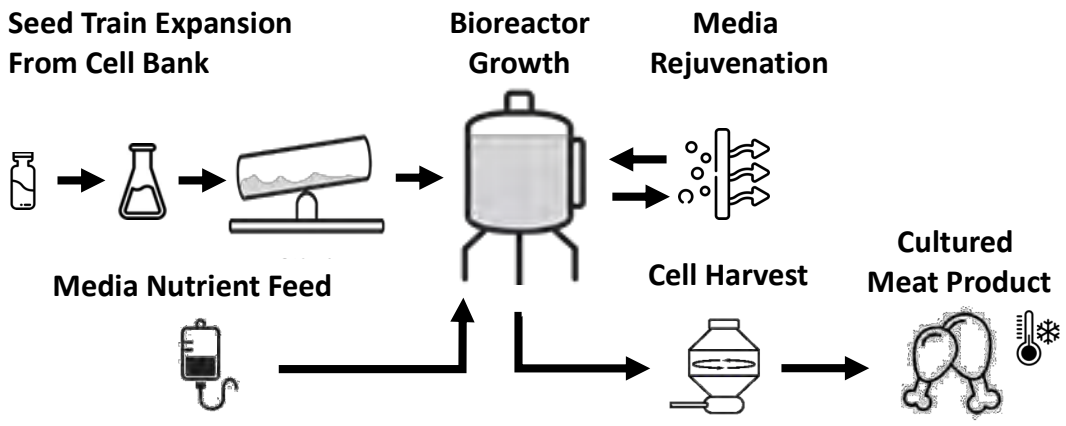
4.0 DESCRIPTION OF THE PRODUCTION PROCESS

4.1 General Overview

The following sections provide an overview of the production process, from the isolation and generation of the commercial cell lines and cell banking steps (Section 4.3) to cell expansion in bioreactors and harvest of Believer Meats' cultured chicken cells (Section 4.4). A pictorial representation of the production process is shown in Figure 4.1-1. Data and information from independent production runs of cultured cells as presented within this dossier (Section 5.0) were produced at Believer Meats' production facility in Rehovot, Israel. The company is building a large-scale production facility in Wilson, North Carolina that will produce commercial products for the U.S. marketplace and will operate in accordance with applicable requirements under 21 CFR Part 117 (U.S. FDA, 2022a) including requirements for Good Manufacturing Practice (GMP) and risk-based preventive controls (Hazard Analysis and Risk-based Preventive Controls [HARPC]), as described in Section 4.2. Risks associated with the new location and scale-up will be assessed and the food safety plan will be revised to account for preventative and critical controls necessary to address any identified hazards. All plants producing commercial products will operate in accordance with 21 CFR Part 117, as described in Section 4.2 (U.S. FDA, 2022a), as well as all other applicable food safety requirements.

Figure 4.1-1 Overview of Believer Meats' Production Process



4.2 Food Safety and Quality Systems

Believer Meats' Food Safety and Quality System is based on 21 CFR Part 117 and Global Food Safety Initiative–recognized standards (U.S. FDA, 2022a). The system includes hazard analysis and risk-based preventive controls, as well as supporting prerequisite programs and practices addressing the production environment's role in producing safe food products. The main components of the systems are summarized below. Additional details are provided in Appendix A confidential supplementary material.

- Current Good Manufacturing Practice (cGMP) programs incorporate the requirements laid down in 21 CFR Part 117, subpart B (U.S. FDA, 2022a), including the following:
    - Personnel GMP are defined in Believer Meats' GMP policy based on requirements set in 21 CFR §117.10 for disease control and cleanliness. Practices include appropriate hand washing and sanitizing, appropriate use and handling of personal protective equipment, and foreign object control, such as smocks, hair nets, and beard nets (if needed), and removing jewelry and watches (U.S. FDA, 2022a).
        - Employees' hands are periodically tested for the presence of *S. aureus*, *E.coli,* and coliforms. These microorganisms were selected for periodic testing as they are indicators of adherence to hygiene protocols. Further details describing the frequency, sample type and size, and limits are located in Appendix A of the Confidential Supplementary Materials.

    - Hygienic zoning of Believer Meats' facility is of high importance in minimizing the potential for contamination with environmental pathogens and other potential chemical cross-contaminations. Every facility zone will have specific donning and doffing requirements based on risk assessment, and personnel entering Believer Meats' facility are required to comply with hygiene requirements. Zoning will include distinction between U.S. FDA production, USDA uncooked production, and ready-to-eat USDA area.

- Environmental monitoring for sanitation control verification as part of evaluating the effectiveness of the prerequisite plan (PRP).
    - Sanitation standard operating procedures (SOPs) and controls will be in place for all production areas and equipment. Including sanitation controls for aseptic work, bioreactor cleaning in place (CIP) and sanitizing in place (SIP), and personal hygiene.
    - Environmental monitoring will be conducted according to 21 CFR §117.165 to verify the effectiveness of the PRP including sanitation, hygienic zoning procedures, *etc*. in controlling environmental pathogens and chemical cross-contamination. Routine sampling sites include food and non-food contact surfaces (U.S. FDA, 2022a). A sampling plan for every zone is based on its defined hygienic level. In case of deviation from Believer Meats' microbial standard set for each sampling point, resampling of the adjacent area is applied, and an investigation is performed to identify the source. In addition to evaluating the root cause, additional sanitation, and other corrective actions to regain control of the environment microbial load will be employed.
        - Environmental monitoring includes periodic testing of equipment cleaned by SIP for presence of aerobic bacteria by total plate count (TPC). TPC was selected as this is an indicator for the effectiveness of the SIP sterilization. Further details describing the frequency, sample type and size, and limits are located in Appendix A of the Confidential Supplementary Materials.

- Supplier qualification plan and effective incoming material control are essential parts of producing a safe product. Believer Meats' supplier qualification corresponding with 21 CFR Part 117 subpart G, will be applicable for raw materials, packaging materials, consumables, food contact materials, and equipment (U.S. FDA, 2022a).
    - Suppliers will be approved and evaluated based on their ability to comply with Believer Meats' food safety and quality requirements. This evaluation includes the following:
        - Finalizing product specifications including attributes of interest identified during food safety risk analysis.
        - Approval and verification activities of suppliers of raw materials and other ingredients may be in the form of a food safety standard audit review, suppliers' food safety policies, plans, and records review, or an onsite inspection by a third party- or by Believer Meats' food safety team.
        - Reviewed Food Safety Plan policies and documentation, which may include Food Safety Plan and risk analysis, supplier food defense programs, suppliers' food fraud vulnerability, and supplier's allergen risk assessment and management program.
    - A supplier approval process will be performed before purchase. Receiving procedure will include checks to ensure that the supplier and specification of the incoming material are approved, the condition of the material is acceptable, and it is delivered with the correct paperwork (*e.g.*, certificates of analysis [COAs]). Sampling and testing may be required according to risk assessment for some materials to confirm their suitability for use in production.
    - If incoming material checks are completed satisfactorily the risks associated will be significantly reduced. If a check fails, the supplier will be notified, and corrective action will be required. Suppliers and ingredients will be re-evaluated periodically, or in the event that any change occurs.

- Documented information will be managed and controlled.
    - Company documented information is managed by the quality management system to ensure document review, approval, and maintenance. Company procedures, work instructions, records, and ingredient specifications are reviewed according to need, and at least once a year.
- Control measures mitigating food safety biological, chemical, and physical hazards.
    - A hazard analysis is conducted to identify and evaluate potential hazards for the product. Hazards are based on experience, material origin, production process, and regulatory guidance. The significance of risk to food safety of the product is defined by a combination of probability and severity and is then used to determine whether preventive controls are required. The hazard analysis and risk evaluation as well as preventive controls are documented in Believer Meats' Food Safety Plan by a Preventive Controls Qualified Individual (PCQI).
- Product compliance and release.
    - Quality assurance process is designed for the release of conforming raw materials, intermediate products, and final products from on-hold status. The process is based on a COA, process and product quality control testing, and production process documentation review. Non-conforming incoming materials or products would be placed on hold until further investigation is performed. Quality compliance status will be managed through an Enterprise Resource Planning (ERP) system that excludes on-hold materials from available inventory.
- PCQI record review.
    - Process records including ingredient use, in-process quality control, and critical control point monitoring are kept. Records are reviewed under the oversight of PCQI to verify compliance of products with food safety specifications and requirements.
- Traceability.
    - Traceability will be maintained through documentation of raw materials, consumables, and primary packaging identification such as a batch number. Traceability will be audited and documented by a PCQI through a recall drill based on the Believer Meats' recall procedure.

Believer Meats' quality management system supports the Food Safety Plan maintenance through the management of the following plans:

- Corrective actions.
    - Believer Meats' quality management supports document and management of non-conformities and following root cause analysis, correction and corrective actions definition and implementation and effectiveness evaluation where applicable. When a food safety nonconformity is identified, the product will be quarantined, until its assessment by the food safety team.
- Internal audits for food safety and quality compliance.
    - Internal audits are planned periodic reviews of prerequisite plans, implementation of procedures and work instructions, employee competence, and qualifications. Compliance with the Food Safety Plan is also audited. Nonconformities stemming from these audits are managed according to Believer Meats' corrective action procedure in the quality management system. Audit observations are analyzed for trends which will be reviewed periodically with the management to facilitate continuous improvement.
- Raw material incoming inspection and final product analysis.
    - Raw materials food safety risk analysis is carried out as part of ingredient approval. The risk analysis is based on regulatory guidance, origin, known issues, supplier and ingredient

history, and other relevant information. Incoming inspection will be based on risk analysis and may be adjusted according to identified trends.
    - In-process quality control is performed according to risk analysis to verify compliance with defined food safety and quality parameters during production.
    - Intermediate and finished products are tested to ensure their safety, according to risk analysis–based quality control. Product risk analysis is based on identified hazards associated with product ingredients, production process, production area, and regulatory guidance. Inventory of raw materials, intermediate and finished products will be managed according to the "best before" date as needed to maintain food safety and quality.
    - Raw materials, intermediate, and finished products will have defined specifications. Specifications will include chemical, biological, and physical attributes relevant to food safety and quality.
- Hygienic design of equipment and tools.
    - Equipment and food contact tools are reviewed to ensure suitability for food contact. Equipment is hygienically designed, allowing access to sanitation operations, as well as minimizing harborage points. Sanitation is validated as part of the equipment acceptance test and approval.
    - Food contact surfaces shall be corrosion-resistant and made of nontoxic materials. Food contact surfaces will be designed to withstand the environment of their functionality, and, if applicable, cleaning compounds and sanitizing agents
- Product recall, food defense, and food fraud plans.
    - A product recall policy was written to define the process of withdrawing products from the market due to possible health hazards. This policy will be implemented as part of Believer Meats' crisis management. The recall policy will be reviewed at least once a year including a mock recall to ensure continuous improvement as part of the food safety and quality management system.
    - A food defense policy was designed to protect raw materials and products from intentional adulteration. Believer Meats' food defense policy will comply with the *Food Safety Modernization Act* Final Rule for Mitigation Strategies to Protect Food Against Intentional Adulteration. As part of the food defense policy implementation incidents are documented and reviewed at least annually to ensure continuous improvement as part of the food safety and quality management system.
    - A food fraud policy will be in place to prevent economically motivated adulteration of raw materials and thereafter the finished product. Raw materials are assessed for food fraud vulnerability. This risk assessment is based on available historical data of both ingredient and supplier, and food fraud associated with them.

- Employee training program.
    - Employees are trained according to their role description and qualification plan, and according to the annual training program to ensure employees are trained for their roles. Additional training may stem from root cause analysis of nonconformities as part of the food safety and quality management system continuous improvement.

Believer Meats' Food Safety Plan complies with 21 CFR Part 117 (U.S. FDA, 2022a). The Food Safety Plan was developed under the guidance of PCQI utilizing the Food Safety Preventive Controls Alliance *Preventive Controls for Human Food Participant Manual v1.2 2016* (FSPCA, 2016).

Believer Meats assesses its processes and ingredients for risks associated with biological, chemical, and physical hazards, as well as vulnerability to food fraud. Each identified potential hazard has a defined

significance based on its severity and probability. The significance could be high or low according to Believer Meats' risk assessment matrix. Potential high-significance hazards are controlled through process preventive controls, allergen preventive controls, sanitation preventive controls, supplier preventive controls, or other preventive controls.

Believer Meats evaluated foreseeable biological hazards, such as yeasts, molds, viruses, and bacteria. Measures for control of identified biological hazards will include, but are not limited to, verified and validated sanitation processes and sanitation controls, GMPs such as personal hygiene and equipment sanitary design and handling, and supplier and raw materials management and inspection.

Believer Meats evaluates chemical hazards, including pesticides, food allergens, and other substances. Measures for control of identified chemical hazards will include, but are not limited to, process controls, verified and validated sanitation processes completely removing cleaning agents used, GMPs, raw materials and food contact consumables management and inspection, and chemicals management and monitoring of usage.

Believer Meats identified and evaluated physical hazards such as plastics, glass, and metals. Measures for control will include GMPs and good warehousing practices, glass and brittle plastic program, incoming inspection for raw materials, and monitoring the final product with either a metal detector or X-ray.

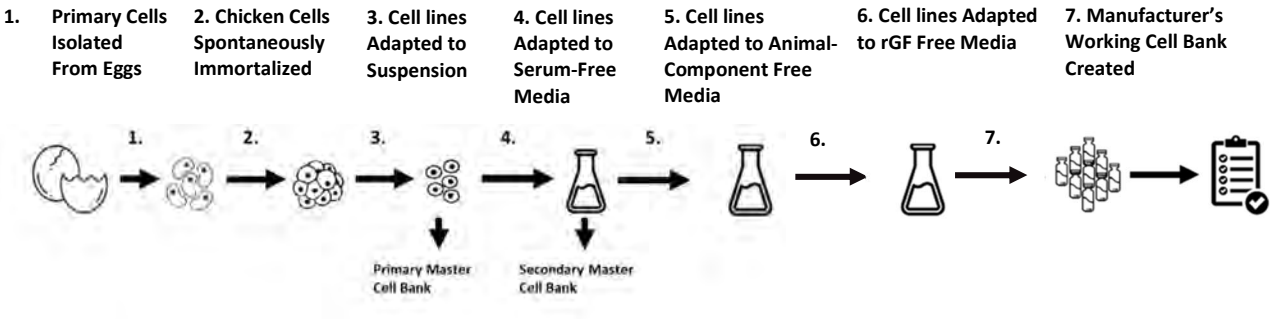
Believer Meats' Food Safety Plan includes a company overview, a description of the product and the process, and a process flow chart. The Food Safety Plan includes biological, chemical, and physical risk analysis, respective preventive controls, and related verification validation, correction, and corrective actions.

Believer Meats' sampling plan is based on the risk analysis within the company's Food Safety Plan. A more detailed outline of Believer Meats' Food Safety Plan Hazard Analysis for production is provided in Appendix A.

4.3 Generation of Cell Banks

Generation of cell lines with a verified species identity, stable and predictable phenotypes, and which are free of contaminating microorganisms is necessary to establish that the cells are food-grade and safe for their intended use in the production of cultured meat products. The following section provides a description of the steps involved in generation of Believer Meats' cell lines. A graphical overview is shown below (see Figure 4.3-1). Believer Meats' cell development process ended with the deposit of suspension adapted chicken cell lines in serum-free medium in the company's MWCB. This process, once completed, is not repeated, as the MWCB are the only source of cells that are used for the production of commercial products entering the food supply.

Figure 4.3-1 Generation of Believer Meats' Manufacturer's Working Cell Banks

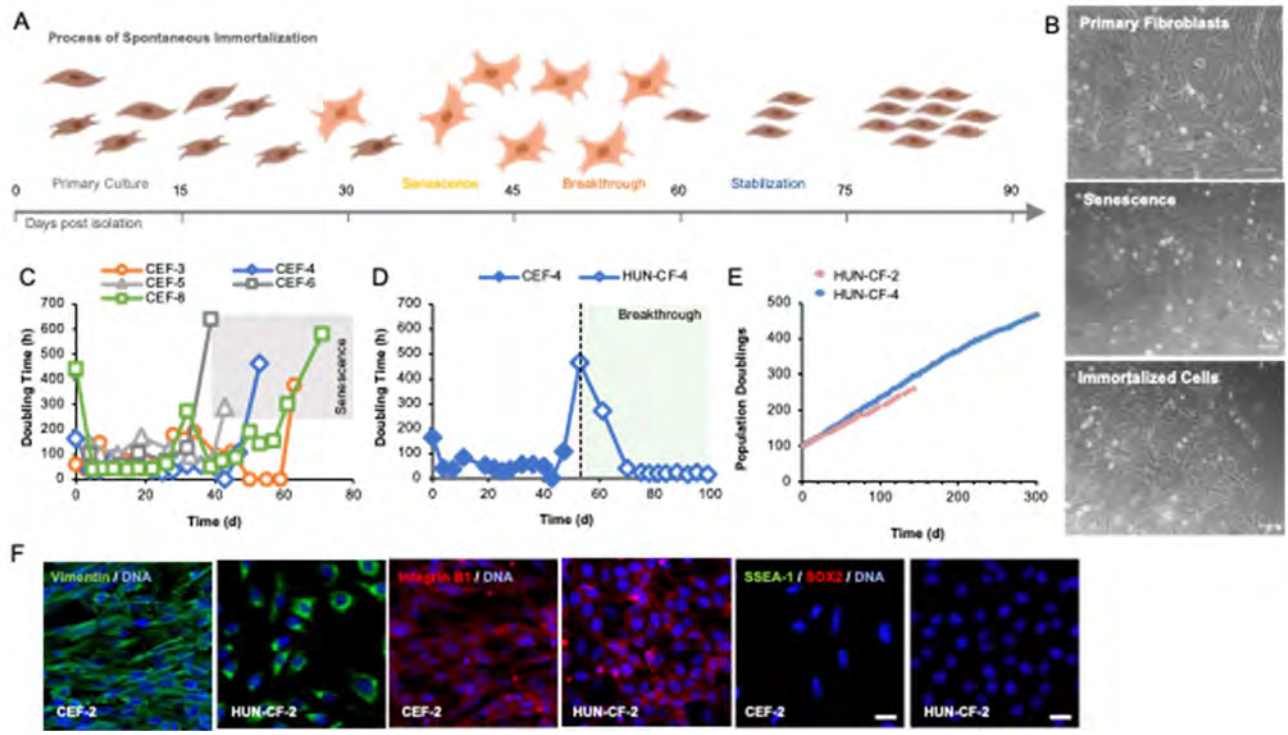


4.3.1 Cell Isolation and Immortalization

Believer Meats has derived the production cell line from an Israeli Baladi chicken, an ancient breed of domestic chicken (*Gallus gallus domesticus)* used for meat and eggs across the Middle East. The procedures used for obtaining primary cells and for the subsequent derivation of cell lines are described in detail in Pasitka *et al*. (2023). In brief, chicken embryonic fibroblasts were isolated from fertilized eggs as previously described (Durkin *et al*., 2013). Isolated tissue was sent for microbiological testing to exclude animal-born contamination (see Section 4.3.4). Primary chicken fibroblasts were expanded using standard culture techniques until the culture underwent replicative senescence within 70 days of isolation. Spontaneous breakthrough occurred in about 3% of isolates, leading to the formation of proliferating colonies of immortalized fibroblasts with a stable doubling time of 20 ± 2 hours (see Figure 4.3.1-1). Immortalized cell lines termed HUN-CF-2[1] and HUN-CF-4 represent cell lines derived from Broiler Ross 308 and Israeli Baladi chickens, respectively (see Figure 4.3.1-1). Immortalized chicken fibroblast lines proliferated exponentially for over 300 PDs showing consistency in doubling time and morphology (see Figure 4.3.3-1). Immortalized chicken fibroblast lines do not undergo reprogramming, remaining negative to pluripotency markers while maintaining expression of classical fibroblast markers (Figure 4.3.1-1).

[1] Note the HUN-CF-2 cell lines derived from Broiler Ross are research and development lines that are not used for cultured chicken production. Since data and information related to characterization of the cell lines has been adopted from studies published by Pasitka et al., (2023), some figures in the supporting references will contain data and information from HUN-CF-2.

Figure 4.3.1-1 Spontaneous Immortalization of Chicken Embryonic Fibroblasts



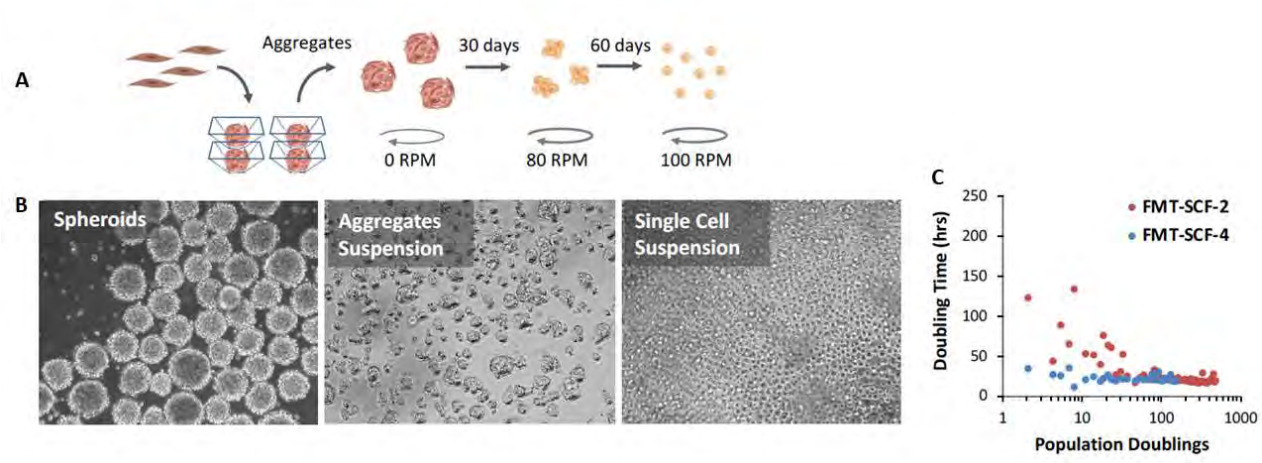
d = day(s); h = hour(s).
(A) Schematic diagram depicting the spontaneous immortalization process of fibroblasts. **(B)** Phase images of chicken embryonic fibroblasts isolated from fertilized chicken eggs. Primary spindle shape is lost during senescence, but spindle morphology is regained in rapidly proliferating colonies following immortalization. Scale bars 100 µm. **(C)** Doubling time of primary cells ranged from 25 to 150 hours. Cells became senescent within 40 to 70 days post-isolation. **(D)** Breakthrough event in Israeli Baladi (CEF-4) chicken. Immortalization occurred within 10 to 30 days post-senescence. Doubling time stabilized within a week to 20 ± 2 hours.
(E) Accumulative cell number of immortalized cells showing stable exponential growth for over 1 year of continuous culture.
(F) Confocal microscopy of primary fibroblasts (CEF-2) and immortalized fibroblasts (HUN-CF-2). Immortalized chicken cells express fibroblast markers vimentin and integrin B1. Immortalized chicken fibroblasts remain negative for pluripotency markers SSEA-1 and Sox2.

4.3.2 Suspension Adaptation

As adherent cells, the growth of fibroblasts in culture is limited by the surface area of the bioreactor. However, current industrial production technologies rely on anchorage-independent growth, that allow cells to grow to high densities limited only by the volume of the bioreactor. The procedures used for suspension adaptation are described in detail in Pasitka *et al*. (2023). In brief, immortalized fibroblast cell lines are allowed to form spheroids on low adhesion surfaces (see Figure 4.3.2-1). Spheroids are mechanically disturbed and transferred to shaker flasks for expansion. Following 30 days of culture, the shaker speed is increased, leading to single-cell suspensions developing over 60 days. Cell lines are considered to be anchorage-independent once the culture showed stable doubling time and viability above 94%.

Suspension adapted chicken cells of each breed were cryopreserved at PDs 120 to 180 and are designated as a primary MCB. The primary MCB consists of frozen ampules stored in liquid nitrogen at 2 independent facilities.

Figure 4.3.2-1 Adaptation of Immortalized Fibroblast Cell Lines to Anchorage-independent Growth



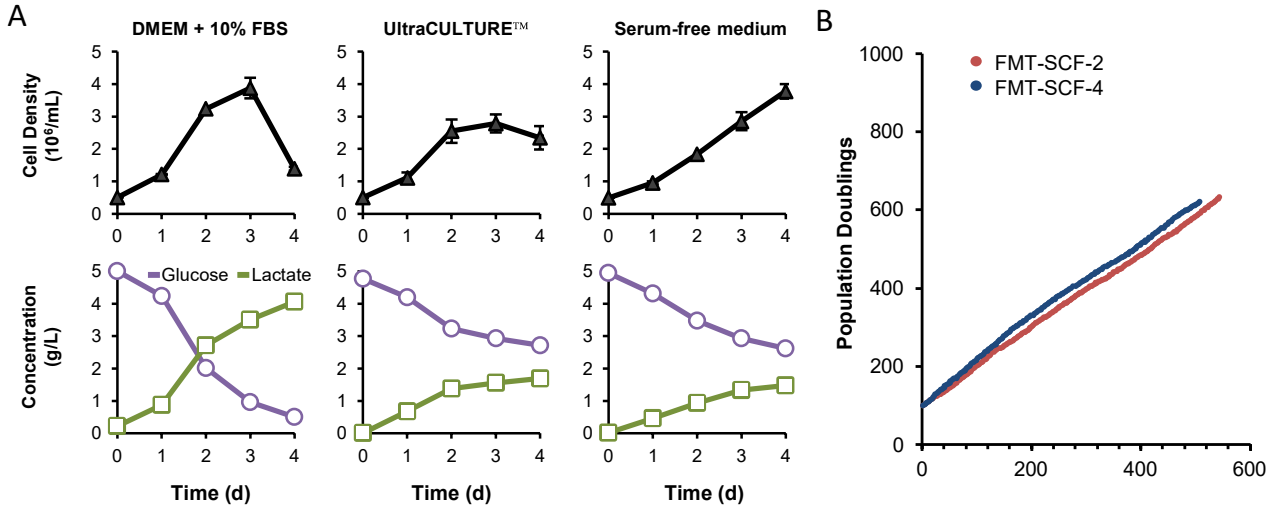
PD = population doubling.

(A) Schematic depiction of adaptation and selection of clones for single-cell suspensions. **(B)** Phase images of spontaneously immortalized fibroblasts during the adaptation and selection process. Fibroblast spheroids sheared into smaller cell aggregates during the adaptation period in shaker flasks. The single-cell suspension formed within 40 to 60 PDs. **(C)** Doubling times tracked during Broiler Ross 308 FMT-SCF-2 (research cell line) and Israeli Baladi FMT-SCF-4 (production cell line). Doubling time decreased from 200 to 50 hours during the adaptation period to 20±2 hours for over 470 PDs in FMT-SCF-2 and 22±2 hours for over 150 PDs in FMT-SCF-4, respectively.

4.3.3 Adaptation to Animal Component-free Media and Generation of Manufacturer's Working Cell Banks

Believer Meats has decided, due to ethical, cost and quality considerations, to utilize culture media that is free of animal derived components (*e.g.*, bovine serum, albumin) in its MWCB. Believer Meats has developed a proprietary animal component free (ACF) culture media formulation that produces cell growth performance that surpasses that of serum or other commercial serum-free media formulations (*e.g.*, UltraCULTURE). Believer Meats' animal component-free media is absent animal serum and other animal derived substances and has been demonstrated to support stable long-term exponential growth of the cells for over 400 days of continuous culture (see Figure 4.3.3-1). Cell growth in ACF media was equivalent to serum-free media formulations. All production runs reported in Section 5.0 of this consultation dossier were carried out with ACF media. The composition of the media is presented in Section 7.1.1 and Appendix B.

Figure 4.3.3-1 Characterization of Suspension-Adapted Chicken Fibroblasts



DMEM = Dulbecco's Modified Eagle Medium; FBS = fetal bovine serum.

(A) Growth kinetics of FMT-SCF-2 research line in FBS-supplemented medium (DMEM10), commercial serum-free medium (UltraCULTURE) and Believer Meats' serum-free medium. Cells in Believer Meats' serum-free medium stable logarithmic growth beyond Day 3. **(B)** Stable growth of FMT-SCF-2 research cell line and FMT-SCF-4 production cell line in suspension for over 500 days of continuous culture.

Cells from the MCBs were adapted for growth in the company's ACF media until a stable doubling time was achieved and then the cells were expanded to construct secondary MCBs. Manufacturer's Working Cell Banks (MWCB) are then generated from these secondary MCBs and have been demonstrated to be stable up to PD 1000. Believer Meats will create MWCBs provided the cells meet the identity and quality characteristics described in this submission and demonstrate genetic stability and stable PD times. All MWCB used for the production of cultured chicken fibroblasts will conform to the specifications set forth in Table 4.3.3-1 below. MWCB's that meet these specifications are defined as 'food grade' and can safely be used as sources of cells in food products manufactured by Believer Meats. Additional discussion of the testing rationale for adventitious agents are presented in the sections that follow and in 7.1.2.

Table 4.3.3-1 Specifications of the MWCB

Cell Source	Pathogen	Specification Limit	Method Used
MWCB	Sterility Test	Negative	USP 71[1]
	Mycoplasma spp.	Not Detected	Nested-PCR; in-house method based on quality standards of 21 CFR 610.30.[1]
	Avian Influenza A	Not Detected	PCR[1]
	Newcastle disease virus	Not Detected	PCR[1]
	Species Determination	Chicken	RapidFinder™ Chicken ID Kit
	Doubling Time	dT < 30h	Culture (in-house method)
	Morphology	Normal	Confocal microscopy
	Tropomyosin Expression	Cq < 26	RT-PCR (in-house)

[1]Methods are conducted using validated test methodologies that are fit for purpose by third party experts in accordance with the organization's internal protocols.

Storage and maintenance of Believer Meats' cell banks are conducted in a manner that will retain the initial characteristics of the organism and ensure freedom from contamination or deterioration. For example, freezers are monitored for temperature changes to control appropriate storage temperatures for preservation. Cell banks are stored in a secured location at Believer Meats' main production facility and at an off-site location to avoid losses from equipment malfunction. The inventory of Believer Meats' cell banks is documented, and records are retained establishing that the MWCB is food-grade (*i.e.*, species verified, stable phenotype, and freedom of microbial or viral contaminants). As discussed previously, only cells from Believer Meats' MWCBs are used for the production of cultivated meat products entering the U.S. marketplace.

4.3.4 Adventitious Agent Testing

As discussed in greater detail in Section 7.1.2, the isolation and cultivation of animal cells can introduce microbial hazards/adventitious agents to the production process. Believer Meats' MWCBs are qualified to be free of contaminating microorganisms/adventitious agents that present a quality or human food safety risk. Believer Meats adventitious agent testing scheme considers four categories of adventitious agents that may be transferred during construction of the MCB and MWCB and included the following:

1. Bacteria and fungi
2. *Mycoplasma*
3. Parasites
4. Zoonotic viruses (*i.e.*, viruses with an avian-human tropism)

Believer Meats risk assessment of adventitious agents recognized that conventional chicken products have a long-history of safe consumption and therefore focused on risk mitigation measures targeted to microorganisms that have a high endemic prevalence in chickens and that are pathogenic to humans and/or may negatively impact the performance of the bioreactor (See Section 7.1.2 for additional background information on the hazard characterization and risk assessment process).

Testing procedures for bacterial pathogens in food products typically requires selective culture-based procedures that require large sample sizes (*e.g.*, 25 g). Alternative procedures for testing of the MWCB's are therefore necessary to establish that the cell banks are free of pathogenic bacteria. Believer Meats notes that the cell culture process must operate under aseptic conditions as contaminating bacteria or fungi would quickly overwhelm the bioreactor leading to a failed production run. The process is therefore inherently self-limiting as any form of bacterial or fungal contamination would render the MWCB's non-viable for food production. Accordingly, the MWCB's are certified to be sterile. Believer Meats tests the MWCB's for sterility using a test protocol validated for cellular biomass substrates (*i.e.*, USP 71). The test conditions for demonstrating sterility of the MWCB are conducive to the growth of all known foodborne pathogens and therefore demonstration of sterility of the MWCB is used as an indirect verification that the cell bank is free of pathogenic bacteria or fungi that may originate from the donor chicken.

The innocuous nature of *Mycoplasma* in humans, animals, and the environment increases the likelihood of introducing these organisms into cell lines or a manufacturing process. *Mycoplasma* contamination is not detected during sterility testing, does not produce turbidity or pH changes during cell culture contamination event, and therefore are not always apparent. *Mycoplasma* also can have adverse effects on cell line growth and characteristics. Although common chicken *mycoplasma* serovars (*e.g.*, *Mycoplasma gallisepticum*, *Mycoplasma synovium*) are not human pathogens, for quality reasons the MWCB must be demonstrated to be absent contamination from all mycoplasma species. Broad-based testing of the MWCB

for *Mycoplasma* spp. was conducted by third-party experts using a validated assay that utilizes nested PCR to amplify the 16S rRNA coding region in the mycoplasma genome.

Although chickens can harbor pathogenic parasitic organisms that are a food safety risk (*e.g.*, *Toxoplasma gondii*), parasites require complex life cycles for propagation that require live animal hosts. The cell culture procedure is not expected to support growth of these organisms and therefore testing of the MWCB for parasites was not considered necessary.

Several viruses are endemic to poultry environments and chickens used in food production (*e.g.*, broilers and layers) may become infected with viruses, which may or may not cause disease in the birds. However, as most poultry viruses do not display avian-human tropism and therefore the majority of avian viruses are not recognized as food safety concerns. The two most prevalent avian zoonotic viruses of economic importance to poultry agriculture include avian influenza A virus subtypes H5, H7, and H9 and *avian paramyxovirus* type 1 responsible for Newcastle Disease (ND) in chickens; however, neither avian influenza nor ND are recognized as human food safety hazards, as transmission to humans from handling or ingestion of poultry meat is not known to occur (EFSA, 2012) (See Section 7.1.2). Although the food safety risk of acquiring avian influenza or ND virus from poultry food products is low, due to the prevalence of these viruses combined with their theoretical risk of human zoonoses, testing of the MWCB was conducted using validated PCR based assays. Believer Meats also considered the theoretical possibility that non-avian zoonotic viruses may have contaminated the MWCB during upstream development of the cell lines where exposure to animal derived components (*e.g.*, bovine serum and porcine trypsin) have occurred. As the MWCB are produced in ACF media, the risk of transferring mammalian viruses from the animal components is low as ovine and porcine viruses are not expected to be share a tropism with avian species that would be conducive to their propagation in chicken cells. Notwithstanding this viewpoint, the MCB was tested for the presence of mammalian polyadenylated viruses using RNA sequencing methods and *in silico* screening of the sequenced genomic information against a data bank of bovine and porcine viruses. No mammalian viruses of human food safety concern were identified (for the list of viruses identified from the literature see Appendix C). An overview of the adventitious agent testing panel, methodology and results of Believer Meats' cell bank testing program are presented in Sections 4.3.4.1 and 4.3.4.2 below.

Believer Meats has incorporated an adventitious agent testing procedure into its Food Safety Plan (see Appendix A). The prevention of adventitious agent contamination uses multiple approaches:

1. Prevention of adventitious agent entry into the meat production system by selecting low-risk raw materials (established through a supplier verification program) for production of the company's cell banks, and during scale up, manufacture, and harvest of the chicken biomass.
2. Use of filter sterilization methods (0.2 µM) for filtration of the media used for cell-line banking and for cultured fibroblast production.
3. Filtration and/or sterilization of water, gases, and food contact surfaces that may come in contact with the cells during the meat production process.
4. Testing of the MCB, MWCB, and harvested biomass to verify the absence of relevant microbial contaminants and other adventitious agents that are of human health risk.

Although not a risk mitigation measure, the requirement for cooking cultured chicken products to safe temperatures (*i.e.*, 165°F) provides a third level of assurance that Believer Meats' products used as food will be safe from viral and bacterial contaminants that would be inactivated at these temperatures. Additional discussion of the adventitious agent testing approach is presented for the MCB and MWCB below.

4.3.4.1 Adventitious Agent Testing from Cell Derivation to Establishment of Master Cell Banks

Believer Meats conducted preliminary quality control screening of the embryonic tissue and MCBs for microbial and viral contamination that may negatively influence worker safety or the performance of the cultures. Samples of the tissues from the donor animal and the MCB were sent for virus and mycoplasma testing. As summarized in Table 4.3.4.1-1, the chicken embryonic tissues and MCBs were absent contamination by Newcastle disease virus and avian influenza. Avian influenza is considered one of the most important avian zoonotic viruses due to its significant potential for severe pathogenicity in humans, and Newcastle disease virus can produce mild conjunctivitis in humans; however, as discussed in Section 7.1.2, neither virus is recognized as a food safety risk for poultry meat. The presence of these viruses in the cell lines would trigger termination of the cell-line from future development. At the MCB stage, *Mycoplasma* was limited to preliminary screening of two common avian species (*Mycoplasma gallisepticum*, and *Mycoplasma synoviae*) for quality control purposes. Complete mycoplasma screening for over 100 common species that may contaminate cell lines is conducted on the secondary MCB and the MWCB.

The MCB was tested for aerobic plate counts, yeast and mold, as well as pathogenic food safety bacteria that are indigenous to the chicken microbiome (*i.e.*, *E. coli*, *Salmonella* sp., and *Campylobacter* sp.). No bacteria, yeast, or mold contamination of the MCB could be detected using validated assays at third party laboratories.

Believer Meats is continually improving its adventitious agent testing program to include more agnostic broad-based testing procedures. For example, Believer Meats recognizes that the use of animal derived components from bovine or porcine species can introduce mammalian viruses to the cell lines, which may be a food safety concern in situations where such viruses display an avian-mammalian tropism. Believer Meats notes that viruses displaying avian-bovine/porcine tropism[2] and that produce documented evidence of foodborne illness are likely extremely rare. Nevertheless, Believer Meats has developed a comprehensive database and algorithm designed for the detection of polyadenylated viruses within sequenced RNA-seq samples. This initiative involved compiling a dataset of 3259 vertebrate viruses sourced from NCBI, consolidating those with multiple segments into a unified segment. The resultant database, referred to as the "vertebrate virus database," was optimized for alignment using the bowtie2 tool (Langmead and Salzberg, 2012). It is important to acknowledge the methodology's limitation, which focuses on viruses undergoing RNA polyadenylation due to the use of poly-T primers during the library preparation of chicken RNA-seq samples. Future iterations may extend this approach to unbiased total RNA-seq libraries for detecting all viral RNA molecules, irrespective of polyadenylation. To address food safety concerns, a compilation of bovine and porcine viruses previously identified in bovine serum or porcine trypsin was created (Zhang *et al.* 2022, Paim *et al.* 2021). The method was validated by using sequence information from external databases of Rabies Lyssavirus (SRR22207407) and Vesicular Stomatitis Indiana virus (SRR17272562) infected samples. The SCF-4 RNA-seq data exhibited no significant alignment with the listed viral genomes (See Appendix C). Consequently, it was concluded that BM's chicken cell line does not contain bovine and porcine viruses posing food safety concerns. Additional discussion of this analyses is presented in Appendix C.

Believer Meats emphasizes that the cryopreserved cell lines that constitute the MCBs are precious samples with limited sample sizes and therefore are only tested for quality purposes to determine the suitability of the cell lines for further development. The MCBs are stored in separate cryovessels from the MWCB to ensure that cross-contamination or accidental use of these cell lines for cultivated meat production does not occur. Negative results of the RNA seq analyses in the MCB will represent a release requirement for

[2] Avian-bovine or avian-porcine Tropism refers to viruses that infect both chickens and cows or chickens and pigs respectively.

preparation of the MWCB. Alternatively, RNA seq can also be incorporated into the specification requirements for the MWCB.

Based on the testing results shown in Table 4.3.4.1-1 below it can be concluded that the MCB was free of bacteria, yeasts and molds, mycoplasma, and viruses that are a food safety concern for cultured chicken cells.

Table 4.3.4.1-1 Viral and Microbiological Testing of Chicken Embryonic Tissue and Master Cell Banks

Israeli Baladi Early Cell Cultures			
Cell Type	**Pathogen**	**Method Used[1]**	**Results**
Embryonic tissue	Avian influenza A	Kylt® Influenza A - H9 RT-PCR	ND
	Newcastle disease virus	Kylt® Paramyxovirus 1 RT-PCR	ND
Master cell banks	Total aerobic count	Pour plate, SOP No. 10-021, based on Harmonized USP/EP Pharmacopeias	ND
	Yeast and Molds	Pour plate, SOP No. 10-021, based on Harmonized USP/EP Pharmacopeias	ND
	E. col	Pour plate, SOP No. 10-021, based on Harmonized USP/EP Pharmacopeias	ND
	Salmonella sp.	Pour plate, SOP No. 10-021, based on Harmonized USP/EP Pharmacopeias	ND
	Campylobacter sp.	VIDAS enzyme-linked fluorescent immunoassay (ELFA)	ND
	Mycoplasma spp.	Nested-PCR; in-house method based on quality standards of 21 CFR 610.30.	ND
	Avian influenza A	PCR	ND
	Newcastle disease virus	PCR	ND
	Select polyadenylated bovine and porcine viruses of human food safety concern.	RNA seq	ND

ND = not detected; RT-PCR = real-time polymerase chain reaction
[1]Methods are conducted using validated test methodologies that are fit for purpose by third party experts in accordance with the organization's internal protocols.

4.3.4.2 Adventitious Agent Testing of the Manufacturer's Working Cell Banks

Believer Meats has generated MWCBs from suspension-adapted immortalized chicken cells growing in serum-free media. No animal derived reagents are introduced to the cells at this stage and therefore there are no new sources of adventitious agents during this step in the production process. Only cells derived from the MWCB's will be used for producing cultured chicken fibroblasts that enter the U.S. food supply; therefore, testing of the MWCB for sterility, absence of mycoplasma, and avian zoonotic viruses, represents the primary risk mitigation measure for ensuring that adventitious agents that originate from the donor animal or media components used during cell-line development do not enter the food supply. The MWCB is tested once for adventitious agents as shown in Table 4.3.4.2-1. These analyses are conducted using test methods that are validated and fit-for-purpose for use on a cellular biomass matrix.

Table 4.3.4.2-1 Adventitious Agent Testing of Manufacturer's Working Cell Bank

Cell Source	Pathogen	Method Used	Results
Manufacturer's Working Cell Bank (MWCB)	Sterility Test	USP 71[1]	No Growth
	Mycoplasma spp.	Nested-PCR; in-house method based on quality standards of 21 CFR 610.30.[1]	ND
	Avian Influenza A	PCR[1]	ND
	Newcastle disease virus	PCR[1]	ND

ND = not detected; RT-PCR = real-time polymerase chain reaction
[1]Methods are conducted using validated test methodologies that are fit for purpose by third party experts in accordance with the organization's internal protocols.

The absence of cultivatable bacteria and fungi in the cell bank was demonstrated using the gold standard for sterility verification of drug substances in accordance with USP 71[3]. USP 71 has been adopted for use in cellular therapies and therefore is validated for use on cell bank samples (Gebo and Lau, 2020). As per the requirements of USP 71, the test method was validated for use on cellular material (*i.e*., Believer Meats' MWCBs) by inoculation with 6 challenge microorganisms, representing aerobic bacteria, anaerobic bacteria, and fungi (see Table 4.3.4.2-2). The sensitivity of the test was established for each microorganism by inoculation of the test article with not more than 100 CFU. Thioglycolate broth (Fluid Thioglycolate Medium, FTM) is used to test bacteria with different degrees of tolerance to oxygen. The media contains basic nutrients to support bacterial growth, sodium thioglycolate, thioglycolic acid, L-cystine, methylene blue, and agar. Sodium thioglycolate, thioglycolic acid, and L-cystine reduce the oxygen to water to create an environment for strict anaerobes. Methylene blue is an indicator that is colorless in an anaerobic environment and greenish blue in the presence of oxygen. The agar helps retard oxygen diffusion and helps maintain the stratification of organisms growing in different layers of the broth. Oxygen is driven out of the broth by autoclaving, but as the broth incubates at room temperature, oxygen begins to diffuse back into the tube producing a thin layer of blue-green staining at the top of the broth. Obligate aerobes will only grow in this oxygen-rich top layer, obligate anaerobes will only grow in the lower areas of the tube, and microaerophiles will grow in a thin layer below the richly oxygenated layer. Facultative or aerotolerant anaerobes will grow throughout the medium but will primarily grow in the middle of the tube, between the oxygen-rich and oxygen-free zones (Austin Community College, 2007).

Tryptic soy broth (TSB) is used for detecting growth of yeast, fungi and spore forming bacteria. TSB is a general-purpose medium. Clostridia and non-sporulating anaerobes grow rapidly in this broth when incubated under anaerobic conditions. TSB is often recommended for testing bacterial contaminants in cosmetics and complies with established standards in the food industry. The broth typically contains an enzymatic digest of casein and soybean meal as nitrogen sources, dextrose as a carbon source, sodium chloride for maintaining osmotic balance and dipotassium phosphate as a buffering agent.

Believer Meats has demonstrated that its MWCBs are sterile using USP 71, the gold standard for sterility testing. All of the most important food-borne pathogens originating from poultry (*e.g*., *Salmonella* sp., *E. coli* sp., *Campylobacter* sp.) were expected to grow under conditions of the sterility testing assay. Based on the findings of the sterility assay combined with the species-specific testing of the MCB for various pathogenic bacteria endemic to chickens (*i.e*., *E. coli*, *Salmonella* sp., and *Campylobacter* sp.) the MWCB was concluded to be absent bacterial contaminants that are a quality or food safety hazard.

[3] Compendial methods for food (*e.g*., Food Chemical Codex) do not exist; however, in this case, pharmacopeia test methods were considered appropriate for food use and are expected to meet or exceed those typically applied to food.

Table 4.3.4.2-2 Challenge Microorganisms and Media

Challenge Microorganism	Medium	Incubation Temperature
Staphylococcus aureus ATCC 6538	Fluid thioglycolate medium	30–35°C
Pseudomonas aeruginosa ATCC 9027		
Clostridium sporogenes ATCC 11437		
Bacillus subtilis ATCC 6633	Tryptic soy broth	20–25°C
Candida albicans ATCC 10231		
Aspergillus brasiliensis ATCC 16404		

Analysis of the MWCB for mycoplasma was conducted using a validated nested-PCR assay developed by an external laboratory. The method utilizes a Nucleic Acid Amplification Technique (NAT) for detection of Mycoplasma that uses a nested (two-stage) PCR process for enhanced sensitivity and specificity. Nested-PCR is used to amplify conserved regions of the Mycoplasma genome using a mix of specially designed primers which allows the detection of more than 100 different Mycoplasma, Acholeplasma and Ureaplasma species including *Mycoplasma gallisepticum* and *Mycoplasma synoviae* as predicted by bioinformatic research and also including the eight most commonly encountered Mycoplasma contaminants which account for more than 96% of cell culture infections.

Believer Meats demonstrated that its MWCB's were absent contamination for Newcastle disease virus and avian influenza A using a validated PCR based assay conducted by the State of Israel Ministry of Agriculture and Rural Development (Table 4.3.4.2-1).

Believer Meats has phased out the use of all animal media components during the production process, including bovine catalase, fetal bovine serum, porcine trypsin, and bovine serum albumin, which effectively eliminates the risk of contamination with bovine and porcine adventitious agents during the production process. In addition, risk mitigation measures for ensuring that bovine/porcine viruses used at upstream points in the cell development process are not introduced to the MWCB were conducted using the RNA seq analyses described in Section 4.3.4.1. In addition, Believer Meats only sources serum from countries with negligible risk of bovine spongiform encephalopathy (BSE) thereby ensuring that sources of pathogenic prions do not contaminate the cell banks at these upstream steps in the cell banking process.

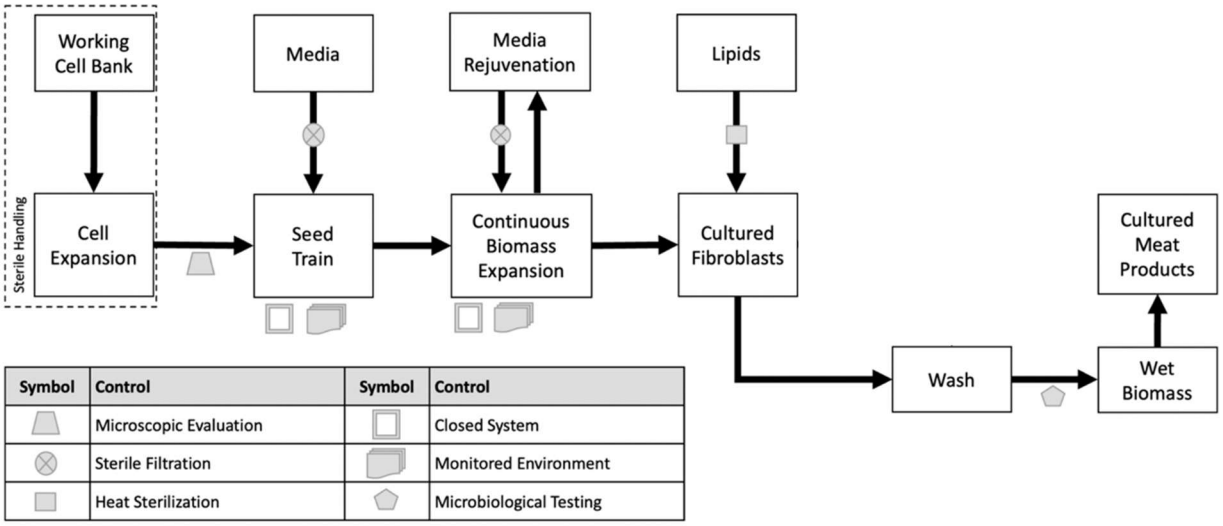
4.4 Manufacturing of Cultured Chicken Cells

Expansion of the immortalized, anchorage-independent, and serum-free adapted chicken cells occurs in bioreactors. This section illustrates the process from the MWCB through the harvesting of cultured chicken cells.

The production Believer Meats' cultured chicken relies on the conversion of nutritional components within the media into edible cultured biomass (see Figure 4.4-1). The process starts by thawing and expanding cells from MWCB under aseptic conditions. Cells are evaluated microscopically and seeded in a closed bioreactor system with a precisely controlled environment that supports cell growth. Batches of cells are cultured successively in a seed train until an adequate number of cells are available to seed large-scale bioreactors. Believer Meats' large-scale bioreactors work under continuous or semi-continuous processes, exchanging waste materials such as ammonia and lactate with rejuvenated media to insure high density cell growth. Rejuvenation of the media involves multiple proprietary filtration steps (including microfiltration through a 0.22 μm filter) of the spent media to remove ammonia and lactate recycles the process nutrients back to the bioreactor. Further details regarding controls for the rejuvenation process may be found in Appendix A-Confidential Supplementary Material.

Cultured cells are harvested directly from the bioreactor. Biomass harvested from the process is washed to remove media residues. Harvested biomass has a minimum of $100x10^6$ cells/gram. Each batch of biomass produced undergoes testing to confirm the product complies with specifications (see Section 5.0). Further food processing combines harvested biomass with plant-based ingredients to generate cultured meat products.

Figure 4.4-1 Production Flow of Cultured Chicken Products



Symbol	Control	Symbol	Control
	Microscopic Evaluation		Closed System
	Sterile Filtration		Monitored Environment
	Heat Sterilization		Microbiological Testing

4.4.1 Contamination Control During Production of Cultured Chicken Fibroblasts

During the production process, the following steps provide complete contamination control (the process controls are also depicted in the flow chart for biomass production, see Figure 4.4-1):

- The growth media, rejuvenated media, and washing agents are sterile filtrated (by 0.2-µm membrane filtration) prior to use in the production process. This step ensures that bacteria and fungi that may originate as contaminants of the water or media ingredients are not transferred to the bioreactor. As no animal components are used during the production process, avoiding the introduction of bacteria, fungi, mycoplasma, or viruses from these sources.
- The bioreactor operates as a closed system and is cleaned prior to use by validated methods. This ensures no bacteria or viruses originating from the environment or worker personnel are introduced to the vessel prior to production.
- A microscopic evaluation to exclude visual contamination is conducted prior to cell inoculation in the seed-train bioreactor.
- In the bioreactor, microbial contamination can be identified based on dissolved oxygen (DO) concentration, which is continuously monitored in-line. Contaminations cause the DO concentration to rapidly drop; in the presence of contamination, confirmation may be acquired by plate count and microscopic evaluation.

Microbial testing is also conducted at the end of the biomass production process as part of biomass release tests. In the case of contamination, the batch will be disqualified, and the vessels will be sterilized according to Believer Meats' internal SOP. In addition, an investigation will be opened to determine the potential cause and to prevent future contamination. The microbes tested on final cell biomass include total aerobic bacterial count, *Enterobacteriaceae*, and *Salmonella* spp.