cell material after it has undergone a hygienic, but not sterile, wash step, and it is an appropriate indicator that hygienic conditions were maintained during this phase of the production process. In support of its argument, the firm referenced the Pasteurized Milk Ordinance for Grade A pasteurized milk products. The firm stated that, as it scales up production at its production facility in North Carolina, it will review microbiological testing data and, if warranted, adjust the specification for total plate count.

- Arsenic (<10 ppb)
- Lead (<50 ppb)
- Mercury (<10 ppb)
- Cadmium (<10 ppb)

Composition

Believer conducted a compositional analysis of six independent batches of the harvested cell material for proximates (i.e., moisture, protein, fat, ash, and carbohydrates), four of which were also analyzed for amino acids, fatty acids, vitamins, and minerals. The firm provided specifications for proximates in the harvested cell material. As points of reference, the firm provided nutrition data from two store-bought chicken samples, one as ground chicken and one as chicken breast, as well as data from a U.S. Department of Agriculture (USDA) database on conventional chicken products. The harvested cell material had higher moisture content and lower protein, ash, and fat content than the conventional comparators. Amino acid, fatty acid, vitamin, and mineral content were lower than or similar to the conventional comparators. The harvested cell material was washed with a saline (sodium chloride) solution, resulting in higher sodium content relative to the conventional chicken reference data.

FDA's Evaluation

FDA evaluated the information provided by Believer 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 000039. 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.

Believer provides information on the establishment of the cell lines used to produce the food that is the subject of CCC 000039. FDA considered the information on the source and lineages of the cell lines, the culture adaptation process, and the harvested cell material. We also considered the information provided by Believer 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 chicken-derived 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 this 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 this 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.

Finally, while ectopic expression of egg protein allergens was a theoretical possibility given that each cell contained the complete chicken genome, including genetic code for the relevant egg

proteins, Believer notes that testing of its cell lines demonstrated an absence of egg proteins. Believer reviews allergen declarations on all raw materials, which are sourced from approved suppliers, verified upon receipt, and documented to ensure traceability. Believer states that it utilizes soy-derived protein during production of the harvested cell material. 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 Believer's assessment of potential sources for introduction of adventitious agents, and the corresponding mitigation and control strategies for each hazard identified. We also considered Believer's use of 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 000039, that indicate an unaddressed food safety risk resulting from microbial, viral, or other contaminants.

FDA considered the general framework that Believer 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 growth factors, including the identity and use of growth factors that are also present in conventional chicken meat, the intended use level, anticipated digestive fate, and corroborative analytical data regarding residual presence of substances used in the production process in the harvested cell material. Furthermore, FDA considered data from a 12-month rat study demonstrating a NOAEL of 500 mg HPβCD/kg bw/day, analytical evidence that HPβCD is below detectable levels in the harvested cell material, a conservative estimated daily intake 2,000 times lower than the NOAEL, and structural similarity to the well-studied β-cyclodextrin to support the safety of HPβCD, a substance new to the U.S. human food supply. We note that the substances described by Believer 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 Believer'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 chicken (discussed above in the "Characterization of the Harvested Cell Material" section); however, the harvested cell material, which is the subject of CCC 000039, 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.[20]

FDA reviewed the information that was provided on the identity and composition of the harvested cell material, including genetic and cellular identity, batch test data for constituents and contaminants, and specifications. We considered the analytical data provided by Believer 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, produce a consistent product, and as relevant information in evaluating the relationship between the production process described in CCC 000039 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 are consistent with levels that are considered safe in food.

We also considered data and information relating to compositional analysis. In all cases, levels of analytes were within the range of those found in commonly consumed foods. 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 Believer'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 000039 or food products that contain this material.

Conclusions

Based on our evaluation of the data and information that Believer provides in CCC 000039, 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 contain any substance or microorganism that would adulterate the food. We have no questions at this time about Believer's conclusion that foods comprised of, or containing, the harvested cell material resulting from the production process defined in CCC 000039 are as safe as comparable foods produced by other methods.

Jessica H. Mills -S

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Jessica Mills

[20] 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)."

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	Labeling, qPCR test in MWCB, visual observation of morphology
Cell Isolation	Carryover of adventitious agents such as bacteria, fungi, viruses, parasites, and prions from source animal	Adventitious agent screening of primary cells, virus screening of source embryonic tissue
Cell Isolation	Introduction of adventitious agents during isolation	Adventitious agent testing in MCB and MWCB, aseptic procedures
Cell Isolation	Introduction of contaminants from animal-derived reagents	Adventitious agent testing in MCB and MWCB, supply chain controls (country of origin requirements, supplier approval protocols)
Cell Isolation	Introduction of contaminants in laboratory reagents	Sterile filtration
Cell Isolation	Facility environment contamination	Adventitious agent testing in MCB and MWCB, aseptic procedures
Establishment of Cell Lines	Cell line identification, cells from different line or species inadvertently used	PCR testing, Genetic species validation in MWCB, testing program
Establishment of Cell Lines	Cells do not display expected growth profile; genetic instability	Karyotyping, measure and discard
Establishment of Cell Lines	Contamination of adventitious agents	Adventitious agent testing in MCB and MWCB, aseptic procedures
Establishment of Cell Lines	Introduction of contaminants in laboratory reagents	Sterile filtration
Establishment of Cell Lines	Introduction of adventitious agents from media components	Sterile filtration, supply-chain controls, testing
Establishment of Cell Lines	Introduction of contaminants from animal-derived reagents	Adventitious agent testing in MCB and MWCB, country of origin requirements, supply-chain controls
Establishment of Cell Lines	Introduction of chemical hazards	Allergen controls, cGMP, chemicals management and monitoring of usage, document control and training, raw materials and food contact consumables management and inspection,

		sanitation controls, supply-chain controls
Establishment of Cell Lines	Introduction of physical hazards	cGMP and good warehousing practices, glass and brittle plastic program, incoming inspection for raw materials; for the finished food product, use of a metal detector
Establishment of Cell Lines	Facility environment contamination	Adventitious agent testing in MCB and MWCB, aseptic procedures
Establishment of MCBs (Primary and Secondary) and MWCB	Cells from different line or species inadvertently used	PCR Testing
Establishment of MCBs (Primary and Secondary) and MWCB	Genetic instability	Measure population doubling (PD) times and compare to specified limits, RT-qPCR testing
Establishment of MCBs (Primary and Secondary) and MWCB	Introduction of adventitious agents	Aseptic procedures, cGMP, environmental monitoring, filtration and/or sterilization of water, gases, and food contact surfaces, sterilization, supply-chain controls, testing
Establishment of MCBs (Primary and Secondary) and MWCB	Contamination with adventitious agents from original animal source	Testing
Establishment of MCBs (Primary and Secondary) and MWCB	Contamination with adventitious agents from culture media components	Aseptic procedures, cGMP, environmental monitoring, sanitation controls, sterile filter, supply-chain controls, testing
Establishment of MCBs (Primary and Secondary) and MWCB	Introduction of chemical hazards	Allergen controls, cGMP, chemicals management and monitoring of usage, document control and training, raw materials and food contact consumables management and inspection, sanitation controls, supply-chain controls
Establishment of MCBs (Primary and	Introduction of physical hazards	cGMP and good warehousing practices, glass and brittle plastic program, incoming

Secondary) and MWCB		inspection for raw materials; for the finished food product, use of a metal detector
Establishment of MCBs (Primary and Secondary) and MWCB	Facility environment contamination	Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls
Establishment of MCBs (Primary and Secondary) and MWCB	Introduction of physical hazards	cGMP and good warehousing practices, glass and brittle plastic program, incoming inspection for raw materials; for the finished food product, use of a metal detector
Proliferation Phase	Facility environment contamination	Aseptic procedures, environmental monitoring, hygienic condition, sanitation controls
Proliferation Phase	Contamination during transfer	Adventitious agent testing in MWCB, aseptic procedures, cGMP, sanitation controls
Proliferation Phase	Phenotypic stability	RT-qPCR testing for MWCB
Proliferation Phase	Introduction of adventitious agents during proliferation phase (including thawing)	Aseptic procedures, cGMP, environmental monitoring, sterile filtration, MWCB release testing protocol, sanitation controls, testing, visual monitoring
Proliferation Phase	Contamination with adventitious agents from media components	Aseptic procedure, batch release microbial testing, cGMP, filtration of gases and solution, sterile filtration, supply-chain controls
Proliferation Phase	Contamination with adventitious agents through inadequate sterilization of vessels and transferring between vessels	Aseptic welding of solution containers, cGMP, environmental monitoring, sanitation controls, testing
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; introduction of media components	Allergen controls, cGMP, chemicals management and monitoring of usage,

	that could persist as residues in harvested cells	document control and training, food safety assessment, raw materials and food contact consumables management and inspection, sanitation controls, supply-chain controls
Proliferation Phase	Introduction of physical hazards	cGMP and good warehousing practices, glass and brittle plastic program, incoming inspection for raw materials; for the finished food product, use of a metal detector
Proliferation Phase	Facility environment contamination	Aseptic procedures, environmental monitoring, filtration and/or sterilization of water and gases, hygienic condition, preventive maintenance program, sanitation controls
Proliferation Phase	Introduction of physical hazards	cGMP and good warehousing practices, glass and brittle plastic program, incoming inspection for raw materials; for the finished food product, use of a metal detector
Harvest of Cell Material	Presence of adventitious agents from culture process	Culture monitoring, specifications, testing
Harvest of Cell Material	Migration of contaminants from food contact materials	Sterilization of food contact surfaces, 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, chemicals management and monitoring of usage, document control and training, raw materials and food contact consumables management and inspection, sanitation controls, supply-chain controls
Harvest of Cell Material	Introduction of physical hazards	cGMP and good warehousing practices, glass and brittle

		plastic program, incoming inspection for raw materials; for the finished food product, use of a metal detector
Harvest of Cell Material	Facility environment contamination	Aseptic procedures, environmental monitoring, filtration and/or sterilization of water and gases, hygienic condition, preventive maintenance program, sanitation controls