7.0 SAFETY ASSESSMENT

7.1 Safety Evaluation of Production Hazards

7.1.1 Media and Washing Agents

In this section, the media constituents necessary for sustaining cell growth during production of the company's cultured chicken products (*e.g*., nutrients, growth mediators, and phenotypic modulators) are discussed. Most of these components are nutritive substances that are common to the diet, and many are synthesized endogenously within all animals as part of metabolic pathways that are necessary to sustain life. Accordingly, a majority of the media components are expected to be naturally present within chicken--derived products consumed as food. Depending on the stage of biomass production, media constituents may differ slightly, although the basic medium used from isolation of cells from embryos through transition to a serum-free medium consists of Dulbecco's Modified Eagle Medium with 10% fetal calf serum, L-alanine-L-glutamine, and antibiotics (penicillin and streptomycin). These upstream media components are only used to produce the MCBs and therefore residues of these components will not be transferred to the finished products. Once cells have transitioned to Believer Meats' proprietary serum-free medium and deposited in the company MWCB, antibiotics are no longer used and there is no risk of introducing antibiotics to Believer Meats' cultured chicken products.

Media components used in Believer Meats' cell production have been selected following studies that optimized cell growth and differentiation and allowed for cost-effective production at a commercial scale. Believer Meats' food safety assessment was conducted for all constituents of the media used in production of the harvested biomass. Theoretical dietary intakes for each substance were estimated using analytical data where available. To account for variability in the production process, the estimated daily intake (EDI) values were set at the mean + 3x the standard deviation from analytical batch results to estimate the maximum upper ranges of intake. When empirical data was not available, the EDI was calculated based on the conservative assumption of daily *per capita* intake of 72 g biomass per day (see Section 6.1) and the presence of the media component in the biomass product at the same concentration as in the media (e.g., 1,000 mg/L = 1,000 mg/1,000 g = 72 mg/72 g biomass). This calculation did not take into account washing steps. The biomass harvested from the bioreactor contains roughly 95% water. Most of Believer Meats' media components are water-soluble and are readily removed from biomass through an osmotically buffered washing step (see Figure 4.4-1). Believer Meats recognizes that some of the media nutrients will be incorporated into the biomass tissues (*e.g*., minerals, fatty acids, and amino acids). The quantities of these substances have been evaluated through detailed compositional testing of the biomass.

As part of Believer Meats' food safety evaluation procedures, the company developed a three-tier ranking for risk assessment categorization of the media components. Media components in the first two categories (Class 1 and 2) included substances that have been previously evaluated by the U.S. FDA for food use, and therefore it was concluded that these substances are non-genotoxic, and that food grade sources are available on the marketplace. Class 3 substances do not have a history of food use and therefore scientific procedures were applied to the safety evaluation of these substances in a manner that was aligned with scientific procedures requirements for traditional food safety evaluation.

Class 1: Class 1 compounds consist of substances that are currently permitted by federal regulation for food applications that are applicable to use in cultured meat production. Typically, these substances are

permitted by regulation for general food use applications as processing-aids and/or in accordance with cGMP (*i.e.*, minimum levels necessary to achieve a desired technical effect). A summary of Class 1 substances and corresponding procedures for evaluation of their safety is presented in Table 7.1.1-1 below. Table B1 in Appendix B provides additional details related Class 1 media components that are considered trade secret.

Table 7.1.1-1 Class 1 Media Component Risk Assessment

Component	Applicable Regulatory Reference	Safety Reference Level (*e.g.*, DV, UL, ADI, NOAEL)[A]	Safety Conclusions	Reference
Fatty Acids				
Oleic acid	21 CFR §172.860	Total oleic acid levels in cultured chicken cells are approximately equivalent to store bought chicken (Table 5.4-3)	No safety concerns with used in accordance with cGMP.	-
Palmitic acid	21 CFR 172.860	Total palmitic acid levels in cultured chicken cells are approximately equivalent to store bought chicken (Table 5.4-3)	No safety concerns with used in accordance with cGMP.	-
Acids				
Hydrochloric acid	21 CFR §182.1057	Permitted by federal regulation without limitation on use	No safety concerns when used in accordance with cGMP.	FASEB (1979)
Salts				
Calcium chloride	21 CFR §184.1193	DV = 1,300 mg	No safety concerns when used in accordance with cGMP.	IOM (2011)
Magnesium chloride	21 CFR §184.1426	DV = 420 mg	No safety concerns when used in accordance with cGMP.	FASEB (1976b)
Magnesium sulfate	21 CFR §184.1443	DV = 420 mg	No safety concerns when used in accordance with cGMP.	FASEB (1976b)
Manganese (II) chloride	21 CFR §184.1446	DV = 2.3 mg	EDI < DV.	FASEB (1979)
Copper (II) sulfate	21 CFR §184.1261	DV = 900 µg	EDI < DV.	FASEB (1979a); IOM (1998)
Sodium chloride	21 CFR §182.1	No safety concerns, as EDI < levels in common food products and only a small percentage of the DV.		U.S. FDA (2022b)
Sodium phosphate	21 CFR §182.1778	No safety concerns, as EDI for sodium and phosphorus < levels in commonly consumed food products and a small fraction of the DV.		FASEB (1975b)
Sodium hydroxide	21 CFR §184.1763	No safety concerns when used in accordance with cGMP.		FASEB (1976a)
Sodium bicarbonate	21 CFR §184.1736	No safety concerns when used in accordance with cGMP.		FASEB (1975c)

Table 7.1.1-1 Class 1 Media Component Risk Assessment

Component	Applicable Regulatory Reference	Safety Reference Level (*e.g.*, DV, UL, ADI, NOAEL)[A]	Safety Conclusions	Reference
Potassium chloride	21 CFR §184.1622	No safety concerns when used in accordance with cGMP.		FASEB (1979)
Other Substances				
Hydroxypropyl methyl cellulose	21 CFR §172.874	No safety concerns when used in accordance with cGMP.		-
Maltodextrin	21 CFR §184.1444	No safety concerns when used in accordance with cGMP.		-

ADI = acceptable daily intake; bw = body weight; CFR = *Code of Federal Regulations*; EDI = estimated daily intake; FASEB = Federation of American Societies of Experimental Biology; FDA = Food and Drug Administration; FNB = Food and Nutrition Board; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; IOM = Institute of Medicine; JECFA = Joint FAO/WHO Expert Committee on Food Additives; LOAEL = lowest-observed-adverse-effect level; MTDI = maximum tolerable daily intake; N/A = not applicable; NOAEL = no-observed-adverse-effect level; OSL = observed safe level; PTWI = provisional maximum tolerable weekly intake; RDA = recommended dietary allowance; SCOGS = Select Committee on GRAS Substances; UL = tolerable upper intake level; y = years.

[A] When available, the EDI value was derived from the mean+3x the standard deviation of three analytical batch data presented in Tables 5.4-3 or 5.4-5 to estimate the maximum upper ranges of intake. When empirical data was not available, the EDI was calculated based on the conservative assumption of daily *per capita* intake of 72 g biomass per day and the presence of the media component in the biomass product at the same concentration as in the media (*e.g.*, 1,000 mg/L = 1,000 mg/1,000 g = 72 mg/72 g biomass). This calculation does not take into account washing steps following production. EDI is provided as a daily amount (mg per day), and also on a body weight (bw) basis (mg per kg bw per day) for a 60-kg adult. EDI values are presented in Table B-1 of Appendix B.

Class 2: Class 2 substances are those compounds that are permitted for specified food use applications that may not be directly extrapolatable to the intended conditions of use of the substances as a culture media aid, and where hazard characterization of the substance (*e.g.*, toxicology profile, allergenicity concern, anticipated bioaccumulation) suggests a margin of exposure analysis might be needed to compare levels in the final animal cell product with an appropriate safe reference level. In general, the intended technical effect of most Class 2 substances was to provide nutritive components that form the “building blocks” for synthesis of cells and are necessary to sustain cell proliferation and other metabolic processes that are necessary for cell growth and survival. When used in accordance with cGMP (*i.e.*, minimum levels needed to produce optimum cell growth), Class 2 substances will be converted to meat in a manner that is analogous to the dietary conversion of nutritional components within animal feed products, and therefore will be present within the cell cultured chicken meat at levels that are generally similar to levels present within conventional chicken meat obtained from an animal carcass; analytical data comparing the nutrient composition of Believer Meats’ cell cultured products to conventional chicken meat support this viewpoint (See Section 5.4).

Safe reference levels for nutrient substances were established using a tiered approach where the highest confidence level for safety was established relative to reference values from the conventional comparator food or other commonly consumed foods in the diet. When the levels of a nutrient fell within the range that has been reported for conventional chicken products, exposures were concluded to be safe. Where levels of a substance were elevated above the comparator food, comparisons to the Daily Value (DV) were used. Dietary intakes that were below the DV were similarly considered safe. Where dietary intakes were estimated to exceed the DV, reference to the Tolerable Upper Level (UL) established by the Food and Nutrition Board of the Institute of Medicine were used as safe upper limits; however, in these cases further estimation of background intakes from all food uses would be necessary. For non-nutritive substances, safe reference levels were based upon values derived from animal toxicology studies and/or Acceptable Daily Intake (ADI) values derived by authoritative bodies that relied on published data and information.

A summary of Class 2 media components used during the production process described in this consultation submission is provided in Table 7.1.1-2 below along with information on the safe reference levels for each compound and corresponding conclusions on their safety for production of cultured chicken cells. Table B-2 in Appendix B provides the risk assessment information for Category 2 media components.

Table 7.1.1-2 Class 2 Media Component Risk Assessment

Component	Safety Reference Level	Safety Conclusions	Reference
Nutrients			
Fatty acids	Common dietary nutrient	No safety concerns based on hazard profile and widespread presence in diet and fact that levels are < concentrations in conventional poultry meat products.	See Appendix B
Iron salts	DV = 18 mg	No safety concerns, as EDI < DV	See Appendix B
Zinc salts	DV = 15 mg	EDI < DV	See Appendix B
Other mineral salts	PTWI = 14 mg/kg bw	EDI < PTWI	See Appendix B
L-Ascorbic acid and derivatives	DV = 90 mg	EDI < DV	See Appendix B
Choline salts	DV (choline) = 550 mg	EDI < DV	See Appendix B
Myoinositol	"*Orally administered inositol is absorbed slowly and is metabolized. The available information from toxicological studies in animals suggests no adverse effects associated with consumption of inositol at levels considerably in excess of those now consumed by humans*."	No safety concerns based on hazard profile and widespread presence in diet.	FASEB (1975d)
Vitamin B1 (Thiamin)	DV = 1.2 mg	No safety concerns based on hazard profile and widespread presence in diet.	IOM (1998)
Vitamin B2 (Riboflavin)	DV = 1.3 mg/day (19–50 years)	No safety concerns based on hazard profile and widespread presence in diet.	IOM (1998)
Vitamin B3 (Niacin) and derivatives	DV = 15 mg	No safety concerns based on hazard profile and widespread presence in diet.	IOM (1998)
Vitamin B5 (Pantothenic acid)	DV = 5 mg	No safety concerns based on hazard profile and widespread presence in diet.	NIH ODS (2021)
Vitamin B6 (Pyridoxine)	DV = 1.7 mg/day	No safety concerns based on hazard profile and widespread presence in diet.	FASEB (1977)
Vitamin B7 (Biotin)	DV = 30 µg	No safety concerns based on hazard profile and widespread presence in diet.	IOM (1998)
Vitamin B9 (Folic Acid)	DV = 400 µg/day	EDI < DV	IOM (1998)
Vitamin B12 (Cyanocobalamin)	DV = 2.4 µg/day	No safety concerns based on hazard profile and widespread presence in diet.	IOM (1998)
Amino acids (Methionine, alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, histidine, isoleucine,	Free amino acids are water soluble and will be removed from biomass during washing.	Analytical data on amino acid content demonstrate that the amino acid content of the harvested biomass is similar to that of conventional chicken and therefore was considered safe.	IOM (2005)

Table 7.1.1-2 Class 2 Media Component Risk Assessment

Component	Safety Reference Level	Safety Conclusions	Reference
leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, tyrosine, valine)			
Other (*e.g.*, Media Conditioning Agents)			
Methyl cellulose	*"In humans, virtually 100 percent of orally ingested methyl cellulose can be recovered in the feces within four days, indicating that absorption does not occur."*	No safety concerns based on hazard profile.	JECFA (1989)
Cyclodextrins	ADI for βCD = 0 to 5 mg/kg bw/day (JECFA, 1996; EFSA 2016).	No safety concerns based on safety profile and overly conservative estimated level of exposure from cultured chicken cells.	Gould and Scott (2005); JECFA (1996); EFSA (2016)
Alcohols	ADI for ethyl alcohol is limited by cGMP (JECFA, 1970).	The use level is orders of magnitude below the CEDI and use is consistent with cGMP.	JECFA (1970);

ADI = acceptable daily intake; bw = body weight; CFR = *Code of Federal Regulations*; EDI = estimated daily intake; FASEB = Federation of American Societies of Experimental Biology; FDA = Food and Drug Administration; FNB = Food and Nutrition Board; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; IOM = Institue of Medicine; JECFA = Joint FAO/WHO Expert Committee on Food Additives; LOAEL = lowest-observed-adverse-effect level; MTDI = maximum tolerable daily intake; N/A = not applicable; NIH ODS = National Institutes of Health Office of Dietary Supplements; NOAEL = no-observed-adverse-effect level; OSL = observed safe level; PTWI = provisional maximum tolerable weekly intake; RDA = recommended dietary allowance; SCOGS = Select Committee on GRAS Substances; UL = tolerable upper intake level.

Class 3: Class 3 substances include compounds that do not have regulatory status for food use—*i.e.*, substances not currently permitted for any food use application under an appropriate federal regulation, or previously concluded to be GRAS under 21 CFR §170.30 for any food use (U.S. FDA, 2022a). These substances will be evaluated in accordance with Believer Meats' Food Safety Plan to be of suitable food-grade quality (*e.g.,* no impurities of toxicological or allergenic concern) and will be evaluated for safety in accordance with scientific procedures. These substances currently include the following: (A) culture media proteins; (B) hormones; (C) non-essential nutrients; and (D) media conditioning aids.

Substances under categories A and B represent compounds whose functional roles in animals are essential to life and therefore are present in all mammalian cells including tissues derived from agriculturally relevant animals that are consumed as food. Similar to their vital roles *in vivo*, these substances are necessary for the optimal growth, proliferation, and/or differentiation of Believer Meats' cell cultured chicken meat. These substances are inherently self-limiting due to their high cost, which necessitates their use at the minimum levels necessary to achieve an optimal biological effect. In addition, the roles of these substances in critical metabolic pathways are often self-limiting on the basis that when used in excess, they are typically toxic to the cells resulting in cell death or sub-optimal growth. As data and information characterizing the hazards of these substances are often incomplete, a strong emphasis was placed on evaluation of safety *via* comparisons of the measured concentrations of the substance in the cultured chicken meat to levels from an appropriate comparator food that is commonly consumed in the diet (*e.g.*, ground chicken). In some cases, additional hypothesis-based testing studies may be needed and could involve *in vitro* heat stability

and digestion assays, or tests for biological activity thresholds using *in vitro* or *in vivo* assays. The specific types of studies in this regard would be determined on a case-by-case basis.

For Class 3 substances that are not natural constituents of food (*e.g.*, shear protecting and chelation aids), margin of exposure calculations will be required relative to an established safe reference level as described above for Class 2 substances.

Table 7.1.1-3 Class 3 Media Component Risk Assessment

Component	Safety Reference Level	Safety Conclusions	Reference
Trace metals	NOAEL value from a 13week drinking water study in rats.	> 100-fold margin of safety for EDI *vs*. NOAEL from 13-week rat study	See Appendix B
Cholesterol	Common dietary nutrient. One egg (50.3 g) contains 186 mg cholesterol.	EDI value is < quantities safely consumed from other commonly consumed foods in the diet (*i.e.*, one serving of eggs).	USDA Food Data Central (USDA ARS, 2018)
Organosulfur fatty acids	NOAEL value from a 2-year dietary toxicity study in rats.	>100-fold margin of safety for EDI *vs.* NOAEL from 2-year rat study	See Appendix B
Kreb cycle intermediates	Natural metabolites of glycolysis that are produced in all mammalian cells and are not expected to be of safety concern when used at cGMP levels (*i.e.*, levels that are nontoxic to mammalian cells in culture).	No safety concern when used in accordance with cGMP.	See Appendix B
Organic amines	Used as a processing aid in a variety of applications.	EDI values are < levels in commonly consumed foods. >100-fold Margin of safety for EDI *vs.* NOAEL from 90-day rat study.	See Appendix B
Soy-derived enzymes	NOAEL value from a 28-day oral toxicity study in rats.	No safety concern when used in accordance with cGMP.	See Appendix B

ADI = acceptable daily intake; bw = body weight; CFR = *Code of Federal Regulations*; cGMP = current Good Manufacturing Practices; EDI = estimated daily intake; FNB = Food and Nutrition Board; GRAS = Generally Recognized as Safe; GRN = GRAS Notice; JECFA = Joint FAO/WHO Expert Committee on Food Additives; LOAEL = lowest-observed-adverse-effect level; MTDI = maximum tolerable daily intake; N/A = not applicable; NOAEL = no-observed-adverse-effect level; OSL = observed safe level; PTWI = provisional maximum tolerable weekly intake; RDA = recommended dietary allowance; SCOGS = Select Committee on GRAS Substances; UL = tolerable upper intake level.

7.1.2 Adventitious Agents

The use of animal-derived materials as a source of primary cells and media components (*e.g.*, bovine serum, porcine trypsin) to produce cultured chicken cells necessitates consideration of the potential for transmission of infectious viruses, bacteria, and other microorganisms from the source material to the cells. These infectious organisms are collectively referred to as adventitious agents, a term originating from risk

assessment practices for biological products that similarly utilize animal derived materials during the production process. The WHO defines adventitious agents as:

> *Contaminating microorganisms of the cell culture or source materials including bacteria, fungi, mycoplasmas/spiroplasmas, mycobacteria, Rickettsia, protozoa, parasites, transmissible spongiform encephalopathy (TSE) agents, and viruses that have been unintentionally introduced into the manufacturing process of a biological product. The source of these contaminants may be the legacy of the cell line, the raw materials used in the culture medium to propagate the cells (in banking, in production, or in their legacy), the environment, personnel, equipment or elsewhere*. *(WHO, 2013)*

The general principles outlined in WHO[4] and U.S. FDA[5] guidance on the risk assessment practices for adventitious agents contain useful concepts that can be applied to the safety evaluation of adventitious agents during cultured meat production (U.S. FDA, 2010; WHO, 2013); however, human safety risks associated with the presence of adventitious agents in biologic drug products differ from those relevant to food safety. A conclusion that is underscored by the fact that many bacteria, fungi, viruses, and mycoplasma are consumed without apparent harm by humans from a variety of animal and plant derived food sources. For example, mammalian and poultry retroviruses are endemic and consumed in food from animals without apparent harm (DiGiacomo and Hopkins, 1997). Food safety practices for the control of microbial-derived hazards associated with the production of conventional meat products have proven sufficient to mitigate risks associated with transmission of zoonotic diseases from animal tissues to consumers: the destructive physicochemical processes during cooking and food processing and natural barriers within the gastrointestinal tract provide important barriers in this regard. Believer Meats' risk assessment for adventitious agents therefore focused on identifying relevant poultry-derived adventitious agents with established zoonotic potential and that are established food-borne biohazards from conventional poultry products. Zoonosis being defined as any disease or infection that is naturally transmissible from vertebrates to humans. Zoonotic pathogens may be bacterial, viral, or parasitic, or may involve unconventional agents and can spread to humans through direct contact or through food, water, or the environment (WHO, 2020).

[4] *WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks*.
[5] *FDA Guidance for Industry Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications*.

7.1.2.1 Hazard Characterization and Risk Assessment

To identify relevant avian–human zoonoses for risk assessment purposes, Believer Meats conducted literature searches for information from expert bodies (*e.g*., Center for Disease Control; Merck Veterinary Manual, EFSA, 2012), and peer-reviewed reviews of zoonotic diseases in poultry. Relevant zoonosis according to class are presented in Table 7.1.2.1-1 below. Believer Meats notes that cultivation of animal cells requires culture sterility, a technical requirement that is inherently self-limiting. Most food pathogens originating from poultry (*e.g*., *Salmonella* and *Campylobacter*) are heterotrophic facultative anaerobes or microaerophilic bacteria. These species are fastidious in their growth characteristics and where present as a contaminant would be expected to rapidly overtake growth of the cells in the bioreactor; contamination of the bioreactor with bacteria would therefore be readily identified during production of the cell lines or during the meat cultivation process. In this regard, sterility testing conducted using gold standard practices for cell-line sterility (*i.e*., USP 71) would be considered an appropriate approach for ensuring the absence of contaminating bacteria in the cell lines used for food use. In addition to sterility testing, the MCBs were tested for a number of pathogenic bacteria that are endemic in chickens (*i.e*., *E. coli*, *Salmonella* sp., and *Campylobacter*).

Basic sterility testing methods would not detect viral contaminants; however, similar to self-limiting properties of bacterial contamination, propagation of adventitious agents during cell culture would typically be expected to result of lytic or latent viral infections that would have a negative impact on the productive capacity of the cell culture system (Barone *et al*., 2020) and therefore would be identified by the performance characteristics of the cell growth well-prior to the final harvest steps preventing introduction to the food supply as the poor growth performance of the cell culture would trigger quality control checks of the bioreactor. Believer Meats recognized that, in theory, all of the microbial and viral contaminants identified in Table 7.1.2.1-1 below have the potential to contaminate cell-lines derived from chickens. The inherent risk of these adventitious agents should consider that each agent also has the potential to contaminate conventional poultry products consumed as food and therefore testing strategies for risk mitigation should consider the current history of safe consumption of poultry in the food supply. As discussed previously, zoonotic viruses of poultry are not generally considered a food safety risk (EFSA, 2012). Since Believer Meats' cell lines were derived from chicken embryos, adventitious agents were limited to those with demonstrated vertical transmission from hen to egg (*e.g*., *Salmonella* spp., avian influenza). With respect to viruses, avian influenza and Newcastle disease viruses are the only 2 avian-derived pathogens on the World Organization for Animal Health Office International des Epizooties (OIE's) list of transmissible diseases that have the potential for rapid spread, and which pose a serious socio-economic and/or public health consequence (World Organisation for Animal Health, 2023); however, only avian influenza has been associated with rare, documented cases of foodborne illness from consumption of an uncooked product (Swayne, 2019). Avian influenza is however not considered a risk of foodborne illness in poultry (EFSA, 2012). No cases of Newcastle disease from consumption of poultry have ever been reported (USDA, 2023). Other avian zoonotic viruses identified in Table 7.1.2.1-1 included West Nile virus, and Equine Encephalitis viruses (EEE, WEE, VEE); however, because chickens are not natural hosts for these viruses and vertical transmission from hen to egg have not been reported, they were considered of low risk for contamination of the cell lines; similar to avian influenza and Newcastle disease virus, the fact that cultured chicken products will be cooked prior to consumption further reduces any theoretical risks from these viruses.

Based on the above, only avian influenza and Newcastle Disease Virus were considered a viral safety risk[6] that required testing of the cell lines prior to qualification of the MCBs and MWCBs to ensure worker safety. Although neither avian influenza nor Newcastle disease were determined to be food safety hazards, their presence in Believer Meats cell lines is undesirable, therefore for quality reasons mandatory testing of the MCB and MWCB are conducted to ensure the highest possible quality of the company's cell lines for use in food production.

In addition, Believer Meats is also developing an RNA Seq method for unbiased and continual analyses of the company's cell banks and production process to achieve a high level of biohazard control of its products and processes (See Section 4.3.4.1). This method has been used to demonstrate that bovine or porcine derived viruses used during cell-line development were absent from the cells (See Section 4.3.4 and Appendix C).

[6] Safety risk is largely limited to worker safety as risk of foodborne disease was considered to be extremely low.

Table 7.1.2.1-1 Zoonotic Microorganisms of Poultry

Class	Species (Disease)	Zoonosis	Testing Requirement for Assurance of Food Safety
Bacteria	*Campylobacter* spp.	Campylobacteriosis	Yes*
	Salmonella spp.	Salmonellosis	Yes*
	Escherichia coli sp.	Colibacillosis	Yes*
	Chlamydia psittaci	Psittacosis	Yes*
	Erysipelothrix rhusiopathiae	Erysipeloid	Yes*
	Yersinia spp.	Yersiniosis	Yes*
	Mycobacterium avium	Avian tuberculosis	No
	Listeria sp.	Listeriosis	Yes*
Mycoplasma	*Mycoplasma* spp.	Common species associated with poultry infections (*M. gallisepticum*, *M. synoviae*, M. *meleagridis*, *M. iowae*) are not known to be human pathogens. (Lierz *et al.*, 2008).	No; however, for quality purposes the MWCB is tested for mycoplasma using PCR based analyses
Fungi	*Histoplasma capsulatum*	Histoplasmosis	No
	Cryptococcus neoformans	Cryptococcosis	No
Viruses	Avian Influenza virus A	Avian influenza	No as avian influenza is not known to be a food borne hazard; however, mandatory testing of the MWCB using RT-PCR based is applied for quality purposes and to protect workers.
	Newcastle Disease virus (Avian paramyxovirus 1)	Newcastle Disease	No as Newcastle disease is not known to be a food borne hazard; however, mandatory testing of the MWCB using RT-PCR based is applied for quality purposes and to protect workers.
	West Nile virus	West Nile fever	No.
	Eastern, Western, and Venezuelan equine encephalitis virus (EEE, WEE, VEE) alphaviruses	Encephalitis	No.
Parasites	*Giardia duodenalis*, *Giardia intestalis*, *Giardia lamblia*, *Toxoplasma gondii*	Giardiasis, toxoplasmosis	No.

*Species specific testing for these microorganisms was not required where the cell banks are demonstrated to be sterile using USP 71 – Sterility Testing.

7.1.2.2 Overall Risk Mitigation Strategy for Adventitious Agents Across Entire Production Process

Believer Meat has characterized the hazards associated with potential introduction of adventitious agents to the production process, from procurement of the cells from the donor animal through to production of a finished product (*e.g.*, chicken breast). An overview of each step in the production process, the associated hazards as they relate to contamination with adventitious agents, and corresponding risk mitigation measures and testing frequency are outlined below in Table 7.1.2.2-1. It was determined that the introduction of adventitious agents of animal origin (*e.g.*, *Campylobacter*, avian influenza) to the production process would exclusively be limited to the cell line development stage. Once the cell line was demonstrated to be sterile/free from adventitious agents of food safety concern no further testing for these organisms would be necessary as no animal derived components would enter the production process during the cultured meat production stage. Downstream testing would therefore be limited to conventional spoilage organisms and foodborne pathogens common to conventional food production processes (e.g., aerobic plate count, yeast and mold, *Enterobacteriaceae*, *Salmonella*, *E. coli*).

Table 7.1.2.2-1 Identification of Adventitious Agents (AA's), Corresponding Risk Mitigation Measures and Testing Frequency for Cultivated Chicken Production

Stage of Production Process	Source of Adventitious Agents	Hazards Identified	Risk Mitigation Strategy	Frequency of Testing
Cell Line Development	Cells from donor animal (fertilized egg)	*Bacteria: Campylobacter spp.; Salmonella spp.; Escherichia coli sp.; Chlamydia psittaci; Erysipelothrix rhusiopathiae; Yersinia spp.; Listeria sp.*	Sterility testing of MWCB in accordance with USP 71 Species specific testing of the MCB for *E. coli*, *Salmonella* sp., and *Campylobacter* sp.	Testing of MCB for *E. coli*, *Salmonella* sp., and *Camyplobacter* sp. conducted once. Sterility testing of MWCB's conducted once
		Mycoplasma sp.	PCR	Quality screening of tissue samples from donor animal and MCB. Mandatory testing of MWCB's.
		Viruses that are prevalent in chickens and that are a potential food borne safety concern: avian influenza and Newcastle Disease virus	RT-PCR testing	PCR based quality screening of tissue samples from donor animal and MCB. PCR testing during generation of MWCB's.
	Media components	AA's from chemical components. AA's from animal derived components (*e.g.*, bovine serum and porcine trypsin).	Filter sterilization of media and animal serum. Sourcing of bovine serum sources from countries with low BSE risk. *In silico* analyses of RNAseq data. Animal derived components not used during preparation of the MWCB or during the production process.	Filter sterilization and quality screening applied to each lot of material used during generation of cell lines. *In silico* analyses of RNA seq conducted once during preparation of the MCB or MWCB.
	Adventitious agents from environment.	Spoilage organisms and foodborne pathogens from food contact surfaces, air, personnel.	Use of aseptic procedures in a closed biosafety cabinet. Personal hygiene management (*e.g.*, protective gowns)	Routine employee hygiene practices and environmental monitoring.
Cultured Meat Production	Adventitious agents from media components.	Spoilage organisms and foodborne pathogens from media components.	Filtration of air and water sources Filter sterilization of media components.	Conducted on all lots of media.

Table 7.1.2.2-1 Identification of Adventitious Agents (AA's), Corresponding Risk Mitigation Measures and Testing Frequency for Cultivated Chicken Production

Stage of Production Process	Source of Adventitious Agents	Hazards Identified	Risk Mitigation Strategy	Frequency of Testing
			Animal derived media components are not used during the production process.	
	Adventitious agents from environment and food contact surfaces.	Spoilage organisms and foodborne pathogens from food contact surfaces, air, personnel.	Operation conducted in an aseptic closed system. Personal hygiene management (*e.g.*, protective gowns) Steam-in-place sterilization of bioreactor and supply lines/tanks	Environmental monitoring on a periodic basis. Routine employee hygiene practices
Harvested Biomass	Adventitious agents from environment, wash buffer.	Spoilage organisms and foodborne pathogens from wash buffer, food contact surfaces and environment.	Filtration of water sources Sterilization of food contact surfaces Personal hygiene management (*e.g.*, protective gowns)	Each lot of harvested cell material is tested for compliance with food grade specification (Section 5.2). Periodic testing for microbial hazards to generate historical data. Environmental monitoring on a periodic basis. Employee hygiene practices Sterilization of surfaces prior to each harvest

AA = adventitious agents; MWCB = manufacturer's working cell bank; rT-PCR = real-time polymerase chain reaction.

7.1.3 Immortalization

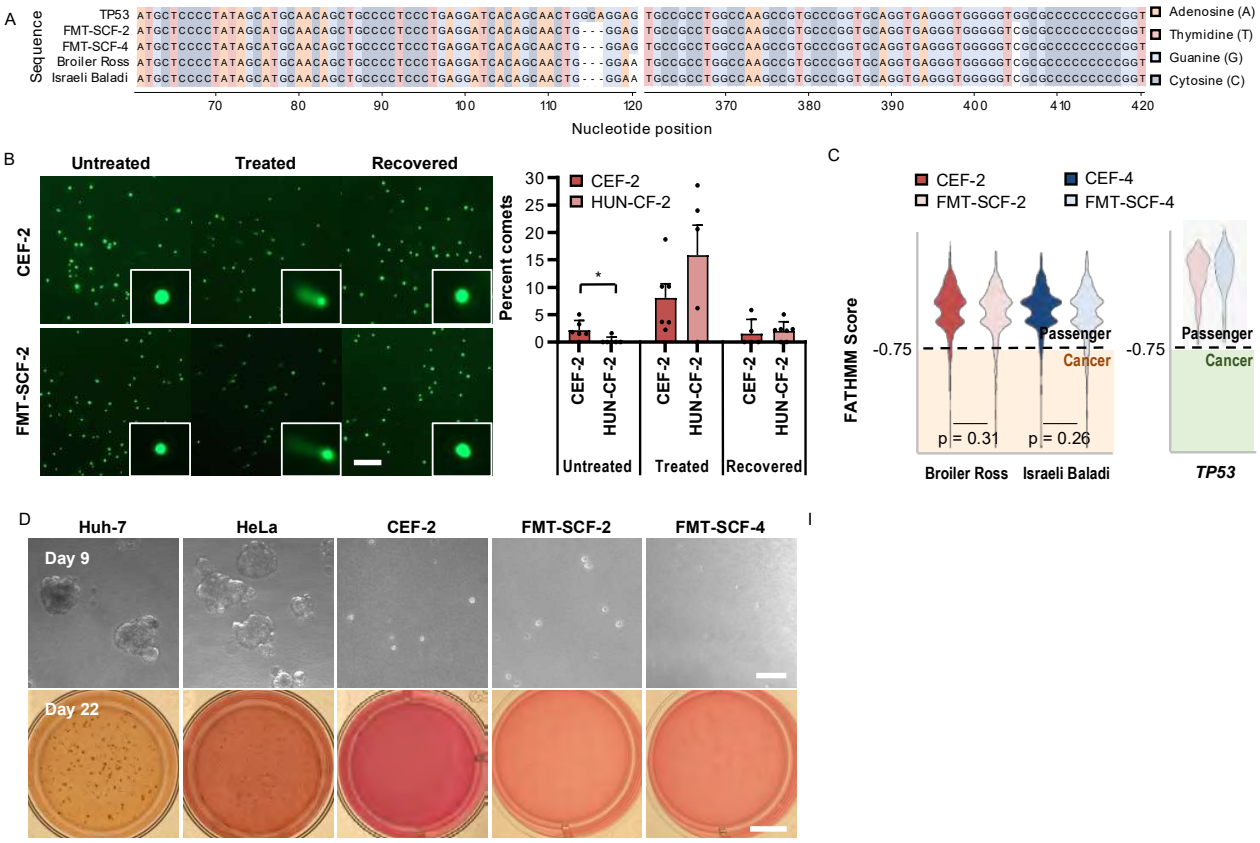
Primary cells display limited lifespans and gradually transition to a state of senescence where the cells display reduced growth rates and cease to divide even in the presence of excess nutrients. This finite replicative lifespan of primary cells was discovered by Leonard Hayflick over 50 years ago and is referred to as the Hayflick limit. The Hayflick limit of a cell is dependent upon numerous factors (species, tissue origin, age of animal) but often restricts primary fibroblasts to about 30 PDs. This limited lifespan of primary cells is not conducive to large scale commercial production of cultured meat, as it would require a continuous source of animal tissues, increasing process variability and risk of introducing animal-derived pathogens into the manufacturing process. For this reason, Believer Meats developed fibroblast cell lines with extended lifespans that display stable phenotypes that are characteristic of the parental cells. The use of immortal cell lines increases process reproducibility and limits the potential exposure to animal-derived pathogens.

Cell lines used by Believer Meats for cultured chicken cell production are spontaneously immortalized. In brief, primary cells are sub-cultured until the main cell populations cease proliferation and become senescent. During this state, a small population of cells escape the senescent state and continue to proliferate indefinitely forming an immortalized cell line. There are typically multiple genetic and epigenetic changes that take place in a cell to facilitate immortalization, including telomere and cell-cycle checkpoint maintenance. However, the Believer Meats spontaneously immortalized cells do not display phenotypes of transformation and remain subject to normal cell growth controls such as contact inhibition and anchorage-dependent colony formation.

Transformed cell lines often show (1) P53 dysfunction, leading to (2) limited DNA repair capability and genetic instability. These changes result in a neoplastic phenotype with a (3) distinct gene expression pattern (Stepanenko and Kavsan, 2012). Transformed cells are ultimately defined by (4) their ability to form colonies in soft agar.

Believer Meats peer-reviewed work demonstrates that its spontaneously immortalized cell lines are genetically stable (Pasitka *et al*. 2023). In brief, Believer Meats performed SNV analysis of TP53 in FMT-SCF-2 and FMT-SCF-4 compared to their respective primary chicken fibroblasts (Figure 7.1.3-1). No new SNV were detected in Believer Meats cell line. To further demonstrate active DNA repair, Believer Meats performed the Comet assay on primary chicken fibroblasts and immortalized cells. The data show no significant difference in DNA repair capability. Believer Meats performed functional analysis through hidden Markov models (FATHMM), a computational method to assess whether genetic variations are related to cancer (Shihab *et al.*, 2013). RNA-Seq signature of FMT-SCF-4 was compared to primary chicken fibroblasts finding the distribution of possible cancer related mutations was not significantly different between primary chicken cells and the immortalized lines ($p>0.25$). Finally, Believer Meats performed a soft agar colony formation assay. Both immortalized lines failed to form colonies on soft agar, demonstrating that the immortalization events are not associated with unstable transformation. These results demonstrate that immortalization events are not associated with genetic changes towards an unregulated unstable cell line and thus do not pose a food safety risk.

Figure 7.1.3-1 Genetic Stability of Spontaneously Immortalized Chicken Fibroblasts



FATHMM = functional analysis through hidden Markov models; SNV = single nucleotide variation.

(A) SNV analysis of mRNA transcripts of *TP53* in primary chicken fibroblasts (CEF-2, CEF-4) and immortalized cell-lines (FMT-SCF-2 and FMT-SCF-4). Uncolored boxes indicate changes from the reference sequence (*TP53*). There germline SNV were identified for primary chicken isolates. One SNV was corrected during immortalization. **(B)** Comet assay performed on CEF-2 and HUN-CF-2 shows no difference in DNA repair capability. **(C)** Analysis of human-specific mutations in the chicken transcriptome. Distribution of chicken genetic regions that correlate to non-synonymous mutations in human genome is not significantly different (p>0.2) between primary chicken cells and the immortalized lines (left). Analysis of the most frequent allele mutations showed no association between these events and common types of human cancer-driving mutations in *TP53* (right). **(D)** Soft agar colony formation assay of CEF-2, FMT-SCF-2 and FMT-SCF-4 showing no colony-forming capabilities, compared with Huh7 and HeLa cell lines as positive controls.

The possibility of horizontal gene transfer of ingested DNA has been the subject of extensive discussion by the Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology (FAO/WHO, 2000). DNA released from ingested foods is exposed to nucleases from animal tissues (salivary gland, pancreas, and intestinal epithelium) and other physical and chemical processes that result in extensive degradation of chromosomal DNA that is ingested from food. Feeding studies of mice administered M13 phage or plasmid DNA have demonstrated that the majority of DNA was degraded to <400 base pairs, and bacterial uptake was not reported. Studies in highly sensitive animal models validated for the ability to detect horizontal gene transfer of exogenous cellular DNA have similarly demonstrated that the transfer and integration of foreign DNA from cell line lysates is not possible even when administered *via* parenteral routes (Sheng-Fowler *et al*., 2014). The uptake and integration of DNA by cells is a very low probability event in the absence of inducing agents (*e.g*., UV light, chemical mutagens) and would represent an event that was not sequence-specific and therefore be subject to competition with the millions of base-pairs of fragmented DNA originating from various food sources in the diet. Believer Meats' process does not involve

recombinant DNA method; thus, genetic differences are limited to deletion or duplication of existing genes and would not contain new alleles that differ from those present in conventional chicken. Accordingly, degraded DNA from cultured chicken would be indistinguishable from degraded DNA from conventional chicken meat.

Overall, Believer Meats has concluded that the risk of horizontal gene transfer is effectively zero, a conclusion that is consistent with view expressed by the U.S. FDA during their evaluation of a cultured fibroblast product where the Agency concluded the following:

> *… once removed from the protected and controlled environment of the bioreactor the cells would quickly die, 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 cellular 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.* (U.S. FDA, 2022c)

7.2 History of Safe Consumption and Comparison to Conventional Chicken

7.2.1 Nutritional Considerations

As demonstrated in Section 5.0, the Believer Meats' cultured chicken cells are nutritionally similar to conventional chicken. Chicken is primarily consumed as a lean source of high-quality protein. Based on the strong congruence of the amino acid composition of Believer Meats' cultured meat product relative to conventional poultry meat products, food products containing Believer Meats' chicken cells would not be nutritionally disadvantageous as a source of dietary protein. Therefore, assuming a substitutive intake, there is no concern regarding nutritional imbalances that may occur due to substitution of conventional chicken in the diet with products containing cultured chicken cells. No nutrients were identified at concentrations that would be considered a food safety concern or would trigger additional regulatory review.

7.2.2 Allergy Considerations

Chicken allergies are rare in humans and are marked by an immune reaction following consumption of poultry meat. The prevalence of poultry meat allergy is not clear, and it may present as a primary (genuine) food allergy or as secondary food allergy resulting from cross-reactivity (Hemmer *et al.*, 2016). Secondary poultry meat allergy may arise in the context of bird-egg-syndrome, which is due to sensitization to serum albumins present in many tissues including muscle tissue and egg yolk (Gal d 5). Due to the heat lability of serum albumins, reactions are often limited to the skin upon contact with raw meat. Symptoms from meat ingestion are rare and mostly mild, whereas systemic reactions are common after ingestion of raw or soft-boiled egg yolk. Primary poultry meat allergy is mainly seen in adolescents and young adults, with egg allergy usually being absent. Typical symptoms of primary poultry meat allergy include oral allergy syndrome (± dyspnea), gastrointestinal complaints, urticaria, and angioedema; notably, severe anaphylaxis with cardiovascular symptoms is rare. The allergens thus far recognized in genuine poultry meat are low molecular weight proteins of 5 to 25 kDa. One of them has been identified as α-parvalbumin. Recently, myosin light chains, including 23 kDa MLC-1 (Gal d 7) and 15 kDa MLC-3, have been recognized as new major allergens in chicken meat. Regarding cultured chicken cells, it is assumed that consumers are aware of potential allergenicity and thus would avoid consumption of chicken products, and the marketing and

sale of cultured chicken cells anticipated to explicitly express similarities to that of conventional chicken products.

One theoretical concern, although extremely unlikely, is that the Believer Meats' cultured chicken cells may be activating genes that express proteins normally found in eggs. Egg allergies are prevalent among infants and children; the *Federal Food, Drug, and Cosmetic Act* requires that foods that contain a "major food allergen" (milk, eggs, finfish and shellfish, tree nuts, peanuts, wheat, sesame, and soybeans) declare its presence on their labels.

In order to ensure that Believer Meats' product does not contain egg or egg proteins, testing was performed to provide evidence for the absence of the proteins. These proteins include ovomucoid (which has been shown to be a dominant allergen in egg), ovalbumin, ovotransferrin, lysozyme (found in the egg white), and *alpha -livetin*, found in yolks. Although oral challenge remains a standard in determining an individual's sensitivity to eggs, there are sensitive molecular test kits (sandwich enzyme-linked immunosorbent assay [ELISA]) that can measure the presence of egg proteins in food matrices[7]. Believer Meats believes that testing for the presence of an egg protein is more relevant than testing transcripts, which may never be translated into proteins. As a result, Believer Meats has tested for these egg allergens and the results indicate that egg allergens are absent from cultured chicken cells at a limit of quantitation of 2.5 ppm.

Where soy ingredients are utilized in the media for production of cultured chicken cells, Believer Meats will declare soy as a potential allergen present in its products.

[7] https://www.neogen.com/solutions/allergens/veratox-egg-allergen

8.0 CONCLUSION

Based on the information and analyses described above, Believer Meats has concluded the following:

1. That cultured chicken fibroblast cells produced from the company's MWCB using culture methods described in this submission display an identity and composition that is similar to conventional chicken meat;

2. That the cultured chicken fibroblasts do not contain added substances the use of which are subject to premarket approval under the *Federal Food, Drug, and Cosmetic Act*; and

3. That the cultured chicken fibroblasts cells do not contain contaminants/microbial hazards originating from the production process.

Believer Meats has therefore concluded that its cultured chicken fibroblast cells are as safe as conventional meat from a chicken carcass.

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Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
117—Current good manufacturing practice, hazard analysis, and risk-based preventive controls for human food	117	[full section]
	Subpart B	Current good manufacturing practice
	117.10	Personnel
	117.165	Verification of implementation and effectiveness
	Subpart G	Supply-chain Program
170—Food additives	170.30	Eligibility for classification as generally recognized as safe (GRAS)
172—Food additives permitted for direct addition to food for human consumption	172.860	Fatty Acids
	172.874	Hydroxypropyl methylcellulose
182—Substances generally recognized as safe	182.1057	Hydrochloric acid
	182.1	Sodium chloride
	182.1778	Sodium phosphate
184—Direct food substances affirmed as generally recognized as safe	184.1193	Calcium chloride
	184.1426	Magnesium chloride
	184.1443	Magnesium sulfate
	184.1261	Copper sulfate
	184.1763	Sodium hydroxide
	184.1736	Sodium bicarbonate
	184.1622	Potassium chloride
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