RESPONSE TO FDA FOR CLARIFICATION OF QUESTIONS RELATING TO BELIEVER MEATS CULTIVATED CHICKEN SUBMISSION CCC 000039

Requests for Information to be added to the DSN

Introduction

This document responds to the Food and Drug Administration's (FDA) request for additional information relating to Believer Meats' safety assessment of its cultivated chicken product, dated May 21, 2024, and designated by the agency as CCC No. 000039. The questions from FDA are presented below in bold text and our responses follow.

Before discussing each question in turn, we believe it is helpful to provide preliminary clarification of two overarching topics that inform several questions below. One is the definition of the point of "harvest" in our particular process addressed by our safety assessment and the other is further clarification of our approach to controlling microbiological and viral adventitious agents during the manufacturing process.

Point of Harvest

FDA and USDA-FSIS each have an important and defined role in the oversight of human food comprised of or containing cell-cultivated meat, as outlined in the March 2019 Formal Agreement between the two agencies. As explained by the agencies, the transition of regulatory oversight from FDA to USDA-FSIS occurs at the point of harvest during the cell-culturing process. Harvest is defined by FSIS as the stage where "cells are removed from the controlled environment and are prepared for additional food processing" (FSIS, 2023). FDA explains that it "oversees cell collection, cell banks, and cell growth and differentiation," and "[a] transition from FDA to FSIS oversight will occur during the cell harvest stage" (FDA, 2019a). Based on feedback provided by the FDA Review Team during the pre-market consultation process, we further understand that a condition of harvest is the point where cells growth stops and cells are removed from the controlled environment.

The process described in our premarket submission contemplates that the transition between FDA and USDA-FSIS oversight is when the expanded cells exit the bioreactors and are washed using Sodium Chloride, all within a continuous closed system. After this preharvest process, the cell growth has ceased and the washed biomass is removed from the controlled environment of the storage tank, marking the point of harvest. From that point, there are two possible downstream production modalities for its cell-cultivated chicken product.

Under the first modality, the biomass is stored in a closed system storage tank prior to being transferred to a mixing tank for use in subsequent processing. When removed from the controlled environment of the closed system storage tanks, the biomass is deposited directly into a mixing tank where it is combined with non-meat ingredients. Under our second or alternative modality, after harvesting the biomass from the closed system storage tanks, instead of being deposited directly into a mixing tank, the biomass is frozen and flaked for storage, pending further downstream mixing and production.

Hazard Controls

The second point of clarification informs our answers to multiple requests from the FDA review team for additional information. As an addition to the DSN, we have updated our risk assessment for bacterial and viral hazards that are common transient hazards from conventional poultry processing and food processing more generally (FDA, 2024a). We referenced the FSIS's Meat and Poultry Hazards and Controls Guide (USDA, 2018) and the FDA's Draft Guidance for Hazard Analysis and Risk-Based Preventive Controls (FDA, 2024a) for a list of adventitious agents to include in the evaluation that is documented in Table 1 beginning on the next page. From this risk assessment, we conclude that none of these adventitious agents are likely to be transient bacterial and viral hazards where species specific testing is deemed necessary.

Table 1. Risk Assessment for Biological Adventitious Agents

Class	Adventitious Agent	Growth Considerations	Usual Sources of Risk						Review of Mitigation Strategies for Believer Meats	Hazard Reasonably Likely to Occur
			Live Animals	Slaughter	Environment	Water	Ingredients	Employee		
Bacteria	*Campylobacter* spp.	*Campylobacter* organisms are commonly found in the intestinal tracts of poultry. The bacteria pass through the body in the feces and cycle through the environment. *Campylobacter* are also found in untreated water (FSIS, 2013).	X	X		X			No poultry slaughter or raw conventional poultry activity on-site. MCB are tested negative for *Campylobacter*. Cells are handled aseptically and expanded in closed-system bioreactors. Process water is city water source and is treated by reverse osmosis. Personnel hygiene and facility GMPs control the introduction of *Campylobacter* to the environment.	No
Bacteria	*Salmonella* spp.	*Salmonella* organisms are commonly found in the intestinal tracts and fecal of poultry and can cycle through the environment (FDA, 2019b).	X	X			X		No poultry slaughter or raw conventional poultry activity on-site. MCB are tested negative for *Salmonella*. Cells are handled aseptically and expanded in closed-system bioreactors. Media is filtered to 0.2 micron prior to addition to bioreactors. No high-risk *Salmonella* ingredients in facility. All lots of downstream ingredients must be negative for *Salmonella.* Personnel hygiene and facility GMPs control the introduction of *Salmonella* to the environment.	No
Bacteria	*Escherichia coli* sp.	Poultry, livestock, and humans are occasional carriers of pathogenic *E. coli*. Contamination is typically spread when feces encounter food or water. Human carriers can spread infections if proper hand washing hygiene after using the restroom is not followed (FDA, 2019a).	X	X		X		X	No poultry slaughter or raw conventional poultry activity on-site. MCB are tested negative for E. Coli. Cells are handled aseptically and expanded in closed-system bioreactors. Employee hands are swabbed monthly for *E. Coli* to verify adherence to hand washing protocols. Process water is city water source and is treated by reverse osmosis. Personnel hygiene practices, handwashing, and facility GMPs control the introduction of E. coli to the environment.	No
Bacteria	*Listeria* spp.	*Listeria spp.* is widespread and found in soil, water, and sewage. *L. monocytogenes* has been shown to persist in food processing environments. In addition to being able to survive and grow at refrigeration temperatures and survives in frozen storage for extended periods (FDA, 2017).	X	X	X				No poultry slaughter or raw conventional poultry activity on-site. MWCB are tested negative for *Listeria* spp. Cells are handled aseptically and expanded in closed-system bioreactors. Wet washdown and temperature-controlled storage areas downstream have highest potential to support *Listeria* growth. Personnel hygiene practices, handwashing, and facility GMPs control the introduction of *Listeria* to the environment.	No

Class	Adventitious Agent	Growth Considerations	Usual Sources of Risk						Review of Mitigation Strategies for Believer Meats	Hazard Reasonably Likely to Occur
			Live Animals	Slaughter	Environment	Water	Ingredients	Employee		
Mycoplasma	*Mycoplasma* spp.	*Mycoplasmas* are frequent contaminants of cell cultures and bioprocessing fluids. *Mycoplasma* contamination can be caused by poor culturing practices or malfunctioning laboratory equipment (ATCC, n.d).	X	X			X		No poultry slaughter or raw conventional poultry activity on-site. MCB and MWCB tested negative for *Mycoplasma*. Good laboratory practices minimize the potential for *Mycoplasma* introduction to cell cultures. Contamination is self-limiting and would not result in cell expansion. Cells are handled aseptically and expanded in closed-system bioreactors. Media is filtered to 0.2 micron prior to addition to bioreactors.	No
Bacteria	*Bacillus cereus*	*Bacillus cereus* is commonly widespread in the environment and is often found in soil. Vegetative cells are readily denatured through heat treatment but spores that produce endotoxins are heat resistant. Cooked foods should be kept hot or rapidly cooled to 41ºF (5ºC) or below (Schneider, 2020).			X				Personnel hygiene practices, handwashing, and facility GMPs control the introduction of adventitious agents to product. Cells are handled aseptically and expanded in closed-system bioreactors. Product is rapidly chilled post-harvest to control spore formation.	No
Bacteria	*Staphylococcus aureus*	*Staphylococcus aureus* is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents. Thus, the presence of this bacterium or its enterotoxins in processed foods or on food processing equipment is generally an indication of poor sanitation (FDA, 2019c).						X	Personnel hygiene practices, handwashing, and facility GMPs control the introduction of viruses to product. Employee hands are swabbed monthly for *Staphylococcus aureus* to verify adherence to hand washing protocols. Cells are handled aseptically and expanded in closed-system bioreactors.	No
Viruses	Norovirus	Norovirus remains the leading cause of foodborne illnesses. Most of these illnesses can be traced back to food contaminated from feces or vomit from soiled hands of infected food employees in the retail food establishment (or restaurant) setting (FDA 2017).						X	Personnel hygiene practices, handwashing, and facility GMPs control the introduction of viruses to product. Employees who exhibit symptoms are not permitted to handle cell cultures or biomass. Cells are handled aseptically and expanded in closed-system bioreactors.	No
Viruses	Influenza Virus A	HPAI viruses can be transmitted by wild birds to domestic poultry and	X	X					No poultry slaughter or raw conventional poultry activity on-site. MCB and MWCB Tested negative for Avian Influenza.	No

Class	Adventitious Agent	Growth Considerations	Usual Sources of Risk						Review of Mitigation Strategies for Believer Meats	Hazard Reasonably Likely to Occur
			Live Animals	Slaughter	Environment	Water	Ingredients	Employee		
		other bird and animal species. Although bird flu viruses do not normally infect humans, sporadic human infections have occurred (FDA, 2024b).								
Viruses	Newcastle Disease	Newcastle disease is a contagious and fatal viral disease of birds and poultry. It attacks their respiratory, nervous, and digestive systems. Many birds and poultry die without showing any clinical signs. Virulent Newcastle disease is not a food safety concern as properly cooked poultry products are safe to eat (APHIS, 2024).	X	X					No poultry slaughter or raw conventional poultry activity on-site. MCB and MWCB Tested negative for Newcastle Disease Virus.	No

Requests for Information to be added to the DSN

Adventitious Agent Hazard Assessment

Animal Health Certificate

Question 1. On Page 12 of the DSN, you state "Believer Meat's proprietary process derives cultured chicken cell lines from fertilized eggs of Gallus gallus domesticus (domestic chicken)." For addition to the DSN, with supporting information in the SCM as needed, please provide documentation supporting the health of the source animal, including any related adventitious agent testing results.

The health of the source chicken embryo from which our cell lines were derived was confirmed through microbiological and viral testing on embryonic tissue cells and isolated primary cells for the following infectious agents known to infect poultry: Newcastle Virus Disease and Avian Influence A. These viruses are identified by Animal and Plant Health Inspection Service as two avian disease that could harm the health or quality of domestic poultry (APHIS, 2024). PCR testing found that the embryonic tissue was negative for Newcastle Virus Disease and Avian Influenza A, confirming that these avian viruses of concern would not be passed to future passages of cell lines. The absence of these two viruses of concern is also incorporated into the specifications for master cell banks and manufacturing working cell banks.

Primary cells isolated from embryonic tissue were also tested for microbiological adventitious agents that are commonly associated with live birds and poultry (USDA, 2018). Primary cells were tested for *E. Coli, S. aureus, P. aeruginosa, Candida, Salmonella,* Aerobic Plate Count, Yeast and Mold with no growth detected for all tests.

Supporting information for all analytical methods used is included in the SCM for FDA Question 1.

Hazard Analysis and Process Controls

Question 2. On page 13 of the DSN, you state, "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." For addition to the DSN:

2a) Please confirm that Section 4.2 "the Food Safety and Quality Systems" and Appendix A are written for the current facility which manufactures the harvested cell material, including all hazards identified and preventative controls described in the food safety plan.

The food safety and quality systems described in Section 4.2 and Appendix A were based on the process followed at Believer Meats' facility located in Rehovot, Israel, where the company has successfully produced cell-cultivated chicken biomass following food safety controls. The food safety and quality systems described in Section 4.2 and Appendix A, including the hazard analysis and preventive controls identified in those sections, are the essential steps required to manage the hazards inherent to our cell-cultivation process. As such, the facility in Wilson, North Carolina, which is designed based on the operation in Rehovot, adopts the food safety and quality systems in Section 4.2 and preventive the controls as outlined in Appendix A, subject to any appropriate modifications.

Question 2b) Please discuss why the results of the analytical testing (e.g., adventitious agent or compositional analyses) performed on the harvested cell material produced in the Rehovot, Israel facility is considered representative of the anticipated production process in Wilson, North Carolina.

The analytical results from harvested cell material produced in Israel are representative of the production process in Wilson, NC because the same exact cell lines, thawing and flask expansion processes, media components, media filtration process, and bioreactor expansion process that was used in Rehovot. Both facilities rely upon the same adventitious agent mitigation strategies, i.e., exclusion of adventitious agents from raw materials, maintaining aseptic conditions during cell expansion, and sanitation controls of equipment. These strategies are discussed in more detail in our response to Question 11.

The two differences between the Rehovot production and Wilson production process are (1) the use of larger sized bioreactors and wash centrifuges at the Wilson, NC facility and (2) the use of more automation in transferring biomass between process steps at large scale at the Wilson, NC facility. Bioreactor and centrifuge operational parameters are subject to be modified based on the larger scale while targeting biomass with the same specification parameters outlined in Table 5.2-1 of the DSN. More automated process steps in the Wilcon, NC facility lowers the risk

of product contamination with adventitious agents but does not have an effect on compositional analysis.

Question 2c) Please indicate whether the controls described in Section 4.2 apply to the facility in Wilson, North Carolina. If so, please discuss the hazard analysis and safety management strategy used in the Rehovot, Israel facility, including a stepwise hazard analysis as well as control and mitigation strategies for hazards identified during cell isolation and in the development of the master cell bank in DSN and with supportive details provided in the SCM as applicable.

The controls in Section 4.2 were based on the manufacturing process used in Rehovot, Israel and serves as the basis for the hazard analysis and safety management strategy for Wilson, NC.

While cell isolation, establishment of cell-lines, and establishment of working cell banks are not slated to take place in Wilson, NC at facility start-up they are critical steps in our supply chain to exclude adventitious agents from the process. If any cell line development activities take place on-site in Wilson, NC in the future, the facility food safety plan will be reassessed, and the appropriate management strategies as outlined in Table 2 will be implemented. Table 2, below, shows the hazard analysis and mitigation strategies for cell line development steps that occur in Rehovot, Israel:

Table 2. Hazard Analysis for Upstream Cell Line Development

Process Step	Potential Issue	Management Strategy
Cell Isolation	Cell source (animal health, species-specific considerations)	Virus screening of source embryonic tissue Pathogen screening of primary cells
	Cells from different line or species inadvertently used	Labeling, visual observation of morphology, genetic species validation via PCR in WCB
	Carryover of adventitious agents such as bacteria, fungi, viruses, parasites, and prions during isolation	Aseptic handing procedures to prevent contamination from employees Adventitious agent testing in MCB and WCB
	Introduction of contaminants in laboratory reagents	Filtration of media components to 0.2 micron
	Introduction of contaminants from animal-derived reagents	Supplier approval protocols, country of origin requirements, adventitious agent testing in MCB and WCB
	Facility environment contamination	Aseptic handling procedures, Adventitious agent testing in MCB and WCB
Establishment of Cell Lines	Cells from different line or species inadvertently used	Genetic species validation in WCB, testing program
	Mycoplasma spp. and other adventitious agent contamination	Aseptic handling procedures, adventitious agent testing in MCB and WCB
	Introduction of contaminants in laboratory reagents	Filtration of media components to 0.2 micron
	Cells do not display expected growth profile	Measure and discard
	Animal-derived reagent contamination	Supplier approval protocols, country of origin requirements, adventitious agent testing in MCB and WCB
	Facility environment contamination	Aseptic handling procedures, Adventitious agent testing in MCB and WCB

Process Step	Potential Issue	Management Strategy
Establishment of WCB	Contamination during transfer	Aseptic handling procedures, Adventitious agent testing in WCB
	Cells from different line or species inadvertently used	Genetic species validation via PCR
	Cells do not display expected growth profile	Measure PD and compare to specified limits WCB that do not meet specified limits are discarded
	Contamination with adventitious agents such as bacteria, fungi, and viruses from original source	Aseptic handling procedures, Adventitious agent testing in WCB
	Contamination with adventitious agents from culture media components	Filtration of media components to 0.2 micron
	Facility environment contamination	Aseptic handling procedures, Adventitious agent testing in WCB

Question 3. On Page 16 of the DSN, traceability is briefly described. For addition to the DSN, please expand the discussion of the traceability program to describe inventory control of cell materials. If there are cell materials from different species or research cell lines present at your production facility, please describe in detail how you ensure that no mislabeled cell lines are used for production.

Cell inventories are maintained by species and lot via an inventory tracking system. We do not permit storing different species of cell lines in the same racks in liquid nitrogen storage dewars. Research cell lines are also stored in separate racks from commercially approved cell lines to prevent use in the production process. Cell vials, including research cell vials, are labeled with lot number and cell line name that is species specific.

The lot number and species on each vial is recorded in accompanying process documentation each time cells are thawed and expanded to verify that the correct cell line is used and to ensure traceability by lot number. This information is included in chain of custody documentation and verified upon receipt at the manufacturing location.

Question 4. On Page 18 of the DSN, you state that "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." For addition to DSN, with supporting information in the SCM as needed, please clarify what process control is applied for chemical hazards and expand the discussion of validated processes used for removing cleaning agents.

Two key process controls are applied for chemical hazards: (1) application of a washing step before harvesting of the biomass and (2) implementation of a clean-in-place process for food contact surfaces.

Media components are removed via a washing step with an NaCl solution before harvesting the biomass. The safety analysis of each media component and presence in washed biomass is

discussed in Appendix B of the SCM. Wash conditions are validated using analytical testing of washed biomass and monitored during ongoing operation. Conditions monitored during the wash step are the concentration of NaCl, centrifuge rotations per minute (RPM), volume of NaCl buffer volume used in the wash, and the number of washes completed.

Food contact surfaces are cleaned via clean-in-place (CIP) processes that use specified chemicals at concentrations verified via titration. After the appropriate CIP program is followed, food contact equipment is rinsed with water to remove residual cleaning agents. The conductivity of post-CIP rinses is used as an indicator that the rinse has effectively removed cleaning agents.

Question 5. On page 23 and 27 of the DSN, you briefly mention temperature monitoring at the production facility. For addition to the DSN, please provide a narrative describing the temperature control program, with supportive details provided in the SCM as applicable.

Cell bank temperature during storage and receiving is critical to ensure cell viability. Cells are transported in liquid nitrogen shipping dewars with temperature logs accompanying each shipment. The temperature of the shipping dewars is verified upon receipt. Liquid nitrogen storage dewars at the facility are also equipped with temperature monitoring and alarms to notify the management team in Rehovot of any temperature deviations. This protocol is in also place for the Wilson, NC operations.

Additionally, bioreactor temperatures are maintained at 39°C to support cell growth and viability. While the cell bank shipping and storage temperatures and temperatures are conditions that will be monitored and controlled, these are not considered steps to control a food safety hazard. Lastly, CIP solution temperature is controlled and monitored to be within the effective temperature ranges for sanitation chemicals used in CIP.

Question 6. On Page 29 of the DSN, you state that "the bioreactor operates as a closed system and is cleaned prior to use by validated methods." For addition to the DSN, with supporting information in the SCM as needed, please provide detailed information about these validated methods.

The CIP procedure was validated as an effective cleaning cycle for removing all residual biomass from the bioreactors. Bioreactor sanitation uses automatic CIP system and caustic, acid, and water to effectively clean food contact surfaces in the bioreactor. The sanitation chemicals are selected based on approved use in food processing applications and are extensively used in food processing applications. The CIP cycle validation study takes into consideration chemical concentrations, temperature, and contact time of cleaning solution. We also use modeling and flow rate calculations to ensure bioreactor food contact surfaces are fully covered during the wash cycle.

After cell-cultivated chicken was produced, the CIP cycle was followed at the prescribed flow rate, chemical concentrations, temperatures, and contact time. Rinse waters post-CIP were sampled from the bioreactor drain and tested for total organic carbon and total plate count to complete the method validation.

Question 7. On Page 57 of the DSN in Table 7.1.2.2-1, "Personal hygiene management" is listed as a risk mitigation strategy. For addition to the DSN, please provide a more detailed summary of your approach to personal hygiene management.

Personnel GMPs are established in Believer Meats' GMP standard operating policy based on requirements set in 21 CFR §117.10 for disease control and cleanliness. Practices required under this policy include appropriate hand washing and sanitizing, appropriate use, and handling of personal protective equipment, prohibiting employees from bringing food, drink, or tobacco products outside of break rooms, and implementation of foreign object control measures, such as the use of smocks, hair nets, and beard nets (if needed), and removing jewelry and watches (U.S. FDA, 2022a). To verify adherence to hygiene protocols, we test employee hands monthly for *S. aureus, E. coli*, and coliforms. Further details describing the frequency, sample type and size, and limits of this testing are in Appendix A of the CSM. Employees are also trained to stay home when they are sick to prevent the introduction of adventitious viruses and human-borne disease to the manufacturing environment.

Question 8. For addition to the DSN, please state whether there is an allergen control program in the facility to address any potential cross-contamination from major food allergens and provide a detailed summary of this program. Also, please confirm that your allergen control program includes all major food allergens identified in the Food Allergen Labeling and Consumer Protection Act (FALCPA), including sesame which was added as a ninth major allergen by the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act effective as of January 1, 2023.

There is an allergen control program to address cross-contamination for milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame as identified by the FALCPA and FASTER Acts. All finished products will contain soy allergen and therefore our product label, which is pending approval with USDA will state, "Contains Soy" so no allergen preventive controls are needed for storage, segregation, or product changeover. Finished product labeling is a critical control in our downstream FSIS HACCP plan to ensure the appropriate allergen declaration is present on finished product. The "Contains Soy" allergen declaration is verified on pre-printed labels upon receipt and regular quality control verification checks are performed to ensure labels are properly applied to finished product.

All raw material allergen declarations are reviewed as part of our supplier qualification process. We only source from approved suppliers and verify supplier and material accuracy upon receipt. For any new ingredients or ingredient suppliers, allergen declarations are reviewed and evaluated for new allergen risks as part of our Supplier and Ingredient Qualification Program. If a new allergen is introduced to the facility, the allergen control program is reviewed and updated with appropriate segregation and changeover procedures to prevent cross-contamination.

For cross-contamination within the facility, no food or drink is permitted outside of break areas. Personal hygiene protocols including use of smocks and hand washing prevent undeclared allergens from being introduced to the production area.

Question 9. Please either move pages 15 and 16 of Appendix A to the DSN or summarize this information in the DSN.

There are four sanitation preventive controls identified for the cell-cultivation and harvest process in Appendix A:

Sanitation Preventive Control #1 – Biosafety Cabinet Sanitation and Disinfection

The purpose of this sanitation control is to mitigate microbial contamination of cell suspension from the laboratory environment and employees. The biosafety cabinet is cleaned and sanitized before and after every use. Sanitation effectiveness is monitored using ATP swabs before use. Cleaning records are maintained, and hygiene audits are used to verify sanitation SOP execution and are included in our response for environmental monitoring in Question 15.

Sanitation Preventive Control #2 – Bioreactor Sterilization using Steam in Place

The purpose of this sanitation control is to prevent contamination of cells in the bioreactors through sterilization of food contact surfaces at 121°C for at least 20 minutes. Bioreactors are sterilized before every use. Sterilization cycles and documented and are monitored for time and temperature. The sterilization is verified through routine testing of SIP water condensate for total plate count and for testing of the biomass for total plate count.

Sanitation Preventive Control #3 – Centrifuge CIP

The purpose of this sanitation control is to mitigate microbial contamination from environment and process equipment in the centrifuge. Centrifuge CIP cycles are completed before use of the equipment. The CIP cycles are documented and monitored by chemical concentration, time, and flow rate. CIP effectiveness is verified through testing of CIP rinse water for total plate count and for testing of the biomass for total plate count.

Sanitation Preventive Control #4

The purpose of this sanitation control is to mitigate microbial contamination from employee hands. Employee hand washing protocols are included as part of the employee hygiene plan which all employees are trained on. Hand hygiene is verified via hand swabbing and testing for *Staphylococcus aureus, E. coli*, and total coliforms.

Question 10. Please move pages 17 and 18 of Appendix A to the DSN.

This table from Appendix A of our SCM summarizes the microbiological control and testing summary:

Table 3. From Appendix A - Microbiological Control and Testing Summary

Process Step	Risk	Controls	Related Microbial Testing	Frequency	Sample size	Specification	Deviation corrective actions
Nutrient Prep	Microbial contamination from raw materials, environment, or packaging	Follow established protocols for storage and handling, sampling program, hygienic zoning, media batch release microbial testing	Media batch release	Every batch	100 ml	TPC<10 CFU/gr	Refilter
Media Filtration	Microbial contamination due to breached or defective filter	Filter integrity checks after use, media batch release microbial testing	Media batch release	Every batch	100 ml	TPC<10 CFU/gr	Refilter
Thaw MWCB	Microbial contamination from environment	Routine mycoplasma testing aseptic technique, employee training, hygienic zoning, MWCB release testing protocol	Routine mycoplasma test for cell culture in process	Monthly	All cell cultures in process	*Mycoplasma* spp. – absent	Discard cell culture
			MWCB release	Every MWCB	1 MWCB vial	Sterility - no growth, Mycoplasma spp. - absent, Highly Pathogenic Avian Influenza (HPAI) - not detected, Virulent Newcastle Disease (vNCD) - not detected.	Discard MWCB
Ingredient Addition	Microbial contamination from the environment	Aseptic technique, employee training, hygienic zoning, Sanitation preventive control #1.	Biosafety cabinet surfaces after sanitation	Monthly	10cmX10cm surface	TPC<10 CFU/ml yeast & molds<10 CFU/ml	Repeat sanitation and retest. Identify and test possibly affected cultures.
			Routine mycoplasma test for cell culture in process	Monthly	All cell cultures in process	*Mycoplasma* spp. - absent	Discard cell culture
	Microbial contamination from employees	Hygiene and PPE SOPs, employee training, employee routine hand sampling, sanitation preventive control #4.	Employee's hands	Monthly	10cmX10cm hand surface	Coliforms<100 CFU/gr *E. coli* <10CFU/gr *S. aureus*≤50 CFU/gr	Repeat sanitation and retest. Identify and test possibly affected cultures.
Expansion in Bioreactor	Microbial contamination from added gases and solutions	Filtration of gases and solutions, aseptic welding of solution containers, employee training, supplier management program batch release microbial testing, process preventive control #1	Batch release specifications			See Table 5.2-1 in Disclosable Narrative	
Cell bleeding and dilution (optional)	Microbial contamination from employees	Hygiene and PPE SOPs, employee training, employee routine hand sampling, aseptic welding of tubing, batch release microbial testing	Employee's hands	Monthly	10cmX10cm hand surface	Coliforms<100 CFU/gr *E. coli* <10CFU/gr *S. aureus*≤50 CFU/gr	Repeat sanitation and retest. Identify and test possibly affected cultures.
			Batch release specifications			See Table 5.2-1 in Disclosable Narrative	
Batch Culture in Bioreactor	Survival of bacteria or spores due to insufficient 'steam in place (SIP)' process	SOP for steaming in place (SIP), verification of SIP process as part of environmental sampling, sanitation preventive control #2	SIP water condensate	Every SIP	100ml	TPC<10 CFU/ml	Repeat SIP and retest.

Process Step	Risk	Controls	Related Microbial Testing	Frequency	Sample size	Specification	Deviation corrective actions
	Microbial contamination from added gases and compressed air	Filtration of gases, filter integrity checks after each use, preventive maintenance for equipment, process preventive control 1, batch release microbial testing	Batch release specifications			See Table 5.2-1 in Disclosable Narrative	
Perfusion Culture in Bioreactor	Increased microbial contamination risk due to perfusion cycle	Filtration of rejuvenated media, filter integrity checks after each use, batch release microbial testing	Batch release specifications			See Table 5.2-1 in Disclosable Narrative	
Rejuvenation	Microbial contamination due to extended hold time	Filtration of rejuvenated media, filter integrity checks after each use, media acidification, batch release microbial testing	Batch release specifications			See Table 5.2-1 in Disclosable Narrative	
Removal from Bioreactors	Bacterial growth due to insufficient cleaning of centrifuge	Centrifuge CIP validated sanitation according to SOP, employee training, sanitation preventive control #3, batch release microbial testing	Last rinse from CIP	Every CIP	100ml	TPC<10 CFU/ml	Repeat CIP and retest.
			Batch release specifications			See Table 5.2-1 in Disclosable Narrative	
	Microbial contamination from environment and employees	Closed system, addition through steam bridge with aseptic welding, hygiene SOP, hygienic zoning, employee training, employee hand routine sampling, batch release microbial testing	Employee's hands	Monthly	10cmX10cm hand surface	Coliforms<100 CFU/gr *E. coli* <10CFU/gr *S. aureus*≤50 CFU/gr	Repeat sanitation and retest. Identify and test possibly affected cultures.
			Batch release specifications			See Table 5.2-1 in Disclosable Narrative	
Wash	Microbial contamination from employees	Hygiene and PPE SOPs, employee training, employee routine hand sampling, aseptic welding of tubing, process preventive control 1, sanitation preventive control 4, batch release microbial testing	Employee's hands	Monthly	10cmX10cm hand surface	Coliforms<100 CFU/gr *E. coli* <10CFU/gr *S. aureus*≤50 CFU/gr	Repeat sanitation and retest. Identify and test possibly affected cultures.
			Batch release specifications				

Sterility Testing

Question 11. Page 23 of the DSN states that the USP <71> sterility testing is "… an indirect verification that the cell bank is free of pathogenic bacteria or fungi," and throughout the dossier, you indicate that you deem your cell lines to be sterile as a result of this test. We note that USP <71> states, "These Pharmacopeial procedures are not by themselves designed to ensure that a batch of product is sterile or has been sterilized. This is accomplished primarily by validation of the sterilization process or of the aseptic processing procedures. The test is applied to substances, preparations, or articles which, according to the Pharmacopeia, are required to be sterile.

However, a satisfactory result only indicates that no contaminating microorganism has been found in the sample examined under the conditions of the test." On page 23 of the DSN, you further state that "The test conditions for demonstrating sterility of the MWCB are conducive to the growth of all known foodborne pathogens." We note that this statement is inherently false, as viral foodborne pathogens are not captured by the USP <71> test. Further, we note that USP<71> is intended for use in pharmaceutical production, which has several notable differences compared to food production environments. We request that you avoid overstating the results of this test, as it is a general test with several caveats and should not be considered a substitute for organism specific testing and is not appropriate as a standalone control measure for any specific organism that has been identified as a hazard in your process.

For example, on page 57 of the DSN in Table 7.1.2.2-1, you identify Listeria spp. as a hazard introduced during cell line development but provide USP <71> sterility testing performed once on the MWCB as the only risk mitigation strategy for Listeria spp. We note that it is not appropriate to rely on the use of USP <71> sterility testing, a non-specific analytical method, for determining absence of a specific adventitious agent. As you have identified Listeria spp. as a hazard in your production process, we request that you consider including Listeria spp. as part of your environmental monitoring as well as include batch release specifications for Listeria spp. for the harvested cell material.

Our approach to microbiological control will rely on three principles: exclude contamination from process inputs like cell lines and media, maintain aseptic conditions during cell-expansion in bioreactors, and maintain sanitary conditions after removal from the bioreactor during the biomass wash and harvest steps.

To exclude contamination from cell-lines, we conduct adventitious agent testing at various points in cell-line development for embryonic tissue, master cell banks, and manufacturer working cell banks as outlined in Table 4.3.3-1 and Table 4.3.4.1-1 of the DSN. To exclude foodborne pathogens from cell lines, master cell banks are tested for species-specific adventitious agents as acceptable criteria for the cell bank, as outlined in Table 4.3.4.1-1 of the DSN. We acknowledge the review team's comment on our mitigation strategy for *Listeria* spp. and we have added *Listeria* spp. testing to our specification for Master Cell Banks in the amended table 4.3.4.1-1, below. The MCB tested negative for *Listeria* spp. with results included in the SCM.

Amended Table 4.3.4.1-1 - Specifications for Embryonic Tissue and Master Cell Banks

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. coli	Pour plate, SOP No. 10-021, based on Harmonized USP/EP Pharmacopeias	ND
	Salmonella spp.	Pour plate, SOP No. 10-021, based on Harmonized USP/EP Pharmacopeias	ND
	Listeria spp.	ISO 11290-1	Negative
	Campylobacter spp.	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	Kylt® Influenza A - H9 RT-PCR	ND
	Newcastle disease virus	Kylt® Paramyxovirus 1 RT-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.

For further passages of cell lines to create manufacturer working cell banks, we use USP <71> sterility testing as an indicator that aseptic conditions were maintained in cell line development and no pathogens were introduced during MWCB creation. Cell line testing is a verification activity to ensure working cell banks intended for cell-cultivated chicken production meet established specifications in Table 4.3.3-1 in the DSN. Passing results for each MCB and each lot of MWCB are a requirement to use cells in the cell-cultivated chicken manufacturing process.

Because bioreactors are a supportive environment for adventitious agents to grow, it is critical that we maintain aseptic handling procedures and environmental sanitation throughout the process. The second mitigation strategy for microbiological hazards involves maintaining aseptic condition during cell expansion. Cells are thawed and flask expanded in a biosafety cabinet per aseptic handling procedures. Large-scale bioreactors are seeded following aseptic handling procedures. Cell proliferation in large-scale bioreactors happens under aseptic conditions until biomass is removed from the reactors. Biomass exits the bioreactor and is washed in a closed system centrifuge before the harvest step.

Sanitation controls the centrifuge to prevent reintroduction of microbiological contamination at this process step. Because any microbiological contamination would be detrimental to the process, and we need to exclude all contamination from the cell proliferation process. We use total plate count as an indicator that sanitary conditions were maintained during cell expansion, removal from the bioreactors, and biomass washing steps.

Question 12. On page 43 of the DSN, you write that "… the harvesting surfaces are dry sterilized surfaces and not conducive to Listeria growth". For addition to the DSN, please include data to demonstrate the validity and applicability of this statement for your processing environment.

Prior to seeding bioreactors, multi-use equipment is cleaned via validated sanitation procedure (see Q6) and steam sterilized at 121°C for 20 minutes. The sterilization temperature and time exceeds the thermal inactivation temperature and time requirements for *Listeria monocytogenes* (FDA, 2000). Cell proliferation in bioreactors is a closed system held under aseptic conditions until biomass is harvested.

Environmental Sampling

Question 13. On page 43 of the DSN, you write, "Testing for viruses that may originate from the environment (e.g., Norovirus) also was not considered necessary as there is no unique hazards at this stage of the production process that would either introduce or propagate these viruses in a manner that differs from conventional food processing." If you intend to use this justification, we request a data-based discussion, with citations to the publicly available literature, as appropriate, on why excluding viruses and other adventitious agents associated with human handling or environmental contamination, such as Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, or other environmental bacteria, from your testing strategy is sufficiently protective of safety for addition to the DSN. Otherwise, we request that you add batch release specifications for microorganisms you identify as potential risks from human handling or environmental contamination.

Our decision not to test the harvested biomass for certain viruses and other adventitious agents is based on our determination that the robust viral and adventitious agent controls and monitoring procedures we implement throughout our production process, and which we summarize herein, are sufficient to ensure that these viruses and adventitious agents are not present in the harvested biomass. This supports our conclusion that additional testing of the harvested biomass would be redundant and is thus not necessary to ensure safety.

As summarized in Table 1 in the Introduction section of this document, we have conducted a detailed risk assessment on the adventitious agents associated with human handling and environmental contamination. This risk assessment evaluated growth considerations for each agent and the usual sources of risk for each agent, and then identified the mitigation strategies Believer Meats will use in cell line development and cell-cultivated chicken production to control for these risks. As summarized in Table [4] below, this risk assessment accounted for the potential risk sources associated with a range of adventitious agents including, but not limited to, norovirus, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria* spp. As summarized in Table 1, we have identified and implemented specific mitigation strategies to control for each of these adventitious agents.

Table 4. Summary of Table 1 Adventitious Agents

Adventitious Agent	Usual Sources of Risk					
	Live Animals	Slaughter	Environment	Water	Ingredients	Employees
Campylobacter spp. (FSIS, 2013)	X	X		X		
Salmonella spp. (FDA, 2019b)	X	X			X	
Escherichia coli spp. (FDA, 2019a)	X	X		X		X
Listeria spp. (FDA, 2017)	X	X	X			
Mycoplasma spp. (ATCC, n.d)	X	X			X	
Bacillus cereus (Schneider, 2020)			X			
Staphylococcus aureus (FDA, 2019c)						X
Norovirus (FDA 2017)						X
Avian Influenza Virus A (FDA, 2024b)	X	X				
Newcastle Disease Virus (APHIS, 2024)	X	X				

As part of our risk mitigation program, and as outlined in Table 4.3.4.1-1 of the DSN, Believer Meats tests each master cell bank (MCB) for species specific adventitious agents that are usually found in live animals and slaughter operations, including *Campylobacter* spp., *Salmonella* spp., *Escherichia coli* spp. *Listeria* spp., *Mycoplasma* spp., Avian Influenza A, and Newcastle disease virus. The fact that there are neither any live chickens present at our Rehovot, Israel or in Wilson, NC facilities nor any poultry slaughter activities conducted at the facilities greatly reduces the likelihood that adventitious agents commonly found in the intestinal tracts of poultry or introduced during slaughter would be reintroduced to cell-lines after establishment of the MCBs or at any other point in Believer Meat's production process. Despite this, we have implemented a series of controls throughout the production process to control for such risks including, as outlined below, controls for (1) potential adventitious agents common in the environment, (2) potential adventitious agents present in water sources, and (3) potential human-introduced viruses.

- For adventitious agents common in the environment such as *Listeria* spp. and *Bacillus cereus*, personnel hygiene practices, handwashing, and facility GMPs limit the introduction to the manufacturing environment. Cells are handled aseptically and expanded in closed-system bioreactors.
- *Salmonella* spp. and *mycoplasma* spp. are two adventitious agents that could be introduced via media components and therefore present a potential transient hazard in the facility. To control for this potential hazard, media is filtered to 0.2 micron prior to addition bioreactors for cell expansion. *Salmonella* spp. range from 2–3 × 0.4–0.6 micrometer in size (Crump, 2017) and *mycoplasma* spp. range from 0.3 to 0.8 x up to 150 micrometers in length (Smith, 1985) and will be controlled via sterile filtration to 0.2 micron.
- For adventitious agents present in water sources, Believer Meats uses city sources of water that are free of pathogens per annual testing reports. Our Rehovot facility further treats water via distillation and our Wilson, NC commercial facility will further treat water via reverse osmosis. The reverse osmosis system filters source water to <0.001 micron thus removing bacteria, viruses, and spores (FDA, 2014).
- For human-introduced viruses like Norovirus and Hepatitis A and other biological hazards, we rely on a range of CGMPs including personal hygiene management (as explained in our response to Question 7), exclusion of sick employees from the production environment, and aseptic handling procedures to prevent contamination of product with viruses from human handling. These are acknowledged as effective control measures to control viruses associated with human handling (FDA, 2024a).
- "[C]ontamination of food with biological hazards that are viruses (e.g., norovirus and hepatitis A virus), parasites (e.g., Cryptosporidium spp., Cyclospora cayetanensis, and Giardia intestinalis) or the bacterial pathogen Shigella spp. by food handlers generally is addressed by following the CGMPs such as those relevant to worker hygiene and disease control (FDA, 2024)." We extend the same cGMP controls for worker hygiene

and disease control as described in our response to Question 7 for bacterial adventitious agents *E. coli* and *staphylococcus aureus* that are usual risks introduced by employees.

We believe that the measures outlined above allow us to adequately control and monitor for the presence of relevant viruses and adventitious agents such that additional testing of the harvested biomass is not necessary to demonstrate safety.

Moreover, we note that any risk to consumers is further mitigated in the post-harvest process, since our cell-cultivated chicken biomass is not intended to be a ready-to-eat food product and will thus undergo further processing at an FSIS-inspected establishment prior to packaging, in compliance with applicable USDA food safety requirements, and will also undergo sampling in accordance with USDA Standards (*see* FSIS Notice 38-24). At the packaging stage, and before receipt by the end consumer, the products will be labeled with validated cooking instructions and safe handling instructions, as required for conventional poultry products, and will be properly cooked by the end consumer prior to consumption.

Question 14. You list environmental monitoring in Table 7.1.2.2-1 in the DSN, but do not provide an accompanying discussion in the text. For addition to the DSN, please provide a detailed summary of your current environmental monitoring program. In your discussion, please include your approach to root cause analysis, corrective action, verification, and validation in the event of contamination. In addition, please explain how you ensure that no biofilm formation would occur on the equipment and elsewhere in the facility. Please also provide a summary of your control and risk mitigation strategy for emerging environmental bacteria contaminants.

In the event of contamination, we initiate an investigation to identify the root cause of the failure. An investigation checklist is followed that prompts the investigation team to review sanitation records, processing records, and filtration records or any deviations that could have led to contamination. After any contamination event, a remediation plan is followed to remove contaminated material from the facility and thoroughly deep-clean equipment.

Following food industry best practices, our biofilm mitigation strategy involves a combination of sanitary design of equipment and effective sanitation protocols for equipment and the production environment (Carrascosa, 2021). The bioreactors are aseptically designed to limit microorganism access and food-contact surfaces inside bioreactors are smooth and impervious to prevent accumulation of food particles. The vessels are cleaned using automated CIP and SIP cycles. These cycle parameters are validated to be effective as described in our responses to Questions 6 (CIP) and Questions 11 (SIP). Caustic and acid sanitation chemicals are used to eliminate organic residues from food contact surfaces and rinse water post CIP and SIP are analyzed for total organic carbon after each sanitation cycle. A master sanitation schedule is in place for non-food contact equipment to maintain hygienic conditions in all areas of the plant. The high-hygiene zones, such as the inoculation room and bioreactor room, employ

environmental monitoring as outlined in Table 3 from Appendix A of the SCM, which was also added to the DSN in our response to Question 10, to additionally address biofilm formation.

For emerging environmental bacteria contaminants, Believer Meats subscribes to FDA and USDA Safety Alerts to always be apprised of foodborne illness outbreaks that may be relevant or pose an emerging risk to cell-cultivated chicken.

Question 15. For addition to the DSN, please provide more detail about your environmental sampling program, including testing locations, frequency of testing, and tests used.

The environmental monitoring program focuses on the most likely routes of product contamination and includes sampling in production areas, employee hands, and utilities. Environmental samples are taken from various locations and tested for indicators ATP and total plate count. Because there is no facility history for the Wilson, NC location, sample locations, frequencies, and target organisms from the Rehovot facility are used as an input to the environmental monitoring plan for the large-scale facility. Our conclusions from Table 1, Risk Assessment for Biological Adventitious Agents from our Introduction show that biological adventitious agents are not likely to be introduced as transient environmental pathogens. Because *Salmonella* and *Listeria monocytogenes* have an extensive history of being "resident environmental pathogens" in processing facilities, they will be initially monitored as part of the environmental monitoring program for the commercial production facility in Wilson, NC (FDA, 2024a).

Table 3. from Appendix A of the SCM (also added to DSN in our response to Question 10) shows the summary of microbial testing performed in Rehovot, IL. Table 3 outlines several environmental sample locations, test frequencies, and tests performed. For test methods, we use 3M™ Clean-Trace™ ATP Surface Tests and for total plate count, we use method SI 885 part 20. While ATP swabs do not directly measure or specify bacterial species, ATP is present in all organic debris and bacteria, so its presence on surfaces is an indicator of organic matter. ATP swabbing does not detect viruses as viruses do not generate or store ATP (Bakke, 2022).

Historical swab data from the Rehovot, IL facility is a valuable tool to use as a baseline for the environmental monitoring plan in Wilson, NC. As the large-scale operation in Wilson, NC is commercialized, we will continue to assess the frequency and exact swab locations and make appropriate modifications. These general environmental sampling locations from Rehovot that will be adapted as appropriate to the operation in Wilson, NC:

- Air in bioreactor room and biosafety cabinets
- Floors and drain locations in bioreactor room
- Walls and ceilings in bioreactor room
- Transition zones between high-care and low-care areas

Substances Used During Cell Culture

Estimated Daily Intake (EDI) Calculation

Question 16. In Section 6.1 of the DSN, you determine the estimated dietary intake for cultured chicken cells using per capita consumption data for adults 20 years and older from the National Health and Nutrition Examination Survey (NHANES) as average 36 g/d and 72 g/d at the 90th percentile.

Please calculate dietary intake estimates using eaters-only data for suitable surrogate foods at the 90th percentile for members of the U.S. population 2 years and older, which is approximately 150 g/d.

We have updated the Estimated Daily Intakes presented in the SCM to use a daily intake of 159g/person/day, which is based on eaters-only data from the National Health and Nutrition Examination Survey (NHANES). Our cultured chicken cells are intended to be consumed in a similar manner to that of conventional chicken products. Thus, as the cultured chicken cells are intended to substitute conventional chicken consumption, the estimated intakes of conventional chicken were calculated to estimate exposure to cultured chicken cells products. Conservatively, 100% substitution of conventional chicken was assumed and therefore all food codes pertaining to unprocessed (chicken breasts, drumsticks, thighs *etc.*) and processed (ground chicken, chicken nuggets, chicken tenders *etc.*) chicken were selected from the NHANES 2017-2018 food consumption database (CDC, 2024b) and where necessary, product-specific adjustment factors were applied for composite foods/mixtures based on data provided in the Food and Nutrient Database for Dietary Studies (USDA ARS, 2023).

The resulting mean and 90th percentile intakes of cultured chicken cells by the total U.S. population from proposed food uses in the U.S. were estimated to be 76 g/person/day (1.2 g/kg body weight/day) and 152 g/person/day (2.4 g/kg body weight/day), respectively. Among the individual population groups, on an absolute basis, teenagers reported the highest mean and 90th percentile intake of cultured chicken cells at 84 g/person/day and 159 g/person/day, respectively (Centers for Disease Control, 2024). We chose to use the highest, most conservative intake levels for our EDI calculations, and have thus updated the SCM to apply an EDI of 159g/person/day.

Question 17. For addition to the DSN, please provide information on the source of the USDA conventional comparator data presented in Table 5.4-1 of the DSN.

USDA conventional comparator data presented in Table 5.4-1 was retrieved from U.S. Department of Agriculture FoodData Central in the SR Legacy Food FDC ID: 171077, Chicken, broiler or fryers, breast, skinless, boneless, meat only, raw (USDA ARS, 2019). The table is accessible at https://fdc.nal.usda.gov/fdc-app.html#/food-details/171077/nutrients.

Question 18. For thiamine (vitamin B1), riboflavin (vitamin B2), niacinamide (a form of vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitamin B6), biotin (vitamin B7), and cobalamin (vitamin B12), the EDI's for the harvested cellular material are stated to be based on theoretical calculations. Please measure the levels of all vitamins in the harvested cellular material that are used in the cell culture media during the production process and provide EDI's based on analytical measurements for vitamins B1, B2, B3, B5, B6, B7, and B12.

EDI's for thiamine (vitamin B1), riboflavin (vitamin B2), niacinamide (a form of vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitamin B6), biotin (vitamin B7), and cobalamin (vitamin B12) have been updated to use analytical measurements. Table B-2 in Appendix B of the SCM reflect the updated EDI values and safety narratives.

Table 5. Amended Table 5.4.5 with B Vitamin Analytical Results for Biomass

Parameter	Cultured Chicken Cells		
	Batch 1	Batch 2	Batch 3
Thiamine (Vitamin B1) (mg/kg)	1.6	1.62	1.69
Riboflavin (Vitamin B2) (ug/100g)	0.75	0.74	0.76
Niacinamide (Vitamin B3) (mg/kg)	3.74	5.24	3.73
Pantothenic Acid (Vitamin B5) (mg/kg)	8.5	11.5	10.8
Pyridoxine (Vitamin B6) (mg/kg)	1.86	0.58	0.43
Biotin (Vitamin B8) (ug/100g)	2.15	2.21	2.15
Folates (µg/100g)	18	28	23
Cobalamin (Vitamin B12) (ug/100g)	3.94	3.67	3.92

Safety Assessment of Media Inputs

Question 19. Lithium chloride is used as a media input in your cell culture production process. We have considered your proposed use of this substance in your cell culture process and find that its use may likely violate Section 301(ll) of the FD&C Act. Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. We do not believe any of the exemptions apply. Therefore, we would advise you to reconsider the use of this substance in your cell culture production process.

Whether the use of lithium chloride (LiCl) as a processing aid in our production media implicates section 301(ll) of the FD&C Act is now moot for purposes of this safety assessment because, following submission of our safety assessment in May 2024, we removed LiCl from our media formulation by adapting the cells to grow in LiCl-free media.

A more fulsome response to this question will follow with supporting analytical data to confirm our continued conclusion in our safety assessment that foods comprised of or containing cultured chicken resulting from our intended production process are as safe as comparable foods produced by other methods.

Question 20. For addition to the DSN, please include a summary of the media inputs used for cell line establishment of the FMT-SCF-4 production cell line as described in the reference you provided, Pasitka et al. 2023. Please also provide a safety argument for these media inputs, which may include a discussion of the expected dilution of any inputs used in cell line establishment.

As part of the safety assessment of media components used at any step in the development of FMT-SCF-4 shown in Figure 4.3-1 of the DSN, Believer Meats determined the dilution levels from the Manufacturer's Working Cell Bank (MWCB) vial to the final washed biomass at the point of harvest. These estimations were based on cell density and volume, factoring in dilution during inoculum, seed train expansion, bioreactor growth, media exchanges, and final washing with sodium chloride. The table below shows the calculated dilution factors:

Table 6. Dilution Calculations for Chemical Components used in Cell-Line Development

Step	Fold dilution of relevant chemical agent present in MWCB vial
Flask Seed-Train Expansion	6 x 10-12
Bioreactor Expansion	1 x 10-15
Washed Biomass	8 x 10-22

Given the extremely high dilution rates of media components used in cell-line development, these substances are not expected to be present in detectable quantities in the biomass.

Notwithstanding the dilution of all media components used during cell-line establishment, Believer Meats has removed the primary components commonly used in primary Master Cell Bank creation, including 10% fetal bovine serum (FBS), trypsin, and recombinant growth factors. These media components were only used during the early steps in cell isolation steps, immortalization, and adaptation to suspension and Believer Meats has adapted the cells to grow without their use.

The steps to remove the animal components are described in section 4.3.3 and in Figure 4.3-1. First, the primary MCB was adapted to grow in serum-free media (up to step 4 in Figure 4.3-1), eliminating the need for FBS. Next, the secondary MCB was adapted to grow in animal component-free (ACF) media (up to step 5 in Figure 4.3-1). In addition, the secondary MCB was further adapted to produce biomass without the use of recombinant growth factors in the growth media (up to step 6 in Figure 4.3-1). To confirm the FMT-SCF-4 cell line is safe from adventitious agents originating from animal derived components, the secondary MCB was tested for polyadenylated viruses using RNA sequencing and in silico genomic screening a mammalian virus database after adaptation to ACF media. No mammalian viruses of concern nor any non-avian DNA was detected in the MCB further supporting our safety conclusion during cell line establishment (refer to Appendix C from the SCM for a list of screened viruses).

The FMT-SCF-4 cell line is now adapted to grow without animal-derived media inputs during the production of cell-cultivated chicken and the creation of additional working cell banks. Therefore, when combined with the significant dilution of the MWCB in the production of biomass under the intended conditions of use, the media components used in cell-line development have no impact on biomass safety.

Question 21. On pages 23-24 of Appendix B, you provide the naturally occurring levels of pantothenates (not specifically pantothenic acid) in food and state that pantothenic acid (vitamin B5) is also used as a supplement. You state that "The EDI may be considered negligible in the context of total dietary intake of pantothenic acid." To support this statement, please provide the total dietary intake of vitamin B5 along with a reference for this value in the DSN. We note that foods could either naturally contain vitamin B5 or be supplemented by this substance.

The total dietary intake of vitamin B5 in United States is estimated to be 6 mg (Iyenga et al, 2000). This reference level will be used in our safety assessment for Vitamin B5 in Appendix B.

Product Characterization

Fatty Acid Profile

Question 22. In Table 5.4-1 of the DSN, you present proximate results and comparisons between the cultured chicken fibroblasts and conventional chicken breast products. In this table, please include total trans-fat alongside the other summarized lipid analysis results. For addition to the DSN, please also present the comparator's fatty acid profile relative to total fat, as was done for the harvested cell material.

We have added analytical trans-fat results for all three batches of cultured chicken fibroblasts, the comparator ground chicken and chicken breast products. The trans-fat values from the USDA Chicken is available from the U.S. Department of Agriculture FoodData Central in the SR Legacy Food FDC ID: 171077, Chicken, broiler or fryers, breast, skinless, boneless, meat only, raw (USDA ARS, 2019). The table is accessible at https://fdc.nal.usda.gov/fdc-app.html#/food-details/171077/nutrients.

Amended Table 5.4-1. Updated Lipid Analysis

Lipid Analysis	Method	Cultured Chicken Fibroblasts			Chicken Products		
		Batch 1	Batch 2	Batch 3	Ground Chicken	Chicken Breast	USDA Chicken
Saturated fat (g/100g)	AOAC 996.06	0.32	0.46	0.16	1.95	0.79	1.01
Mono-unsaturated fat (g/100g)	AOAC 996.06	0.28	0.52	0.24	2.49	0.85	1.26
Poly-unsaturated fat (g/100g)	AOAC 996.06	0.05	0.06	0.02	1.27	0.44	0.77
Cholesterol (mg/100g)	AOAC 994.10	57.2	67.8	56.8	93.5	73.4	42
Trans Fat Acids (g/100g)	AOAC 996.06	0.020	0.034	0.014	0.053	0.025	0.007

To address the second part of Question 22, the full fatty acid profile for the comparator ground chicken and chicken breast samples were presented in Table 5.4-3 in the DSN.

Question 23. In Table 5.4-3 of the DSN, you present the fatty acid content of three batches of cultured chicken cells as well as two conventional chicken products. The fatty acid levels for the three batches of cultured chicken cells are presented as both g/100 g oil and g/100 g cultured chicken. However, the fatty acid data for the conventional products are only provided on g/100 g wet basis. For addition to the DSN, please also provide the data for the two conventional chicken products on a g/100 g oil basis so that these values can be compared with the data for cultured chicken cells provided on a g/100 g oil basis.

Based on the added trans-fat results to Table 5.4-1 from Question 22, we have updated all fatty acid calculations from Table 5.4-3 of our submission and presented the calculations as both g/100g oil and g/100g chicken. The total lipids used for these calculations are the sum of saturated fat, mono-unsaturated fat, and poly-unsaturated fat.

Cultured Chicken Cells Batch 1 – 0.65%
Cultured Chicken Cells Batch 2 – 1.04%
Cultured Chicken Cells Batch 3 – 0.42%
Ground Chicken Sample – 5.71%
Chicken Breast Sample – 2.08%

Amended Table 5.4-3 Fatty Acid Profile of Cultured Chicken Cells and Store-Bought Chicken Samples

Fatty Acid	Cultured Chicken Cells (g/100g oil)			Cultured Chicken Cells (g/100g)			Store Bought Chicken (g/100 g oil)		Store Bought Chicken (g/100g)	
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Ground Chicken Sample	Chicken Breast Sample	Ground Chicken Sample	Chicken Breast Sample
C4:0	ND	0.035	ND	ND	0.0004	ND	ND	ND	ND	ND
C6:0	ND	0.046	ND	ND	ND	ND	ND	ND	ND	ND
C8:0	0.058	0.543	ND	0.0004	0.0056	ND	0.0200	ND	0.0011	ND
C10:0	0.207	0.442	ND	0.0013	0.0046	ND	0.0350	0.0070	0.0020	0.0001
C11:0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C12:0	12.571	5.644	1.149	0.0817	0.0587	0.0048	0.2570	0.1720	0.0147	0.0036
C14:0	3.158	2.655	1.114	0.0205	0.0276	0.0047	0.6840	0.5640	0.0391	0.0117
C14:1 c9	ND	0.053	ND	ND	0.0006	ND	0.1650	0.0910	0.0094	0.0019
C15:0	0.157	0.065	0.117	0.0010	0.0007	0.0005	0.1160	0.1190	0.0066	0.0025
C16:0	20.46	18.902	20.489	0.1330	0.1966	0.0861	24.2050	24.9590	1.3821	0.5191
C16:1 c9	2.657	4.192	3.988	0.0173	0.0436	0.0167	5.6530	4.5410	0.3228	0.0945
C17:0	0.102	0.055	0.06	0.0007	0.0006	0.0003	0.1780	0.1560	0.0102	0.0032
C18:0	9.158	11.836	10.329	0.0595	0.1231	0.0434	7.5840	10.1220	0.4330	0.2105
C18:1 trans	1.841	1.803	1.946	0.0120	0.0188	0.0082	0.5780	0.7780	0.0330	0.0162
C18:1	36.203	39.521	46.916	0.2353	0.4110	0.1970	36.9300	34.7700	2.1087	0.7232
C18:2 trans	0.66	0.644	0.641	0.0043	0.0067	0.0027	0.2400	0.1390	0.0137	0.0029

Fatty Acid	Cultured Chicken Cells (g/100g oil)			Cultured Chicken Cells (g/100g)			Store Bought Chicken (g/100 g oil)		Store Bought Chicken (g/100g)	
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Ground Chicken Sample	Chicken Breast Sample	Ground Chicken Sample	Chicken Breast Sample
C18:2 all cis-9,12	4.505	3.075	0.506	0.0293	0.0320	0.0021	18.5800	14.4400	1.0609	0.3004
C18:3 trans	0.478	0.72	0.77	0.0031	0.0075	0.0032	0.0980	0.2610	0.0056	0.0054
C18:3 all cis 6,9,12 G	ND	ND	ND	ND	ND	ND	0.1970	0.1350	0.0112	0.0028
C18:3 all cis 9,12,15 ALA	0.239	0.335	0.3	0.0016	0.0035	0.0013	0.9310	0.5280	0.0532	0.0110
C20:0	0.308	0.351	ND	0.0020	0.0037	ND	0.9100	0.1820	0.0520	0.0038
C20:1 c11	0.133	1.637	1.743	0.0009	0.0170	0.0073	0.3170	0.3520	0.0181	0.0073
C20:2 all cis-11,14	ND	ND	ND	ND	ND	ND	0.2600	0.4300	0.0148	0.0089
C20:3 all cis-8,11,14	0.051	0.274	0.732	0.0003	0.0028	0.0031	0.2450	0.6430	0.0140	0.0134
C20:3 all cis-11,14,17	0.19	ND	ND	0.0012	ND	ND	0.0320	0.0500	0.0018	0.0010
C20:4 all cis-5,8,11,14	0.306	0.226	0.163	0.0020	0.0024	0.0007	1.0030	2.7390	0.0573	0.0570
C20:5 all cis-5,8,11,14,17 EPA	0.336	0.493	0.385	0.0022	0.0051	0.0016	0.0390	0.0700	0.0022	0.0015
C21:0	ND	0.058	ND	ND	0.0006	ND	0.6260	1.5690	0.0357	0.0326
C22:0	1.683	2.088	2.728	0.0109	0.0217	0.0115	0.0370	0.0930	0.0021	0.0019
C22:1 n11	0.472	0.155	0.191	0.0031	0.0016	0.0008	0.0400	0.1170	0.0023	0.0024
C22:1 c11	0.154	0.446	0.349	0.0010	0.0046	0.0015	0.0220	0.0520	0.0013	0.0011
C22:1 c13	0.41	0.127	ND	0.0027	0.0013	ND	0.0050	ND	0.0003	ND
C22:2 c-13,16	0.288	0.154	0.07	0.0019	0.0016	0.0003	0.0210	0.0420	0.0012	0.0009
C22:4 all cis-7,10,13,16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:3 all cis-13,16,19	0.456	0.245	0.78	0.0030	0.0025	0.0033	0.0300	0.0940	0.0017	0.0020
C22:4 n6	ND	0.033	ND	ND	0.0003	ND	0.3480	0.8920	0.0199	0.0186
C22:5 all cis-4,7,10,13,16	ND	ND	ND	ND	ND	ND	0.0350	0.0890	0.0020	0.0019
C22:4 n3	ND	0.126	ND	ND	0.0013	ND	0.0530	0.1460	0.0030	0.0030
C22:5 n3	0.179	0.172	0.152	0.0012	0.0018	0.0006	0.1460	0.4000	0.0083	0.0083
C22:6 n3 DHAC 22:6 all cis-4,7,10,13,16,19 DHA	ND	ND	0.639	ND	ND	0.0027	0.0740	0.2650	0.0042	0.0055
C23:0	0.099	0.077	0.048	0.0006	0.0008	0.0002	NA	ND	ND	ND
C24:0	0.348	0.314	0.511	0.0023	0.0033	0.0021	0.0300	0.0710	0.0017	0.0015
C24:1 c15	2.009	1.961	2.761	0.0131	0.0204	0.0116	0.0870	0.2840	0.0050	0.0059

Question 24. On page 39 of the DSN, you provide information on at least three trans fatty acids for three batches of your harvested chicken cells. For addition to the DSN, please also provide information on the total amount of trans fat in the harvested cells. Please compare and comment on the total amount of trans fat in the cultured cells on a g/100 g oil basis with the total amount of trans fat in the two conventional comparators on a g/100 g oil basis.

We have presented trans fatty acid results as g/100g oil and g/100g product in Table 8, below. On a g/100g oil basis, the average trans fatty acids for the three batches of harvested chicken cells is 3.23 g/100g oil. This level is almost 2.5 times the trans-fat in the ground chicken and over 1.5 times the trans-fat in g/100g oil compared to the conventional chicken breast comparator. The average total fat content of harvested chicken cells is 0.70% on a wet matter basis while the ground chicken comparator is 9.06% fat, and the chicken breast comparator is 1.81% fat on a wet matter basis.

Table 7. Trans Fat Acid Comparison

Parameter	Method	Cultured Chicken Fibroblasts			Comparator Chicken Products	
		Batch 1	Batch 2	Batch 3	Ground Chicken	Chicken Breast
Trans Fat Acids (g/100g)	AOAC 996.06	0.02	0.034	0.014	0.053	0.025
Trans Fat Acids (g/100g oil)	AOAC 996.06	3.08	3.27	3.33	0.93	1.20

Compositional Analysis

Question 25. For addition to the DSN, please report nutritional profile batch data (i.e., protein, fat, lipid, amino acid, fatty acid, vitamins, minerals) and specifications for both a dry matter basis and wet matter basis for both the cultured cells and comparators to allow for consistency in comparing levels of compounds between batches.

We have updated the following tables to report analytical results as dry matter basis and wet matter basis to allow consistency in comparing levels between batches. The fatty acid nutritional data is shown in g/100g oil and g/100g dry basis as part of the response for Question 23.

Amended Table 5.4.1 – Comparison of Proximate Results for Believer Meats Cultured Fibroblasts and Conventional Chicken Breast Products – Dry Matter Basis

Parameter (Dry Matter Basis)	Method	Cultured Chicken Fibroblasts			Chicken Products		
		Batch 1	Batch 2	Batch 3	Ground Chicken	Chicken Breast	USDA Chicken
Moisture (%)	AOAC 950.46	96.45	95.6	96.39	71	74.9	73.9
Protein (%DMB)	In-house procedure, based on 976.05, 950.36, 991.3	71.5	66.1	73.3	63.2	85.4	86.2
Ash (%DMB)	AOAC 923.03	7.5	7.6	8.6	4.4	5.0	4.3
Fat (%DMB)	Based on Nestle LI 00.527-1	18.2	23.7	11.6	31.3	7.2	10
Carbohydrates (%DMB)	By difference	<2.8	<2.3	6.4	1.1	2.4	0

Amended Table 5.4.1 – Comparison of Proximate Results for Believer Meats Cultured Fibroblasts and Conventional Chicken Breast Products – Wet Matter Basis

Parameter	Method	Cultured Chicken Fibroblasts			Chicken Products		
		Batch 1	Batch 2	Batch 3	Ground Chicken	Chicken Breast	USDA Chicken
Moisture (%)	AOAC 950.46	96.45	95.6	96.39	71	74.9	73.9
Protein (g/100g)	In-house procedure, based on 976.05, 950.36, 991.3	2.57	2.84	2.64	21.44	18.30	22.50
Ash (g/100g)	AOAC 923.03	0.27	0.33	0.31	1.25	1.26	1.13
Fat (g/100g)	Based on Nestle LI 00.527-1	0.67	1.22	0.43	9.06	1.81	2.62
Carbohydrates (g/100g)	By difference	<0.10	<0.10	0.23	0.04	0.10	0.00

Amended Table 5.4.2 - Amino Acid Profile for Cultured Chicken Fibroblasts – Wet Matter and Dry Matter Basis

Amino Acids (g/100 g)	Cultured Chicken Fibroblasts (Wet Weight)			USDA Chicken Breast (Wet Weight)	Cultured Chicken Fibroblasts (DMB)			USDA Chicken Breast (DMB)
	Batch 1	Batch 2	Batch 3		Batch 1	Batch 2	Batch 3	
Tryptophan (g/100g)	0.0189	0.0377	0.0337	0.283	0.532	0.857	0.934	1.084
Threonine (g/100g)	0.067	0.123	0.122	1.01	1.887	2.795	3.380	3.87
Isoleucine (g/100g)	0.067	0.127	0.101	1.1	1.887	2.886	2.798	4.215
Leucine (g/100g)	0.116	0.223	0.18	1.86	3.268	5.068	4.986	7.126
Lysine (g/100g)	0.102	0.219	0.182	2.16	2.873	4.977	5.042	8.276
Methionine (g/100g)	0.0308	0.067	0.0552	0.585	0.868	1.523	1.529	2.241
Cystine + Cysteine (g/100g)	0.0298	0.0393	0.0352	0.236	0.839	0.893	0.975	0.904
Phenylalanine (g/100g)	0.06	0.125	0.101	0.908	1.69	2.841	2.798	3.479
Tyrosine (g/100g)	0.0511	0.111	0.075	0.81	1.439	2.523	2.078	3.103
Valine (g/100g)	0.073	0.147	0.122	1.16	2.056	3.341	3.380	4.444
Arginine (g/100g)	0.093	0.195	0.161	1.52	2.62	4.432	4.460	5.824
Histidine (g/100g)	0.0289	0.066	0.0514	0.839	0.814	1.500	1.424	3.215
Alanine (g/100g)	0.073	0.149	0.129	1.31	2.056	3.386	3.573	5.019
Aspartic acid (g/100g)	0.133	0.265	0.222	2.12	3.746	6.023	6.150	8.123
Glutamic acid (g/100g)	0.198	0.368	0.306	3.33	5.577	8.364	8.476	12.759
Glycine (g/100g)	0.067	0.133	0.112	0.996	1.887	3.023	3.102	3.816
Proline (g/100g)	0.0534	0.121	0.117	0.712	1.504	2.750	3.241	2.739
Serine (g/100g)	0.076	0.137	0.119	0.858	2.141	3.114	3.296	3.287
Total amino acids (g/100g)	1.3379	2.653	2.2245	NR	37.687	60.295	61.620	NR

Table 8. Amended Table 5.4.5 Vitamins and Minerals

Parameter	Cultured Chicken Cells			Store Bought		Cultured Chicken Cells			Store Bought	
	Batch 1	Batch 2	Batch 3	Ground Chicken Sample	Chicken Breast Sample	Batch 1	Batch 2	Batch 3	Ground Chicken Sample	Chicken Breast Sample
Calculations	**Wet Matter Basis**					**Dry Matter Basis**				
Vitamins										
Vitamin E (mg/100g)	0	0	0	<1	<1	0.00	0.00	0.00	3.45	3.98
Vitamin D3 (µg /kg)	ND	ND	ND	1.09	1.58	28.17	22.73	27.70	3.76	6.29
Vitamin C (mg/100g)	<1	<1	<1	1.91	2.7	28.17	22.73	27.70	6.59	10.76
Folates (µg/100g)	78.6	40	37	138	76	2,214	909.09	1,025	475.86	302.79
Thiamine (Vitamin B1) (mg/kg)	1.6	1.62	1.69	N/A	N/A	45.07	36.82	46.81	N/A	N/A
Riboflavin (Vitamin B2) (µg/100g)	0.75	0.74	0.76	N/A	N/A	21.13	16.82	21.05	N/A	N/A
Niacinamide (Vitamin B3) (mg/kg)	3.74	5.24	3.73	N/A	N/A	105.35	119.09	103.32	N/A	N/A

Parameter	Cultured Chicken Cells			Store Bought		Cultured Chicken Cells			Store Bought	
	Batch 1	Batch 2	Batch 3	Ground Chicken Sample	Chicken Breast Sample	Batch 1	Batch 2	Batch 3	Ground Chicken Sample	Chicken Breast Sample
Pantothenic Acid (Vitamin B5) (mg/kg)	8.5	11.5	10.8	N/A	N/A	239.44	261.36	299.17	N/A	N/A
Pyridoxine (Vitamin B6) (mg/kg)	1.86	0.58	0.43	N/A	N/A	52.39	13.18	11.91	N/A	N/A
Biotin (Vitamin B8) (ug/100g)	2.15	2.21	2.15	N/A	N/A	60.56	50.23	59.56	N/A	N/A
Folates (µg/100g)	18	28	23	N/A	N/A	507.04	636.36	637.12	N/A	N/A
Cobalamin (Vitamin B12) (µg/100g)	3.94	3.67	3.92	N/A	N/A	110.99	83.41	108.59	N/A	N/A
Minerals										
Ag-Silver (mg/kg)	<0.05	<0.05	<0.05	<0.01	<0.01	1.41	1.14	1.39	0.03	0.04
Al-Aluminum (mg/kg)	<1	<1	<1	<1	<1	28.17	22.73	27.70	3.45	3.98
B-Boron (mg/kg)	<2	<2	<2	2*	<2	56.34	45.45	55.40	6.90	7.97
Ba-Barium (mg/kg)	<0.10	<0.10	<0.50	<0.5	<0.5	2.82	2.27	13.85	1.72	1.99
Be-Beryllium (mg/kg)	<0.05	<0.05	<0.05	<0.05	<0.05	1.41	1.14	1.39	0.17	0.20
Ca-Calcium (mg/kg)	9.0	22.58	11.90	52.17	51.2	254.34	513.20	329.64	179.90	203.98
Co-Cobalt (mg/kg)	<0.050	<0.050	<0.010	<0.01	<0.01	1.41	1.14	0.28	0.03	0.04
Cr-Chromium (mg/kg)	<0.050	<0.050	<0.040	0.12	0.04	1.41	1.14	1.11	0.41	0.16
Cu-Copper (mg/kg)	0.34	0.38	0.48	0.34	0.24	9.55	8.70	13.30	1.17	0.96
Fe- Iron (mg/kg)	3.35	4.22	1.96	5.49	4.07	94.37	96.00	54.29	18.93	16.22
Li-Lithium (mg/kg)	<0.05	<0.05	<0.05	<0.03	<0.03	1.41	1.14	1.39	0.10	0.12
Mn-Manganese (mg/kg)	0.07	0.08	0.08	0.13	0.12	1.94	1.77	2.16	0.45	0.48
Mo-Molybdenum (mg/kg)	<0.01	<0.05	<0.01	0.03	0.03	0.28	1.14	0.28	0.10	0.12
Ni-Nickel (mg/kg)	<0.05	<0.05	<0.05	0.05	0.06	1.41	1.14	1.39	0.17	0.24
P-Phosphorus (mg/kg)	684	516	469	1848	2176	19,277	11,731	12,992	6,372	8,669
Sb-Antimony (mg/kg)	<0.01	<0.050	<0.010	<0.01	<0.01	0.28	1.14	0.28	0.03	0.04
Se-Selenium (mg/kg)	<0.05	<0.05	0.03	0.32	0.3	1.41	1.14	0.94	1.10	1.20
Sr-Strontium (mg/kg)	0.24	0.41	<0.20	0.29	0.2	6.65	9.39	0.55	1.00	0.80
Sn- Tin (mg/kg)	0.60	1.02	2.53	<0.1	<0.1	16.85	23.11	70.08	0.34	0.40
Ti-Titanium (mg/kg)	<0.10	<0.10	0.40	1.25	1.7	2.82	2.27	11.08	4.31	6.77
V-Vanadium (mg/kg)	<0.10	<0.10	<0.01	<0.01	<0.01	2.82	2.27	2.77	0.34	0.40
Zn-Zinc (mg/kg)	3.17	2.60	0.98	9.04	6.75	89.32	59.16	27.15	31.17	26.89
K-Potassium (mg/kg)	534	384	528	3,428	3,953	15,037	8,735	14,626	11,821	15,749
Mg-Magnesium (mg/kg)	47	38	43	288	360	1,319	868	1,202	993	1,436
Na-Sodium (mg/kg)	835	905	773	2,386	1,634	23,507	20,561	21,413	8,228	6,510
Si-Silicon -ICP (mg/kg)	35	27	27	7.09	8.81	985.55	609.52	736.84	24.45	35.10

Question 26. In Table 5.4-5 of the DSN indicates the presence of measurable amounts of strontium and silicon in the harvested cultured cell material. For addition to the DSN, please comment on the likely source of measurable amounts of these elements in the harvested cultured cell material.

Strontium (Sr) is a natural element, ubiquitous in the environment and known to occur in water, food, air, and soils. Surface and underground water, air, and plants all contain varying amounts of strontium. Food and drinking water are the largest sources of strontium exposure (Agency for Toxic Substances and Disease Registry, 2014). Due to the ubiquitous nature of Strontium, the most likely sources in our production process are from any of our plant-based media ingredients or from water. We also note that the levels of elemental strontium present in our cell-cultivated chicken is in-line with the analytical values of strontium in the comparator ground chicken and chicken breast products reported in Table 5.4-5.

In addition, we note that at commercial scale, process water will be treated with reverse osmosis. which expect will reduce amounts of minerals and elements in source water, including strontium. Reverse osmosis filters contaminants from source water to <0.001 micron removing bacteria, viruses, and most organic substance with a molecular weight above 100 (FDA, 2014). The U.S. Environmental Protection Agency has identified reverse osmosis as one of the best available technologies for the control of strontium in drinking water (EPA, 2014).

The measurable silicon present in the harvested biomass most likely comes from two sources, a silicon-based defoaming agent used as a processing aid in cell expansion and salt used in the saltwater wash. A silicon-based defoaming agent is used in the cell expansion process, in accordance with current good manufacturing practices. Notwithstanding, analytical data shows that there is measurable elemental silicon present in biomass where the defoaming agent was not used. The levels of elemental silicon in biomass post-wash are higher than the levels of silicon in biomass pre-wash, showing that elemental silicon is present in the saltwater wash.

Points of Clarification

Question 27. On page 22 of the DSN, you state that "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." On Page 25, you state that "Complete mycoplasma screening for over 100 common species that may contaminate cell lines is conducted on the secondary MCB and the MWCB." However, on the same page, you state that "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.)." Please clarify whether each test is performed on the secondary MCBs or the initial MCB.

The primary MCB was tested for two species of Mycoplasma that are of avian concern. The secondary 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* spp., and *Campylobacter* spp.). The secondary MCB was also tested for over 100 species of Mycoplasma using a nested PCR assay.

Question 28. On pages 30-31 of the DSN, you describe your method for species identification testing, and we note that you verify the absence of ovine, pork, and bovine cells. We recommend that you use an unbiased method (e.g., DNA barcoding) to confirm that your cell line is not contaminated with cells from any other species. Alternatively, please discuss why there is no risk of contamination from any other species (e.g., are cell lines made from other species present at your facility, if multi-species please describe the steps taken to ensure there is no cross contamination of cell lines, including any inventory controls)

The cell-line development facility handles multiple species of cells and has inventory controls procedures and DNA verification testing to confirm cell lines are not contaminated with other species. All cell vials are labeled with, at a minimum, lot number and cell line name that is species-specific. Cell inventories are maintained by species and lot. We do not permit storing different species of cell lines on the same nitrogen racks. This lot number and cell line information is included in chain-of-custody documentation that is verified upon receipt at the manufacturing location.

To qualify each MCB, we test all cell lines using species specific Thermo Scientific™ RapidFinder™ Chicken ID test kits to validate the cell line is of chicken origin as outlined in Table 4.3.3-1 of the DSN. We also use Thermo Scientific™ RapidFinder™ DNA testing kits for all other species handled in the cell-development facility to verify no contamination from other species has occurred:

Thermo Scientific™ RapidFinder™ Chicken ID Kit A24393
Thermo Scientific™ RapidFinder™ Sheep ID Kit - A24395
Thermo Scientific™ RapidFinder™ Beef ID Kit - A24391
Thermo Scientific™ RapidFinder™ Pork ID Kit - A24392

29. On page 50 of the DSN, you state that "Class 3 substances currently include the following: (A) culture media proteins; (B) hormones; (C) non-essential nutrients; and (D) media conditioning aids." FDA notes that no Class 3 hormones were discussed in your submission, either in the DSN or the SCM. For addition to the DSN, please state whether any Class 3 hormones are used during manufacturing. If so, please identify these hormones and provide a safety assessment for them.

No Class 3 hormones were used during manufacturing, this was an error on page 50 of the DSN.

30. On page 60 of the DSN, you provide comet assay data collected from the FMT-SCF-2 research cell line derived from a Broiler Ross chicken, but not for the FMT-SCF-4 production cell line derived from the Israeli Baladi chicken. For addition to the DSN, please discuss why only data from the FMT-SCF-2 cell line was presented.

To assess DNA repair activity, we conducted a Comet assay on both primary chicken fibroblasts (CEF-4, Fig. 1A) and immortalized production fibroblasts (FMT-SCF-4, Fig. 1B). The results demonstrated efficient induction of DNA damage following treatment with etoposide, followed by a significant decrease in comet formation after the recovery period in both immortalized and primary fibroblasts (Fig. 1C). This reduction indicates the presence of an active DNA repair mechanism in both cell lines. These results demonstrate that the DNA repair mechanism remains intact in the immortalized SCF-4 cell line.

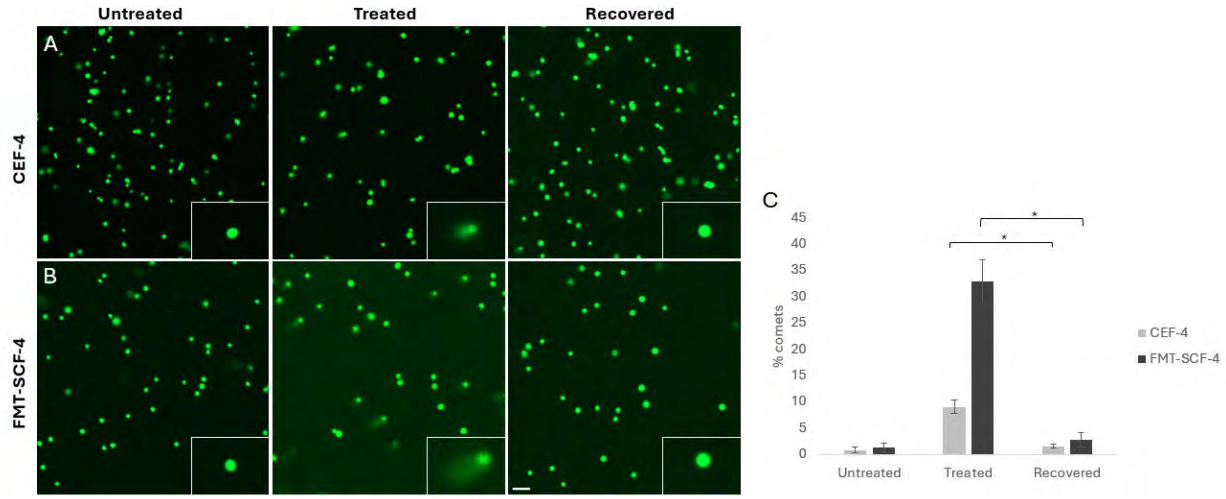

Figure 1. Comet assay analysis of CEF-4 and SCF-4 fibroblast cell lines

The assay was performed using the Comet Assay Kit (Abcam, ab238544) following the manufacturer's protocol. DNA damage was induced by exposing cells to 100 µM etoposide in growth medium, with an incubation period of 1 hour at 39°C in a cell culture incubator. The medium was subsequently replaced with fresh growth medium, and recovery was allowed for additional 5 hours. Control, treated and recovered wells were then processed and analyzed. Results are based on 100 cells per condition, three technical replicates, and a significance level of $p < 0.05$. Imaging was performed using a Zeiss AxioObserver 7 microscope. Scale bars represent 100 µm.

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19. Lithium chloride is used as a media input in your cell culture production process. We have considered your proposed use of this substance in your cell culture process and find that its use may likely violate Section 301(ll) of the FD&C Act. Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. We do not believe any of the exemptions apply. Therefore, we would advise you to reconsider the use of this substance in your cell culture production process.

Following submission of our safety assessment in May 2024, Believer Meats adjusted the production media formulation for its cell-cultivated chicken to remove LiCl. Thus, as stated in our October 4, 2024 response to FDA's August 1, 2024 request for information, the question of whether the use of LiCl as a processing aid in our production media implicates section 301(ll) of the FD&C Act is now moot for purposes of this safety assessment.

We have confirmed that none of the adjustments to the production media formulation will affect cell growth and performance or introduce any safety or other regulatory concerns. The adjustments to the media formulation to support the removal of LiCl may be summarized as follows:

- For the Class 1 media components identified in Table 7.1.1-1 of the DSN, we reduced salt levels, except for an increase in manganese and potassium. Even with these increases, the EDI for both manganese and potassium remains under the DV.
- For the Class 2 media components identified in Table 7.1.1-2 of the DSN, we slightly increased fatty acid levels, but the levels remain below concentrations in conventional poultry meat products. Slight increases were also made in Vitamin B6 (pyridoxine) and myoinositol.
- For the Class 3 media components identified in Table 7.1.1-3 of the DSN, we have removed an organic amine and reduced levels of a trace metal.

These modest adjustments to the media formulation, which allow for the removal of LiCl from our production process, do not materially change our commercial process or the product's safety or identity, and are consistent with our continued conclusion that foods comprised of or containing cultured chicken resulting from our intended production process

are as safe as comparable foods produced by other methods. This assertion is confirmed through analytical testing, as presented in the amended tables below. These tables present analytical data on one batch produced using our current, LiCl-free process (Batch 4) and three batches produced using our prior process (Batches 1-3) that were shared with the review team in May 2024.

Amended Table 5.3-1 – Proximates and Heavy Metals

Specification Parameter	Specification Limit	Batch 1	Batch 2	Batch 3	Batch 4
Protein (%DMB)	>65	71.5	66.1	73.3	64.0*
Moisture (g/100g)	>95	96.45	95.6	96.39	96.07
Ash (%DMB)	>2.5	7.5	7.6	8.6	24.8
Fat by hydrolysis (%DMB)	>10	18.2	23.7	11.6	8.8
Carbohydrates (g/100g)	<10	<0.10	<0.10	0.23	<0.10
Pb-Lead (mg/kg)	<0.05	<0.005	<0.005	0.011	0.007
As-Arsenic (mg/kg)	<0.01	<0.01	<0.01	<0.01	<0.01
Cd-Cadmium (mg/kg)	<0.01	<0.003	<0.003	<0.005	<0.005
Hg-Mercury (mg/kg)	<0.01	<0.003	<0.003	<0.005	<0.005
Total count (CFU/g)	<5,000	<10	<10	10	<10
Yeasts (CFU/g)	<100	<10	<10	<10	<10
Molds (CFU/g)	<200	<10	<10	<10	<10
E. coli TBX (CFU/g)	<10	<10	<10	<10	<10
Salmonella (CFU/25g)	Negative	Negative	Negative	Negative	Negative
Enterobacteriaceae (CFU/g)	<100	<10	<10	<10	<10
TPM1 (Tropomyosin) rt-PCR, in-house (Mean Cq)	<26	20.3	21.7	20.6	19.5

*Protein is 66.41% on Dry Matter Basis and 64.0% when normalized to 100% based on rounding from laboratory results

Amended Table 5.4-1 – Proximate and Lipid Analysis – Dry Matter Basis

Parameter	Method	Cultured Chicken Fibroblasts				Chicken Products		
		Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken	Chicken Breast	USDA Chicken
Moisture (%)	AOAC 950.46	96.45	95.6	96.39	96.07	71	74.9	73.9
Protein (%DMB)	In-house procedure, based on 976.05, 950.36, 991.3	71.5	66.1	73.3	64.0	63.2	85.4	86.2
Ash (%DMB)	AOAC 923.03	7.5	7.6	8.6	24.8	4.4	5	4.3
Fat (%DMB)	Based on Nestle LI 00.527-1	18.2	23.7	11.6	8.8	31.3	7.2	10
Carbohydrates (%DMB)	By difference	<2.8	<2.3	6.4	<2.5	1.1	2.4	0

Amended Table 5.4-1 – Proximate and Lipid Analysis – Wet Matter Basis

Parameter	Method	Cultured Chicken Fibroblasts				Chicken Products		
		Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken	Chicken Breast	USDA Chicken
Moisture (%)	AOAC 950.46	96.45	95.6	96.39	96.07	71	74.9	73.9
Protein (g/100g)	In-house procedure, based on 976.05, 950.36, 991.3	2.57	2.84	2.64	2.61	21.44	18.30	22.50
Ash (g/100g)	AOAC 923.03	0.27	0.33	0.31	1.01	1.25	1.26	1.13
Fat (g/100g)	Based on Nestle LI 00.527-1	0.67	1.22	0.43	0.36	9.06	1.81	2.62
Carbohydrates (g/100g)	By difference	<0.10	<0.10	0.23	<0.10	0.04	0.10	0.00
Saturated fat (g/100g)	AOAC 996.06	0.32	0.46	0.16	0.15	1.95	0.79	1.01
Mono-unsaturated fat (g/100g)	AOAC 996.06	0.28	0.52	0.24	0.17	2.49	0.85	1.26
Poly-unsaturated fat (g/100g)	AOAC 996.06	0.05	0.06	0.02	0.04	1.27	0.44	0.77
Cholesterol (mg/100g)	AOAC 994.10	57.2	67.8	56.8	64.2	93.5	73.4	42
Trans Fat Acids (g/100g)	AOAC 996.06	0.02	0.034	0.014	0.01	0.053	0.025	0.007

Amended Table 5.4-2 – Amino Acid Profile

Amino Acids (g/100 g)	Cultured Chicken Fibroblasts (Wet Weight)				USDA Chicken Breast (Wet Weight)	Cultured Chicken Fibroblasts (DMB)				USDA Chicken Breast (DMB)
	Batch 1	Batch 2	Batch 3	Batch 4		Batch 1	Batch 2	Batch 3	Batch 4	
Tryptophan (g/100g)	0.0189	0.0377	0.0337	0.0293	0.283	0.532	0.857	0.934	0.746	1.084
Threonine (g/100g)	0.067	0.123	0.122	0.106	1.01	1.887	2.795	3.380	2.697	3.87
Isoleucine (g/100g)	0.067	0.127	0.101	0.109	1.1	1.887	2.886	2.798	2.774	4.215
Leucine (g/100g)	0.116	0.223	0.18	0.191	1.86	3.268	5.068	4.986	4.860	7.126
Lysine (g/100g)	0.102	0.219	0.182	0.196	2.16	2.873	4.977	5.042	4.987	8.276
Methionine (g/100g)	0.0308	0.067	0.0552	0.048	0.585	0.868	1.523	1.529	1.221	2.241
Cystine + Cysteine (g/100g)	0.0298	0.0393	0.0352	0.034	0.236	0.839	0.893	0.975	0.865	0.904
Phenylalanine (g/100g)	0.06	0.125	0.101	0.104	0.908	1.69	2.841	2.798	2.646	3.479
Tyrosine (g/100g)	0.0511	0.111	0.075	0.09	0.81	1.439	2.523	2.078	2.290	3.103
Valine (g/100g)	0.073	0.147	0.122	0.114	1.16	2.056	3.341	3.380	2.901	4.444
Arginine (g/100g)	0.093	0.195	0.161	0.029	1.52	2.62	4.432	4.460	0.738	5.824

Amino Acids (g/100 g)	Cultured Chicken Fibroblasts (Wet Weight)				USDA Chicken Breast (Wet Weight)	Cultured Chicken Fibroblasts (DMB)				USDA Chicken Breast (DMB)
	Batch 1	Batch 2	Batch 3	Batch 4		Batch 1	Batch 2	Batch 3	Batch 4	
Histidine (g/100g)	0.0289	0.066	0.0514	0.053	0.839	0.814	1.500	1.424	1.349	3.215
Alanine (g/100g)	0.073	0.149	0.129	0.016	1.31	2.056	3.386	3.573	0.407	5.019
Aspartic acid (g/100g)	0.133	0.265	0.222	0.147	2.12	3.746	6.023	6.150	3.740	8.123
Glutamic acid (g/100g)	0.198	0.368	0.306	0.276	3.33	5.577	8.364	8.476	7.023	12.759
Glycine (g/100g)	0.067	0.133	0.112	0.115	0.996	1.887	3.023	3.102	2.926	3.816
Proline (g/100g)	0.0534	0.121	0.117	0.088	0.712	1.504	2.750	3.241	2.239	2.739
Serine (g/100g)	0.076	0.137	0.119	0.027	0.858	2.141	3.114	3.296	0.687	3.287
Total amino acids (g/100g)	1.3379	2.653	2.2245	1.874	NR	37.687	60.295	61.620	47.684	NR

Amended Table 5.4-3 – Fatty Acid Profile

Fatty Acid	Cultured Chicken Cells (g/100g oil)				Cultured Chicken Cells (g/100g)				Store Bought Chicken (g/100 g oil)		Store Bought Chicken (g/100g)	
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken	Chicken Breast	Ground Chicken	Chicken Breast
C4:0	ND	0.035	ND	ND	ND	0.0004	ND	ND	ND	ND	ND	ND
C6:0	ND	0.046	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C8:0	0.058	0.543	ND	ND	0.0004	0.0056	ND	ND	0.0200	ND	0.0011	ND
C10:0	0.207	0.442	ND	ND	0.0013	0.0046	ND	ND	0.0350	0.0070	0.0020	0.0001
C11:0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C12:0	12.571	5.644	1.149	2.79	0.0817	0.0587	0.0048	0.0100	0.2570	0.1720	0.0147	0.0036
C14:0	3.158	2.655	1.114	1.589	0.0205	0.0276	0.0047	0.0057	0.6840	0.5640	0.0391	0.0117
C14:1 c9	ND	0.053	ND	ND	ND	0.0006	ND	ND	0.1650	0.0910	0.0094	0.0019
C15:0	0.157	0.065	0.117	ND	0.0010	0.0007	0.0005	ND	0.1160	0.1190	0.0066	0.0025
C16:0	20.46	18.902	20.489	28.038	0.1330	0.1966	0.0861	0.1009	24.2050	24.9590	1.3821	0.5191
C16:1 c9	2.657	4.192	3.988	3.242	0.0173	0.0436	0.0167	0.0117	5.6530	4.5410	0.3228	0.0945
C17:0	0.102	0.055	0.06	ND	0.0007	0.0006	0.0003	ND	0.1780	0.1560	0.0102	0.0032
C18:0	9.158	11.836	10.329	10.017	0.0595	0.1231	0.0434	0.0361	7.5840	10.1220	0.4330	0.2105
C18:1 trans	1.841	1.803	1.946	2.668	0.0120	0.0188	0.0082	0.0096	0.5780	0.7780	0.0330	0.0162
C18:1	36.203	39.521	46.916	42.594	0.2353	0.4110	0.1970	0.1533	36.9300	34.7700	2.1087	0.7232
C18:2 trans	0.66	0.644	0.641	ND	0.0043	0.0067	0.0027	ND	0.2400	0.1390	0.0137	0.0029
C18:2 all cis-9,12	4.505	3.075	0.506	9.003	0.0293	0.0320	0.0021	0.0324	18.5800	14.4400	1.0609	0.3004

Fatty Acid	Cultured Chicken Cells (g/100g oil)				Cultured Chicken Cells (g/100g)				Store Bought Chicken (g/100 g oil)		Store Bought Chicken (g/100g)	
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken	Chicken Breast	Ground Chicken	Chicken Breast
C18:3 trans	0.478	0.72	0.77	ND	0.0031	0.0075	0.0032	ND	0.0980	0.2610	0.0056	0.0054
C18:3 all cis 6,9,12 G	ND	ND	ND	ND	ND	ND	ND	ND	0.1970	0.1350	0.0112	0.0028
C18:3 all cis 9,12,15 ALA	0.239	0.335	0.3	0.954	0.0016	0.0035	0.0013	0.0034	0.9310	0.5280	0.0532	0.0110
C20:0	0.308	0.351	ND	ND	0.0020	0.0037	ND	ND	0.9100	0.1820	0.0520	0.0038
C20:1 c11	0.133	1.637	1.743	ND	0.0009	0.0170	0.0073	ND	0.3170	0.3520	0.0181	0.0073
C20:2 all cis-11,14	ND	ND	ND	ND	ND	ND	ND	ND	0.2600	0.4300	0.0148	0.0089
C20:3 all cis-8,11,14	0.051	0.274	0.732	ND	0.0003	0.0028	0.0031	ND	0.2450	0.6430	0.0140	0.0134
C20:3 all cis-11,14,17	0.19	ND	ND	ND	0.0012	ND	ND	ND	0.0320	0.0500	0.0018	0.0010
C20:4 all cis-5,8,11,14	0.306	0.226	0.163	ND	0.0020	0.0024	0.0007	ND	1.0030	2.7390	0.0573	0.0570
C20:5 all cis-5,8,11,14,17 EPA	0.336	0.493	0.385	ND	0.0022	0.0051	0.0016	ND	0.0390	0.0700	0.0022	0.0015
C21:0	ND	0.058	ND	ND	ND	0.0006	ND	ND	0.6260	1.5690	0.0357	0.0326
C22:0	1.683	2.088	2.728	0.425	0.0109	0.0217	0.0115	0.0015	0.0370	0.0930	0.0021	0.0019
C22:1 n11	0.472	0.155	0.191	ND	0.0031	0.0016	0.0008	ND	0.0400	0.1170	0.0023	0.0024
C22:1 c11	0.154	0.446	0.349	ND	0.0010	0.0046	0.0015	ND	0.0220	0.0520	0.0013	0.0011
C22:1 c13	0.41	0.127	ND	ND	0.0027	0.0013	ND	ND	0.0050	ND	0.0003	ND
C22:2 c-13,16	0.288	0.154	0.07	ND	0.0019	0.0016	0.0003	ND	0.0210	0.0420	0.0012	0.0009
C22:4 all cis-7,10,13,16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:3 all cis-13,16,19	0.456	0.245	0.78	ND	0.0030	0.0025	0.0033	ND	0.0300	0.0940	0.0017	0.0020
C22:4 n6	ND	0.033	ND	ND	ND	0.0003	ND	ND	0.3480	0.8920	0.0199	0.0186
C22:5 all cis-4,7,10,13,16	ND	ND	ND	ND	ND	ND	ND	ND	0.0350	0.0890	0.0020	0.0019
C22:4 n3	ND	0.126	ND	ND	ND	0.0013	ND	ND	0.0530	0.1460	0.0030	0.0030
C22:5 n3	0.179	0.172	0.152	ND	0.0012	0.0018	0.0006	ND	0.1460	0.4000	0.0083	0.0083
C22:6 n3 DHAC 22:6 all cis-4,7,10,13,16,19 DHA	ND	ND	0.639	ND	ND	ND	0.0027	ND	0.0740	0.2650	0.0042	0.0055
C23:0	0.099	0.077	0.048	ND	0.0006	0.0008	0.0002	ND	NA	ND	ND	ND
C24:0	0.348	0.314	0.511	0.509	0.0023	0.0033	0.0021	0.0018	0.0300	0.0710	0.0017	0.0015
C24:1 c15	2.009	1.961	2.761	1.17	0.0131	0.0204	0.0116	0.0042	0.0870	0.2840	0.0050	0.0059

Amended Table 5.4-5 – Vitamins and Minerals

Parameter	Cultured Chicken Cells				Store Bought		Cultured Chicken Cells				Store Bought	
	Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken	Chicken Breast	Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken	Chicken Breast
Calculations	**Wet Matter Basis**						**Dry Matter Basis**					
Vitamins												
Vitamin E (mg/100g)	0	0	0	0	<1	<1	0.00	0.00	0.00	0.00	3.45	3.98
Vitamin D3 (µ/kg)	ND	ND	ND	ND	1.09	1.58	28.17	22.73	27.70	25.45	3.76	6.29
Vitamin C (mg/100g)	<1	<1	<1	<1	1.91	2.7	28.17	22.73	27.70	25.45	6.59	10.76
Folates (µg/100g)	78.6	40	37	57	138	76	2,214	909	1,025	1450	475.86	302.79
Thiamine (Vitamin B1) (mg/kg)	1.6	1.62	1.69	1.19	N/A	N/A	45.07	36.82	46.81	30.28	N/A	N/A
Riboflavin (Vitamin B2) (ug/100g)	0.75	0.74	0.76	0.46	N/A	N/A	21.13	16.82	21.05	11.70	N/A	N/A
Niacinamide (Vitamin B3) (mg/kg)	3.74	5.24	3.73	5.4	N/A	N/A	105.35	119.09	103.32	137.40	N/A	N/A
Pantothenic Acid (Vitamin B5) (mg/kg)	8.5	11.5	10.8	11.7	N/A	N/A	239.44	261.36	299.17	297.71	N/A	N/A
Pyridoxine (Vitamin B6) (mg/kg)	1.86	0.58	0.43	0.71	N/A	N/A	52.39	13.18	11.91	18.07	N/A	N/A
Biotin (Vitamin B8) (ug/100g)	2.15	2.21	2.15	2.23	N/A	N/A	60.56	50.23	59.56	56.74	N/A	N/A
Cobalamin (Vitamin B12) (ug/100g)	3.94	3.67	3.92	3.92	N/A	N/A	110.99	83.41	108.59	99.75	N/A	N/A
Minerals												
Ag-Silver (mg/kg)	<0.05	<0.05	<0.05	<0.01	<0.01	<0.01	1.41	1.14	1.39	1.27	0.03	0.04
Al-Aluminum (mg/kg)	<1	<1	<1	3.70	<1	<1	28.17	22.73	27.70	94.15	3.45	3.98
B-Boron (mg/kg)	<2	<2	<2	<2	2*	<2	56.34	45.45	55.40	50.89	6.90	7.97
Ba-Barium (mg/kg)	<0.10	<0.10	<0.50	<0.5	<0.5	<0.5	2.82	2.27	13.85	12.72	1.72	1.99
Be-Beryllium (mg/kg)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	1.41	1.14	1.39	1.27	0.17	0.20
Ca-Calcium (mg/kg)	9.0	22.58	11.90	23.50	52.17	51.2	254.34	513.20	329.64	597.96	179.90	203.98
Co-Cobalt (mg/kg)	<0.050	<0.050	<0.010	<0.01	<0.01	<0.01	1.41	1.14	0.28	0.25	0.03	0.04
Cr-Chromium (mg/kg)	<0.050	<0.050	<0.040	0.11	0.12	0.04	1.41	1.14	1.11	2.80	0.41	0.16
Cu-Copper (mg/kg)	0.34	0.38	0.48	0.20	0.34	0.24	9.55	8.70	13.30	5.09	1.17	0.96
Fe- Iron (mg/kg)	3.35	4.22	1.96	3.59	5.49	4.07	94.37	96.00	54.29	91.35	18.93	16.22
Li-Lithium (mg/kg)	<0.05	<0.05	<0.05	<0.025	<0.03	<0.03	1.41	1.14	1.39	1.27	0.10	0.12
Mn-Manganese (mg/kg)	0.07	0.08	0.08	0.10	0.13	0.12	1.94	1.77	2.16	2.65	0.45	0.48
Mo-Molybdenum (mg/kg)	<0.01	<0.05	<0.01	<0.01	0.03	0.03	0.28	1.14	0.28	0.25	0.10	0.12
Ni-Nickel (mg/kg)	<0.05	<0.05	<0.05	<0.05	0.05	0.06	1.41	1.14	1.39	1.27	0.17	0.24
P-Phosphorus (mg/kg)	684	516	469	405	1848	2176	19,277	11,731	12,992	10,305	6,372	8,669
Sb-Antimony (mg/kg)	<0.01	<0.050	<0.010	<0.01	<0.01	<0.01	0.28	1.14	0.28	0.25	0.03	0.04
Se-Selenium (mg/kg)	<0.05	<0.05	0.03	<0.01	0.32	0.3	1.41	1.14	0.94	0.025	1.10	1.20
Sr-Strontium (mg/kg)	0.24	0.41	<0.20	0.57	0.29	0.2	6.65	9.39	0.55	0.51	1.00	0.80

Parameter	Cultured Chicken Cells				Store Bought		Cultured Chicken Cells				Store Bought	
	Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken	Chicken Breast	Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken	Chicken Breast
Sn- Tin (mg/kg)	0.60	1.02	2.53	0.21	<0.1	<0.1	16.85	23.11	70.08	5.34	0.34	0.40
Ti-Titanium (mg/kg)	<0.10	<0.10	0.40	0.38	1.25	1.7	2.82	2.27	11.08	9.67	4.31	6.77
V-Vanadium (mg/kg)	<0.10	<0.10	<0.01	<0.01	<0.01	<0.01	2.82	2.27	2.77	2.54	0.34	0.40
Zn-Zinc (mg/kg)	3.17	2.60	0.98	2.33	9.04	6.75	89.32	59.16	27.15	59.29	31.17	26.89
K-Potassium (mg/kg)	534	384	528	319	3,428	3,953	15,037	8,735	14,626	8,117	11,821	15,749
Mg-Magnesium (mg/kg)	47	38	43	33	288	360	1,319	868	1,202	840	993	1,436
Na-Sodium (mg/kg)	835	905	773	3,690	2,386	1,634	23,507	20,561	21,413	93,893	8,228	6,510
Si-Silicon -ICP (mg/kg)	35	27	27	19	7.09	8.81	985.55	609.52	736.84	488.55	24.45	35.10

As demonstrated in the tables above, the analytical data show that there are few material differences between Batch 4 (i.e., the batch produced using Believer's current LiCl-free process) and Batches 1-3 (i.e., those produced using Believer's prior LiCl-containing process), thus supporting Believer's conclusion that the removal of LiCl from our production process does not affect our overall safety conclusion.

Below, we provide further context for (a) the increased sodium levels observed in the Batch 4 biomass, (b) the increase in ash levels in in the Batch 4 biomass, and (c) the increased use of vitamin B6 in our LiCl-free media formulation, and we explain why these specific changes do not affect our overall safety conclusion.

a. Increased Sodium Levels in the Batch 4 Washed Biomass

As reflected in Table 5.4-5, analytical testing shows expected higher levels of sodium in the Batch 4 washed biomass (3690 mg/kg) compared to the Batch 1-3 washed biomasses (835 mg/kg, 905 mg/kg, 773 mg/kg). Even with this increase in sodium levels, EDI levels for sodium will still fall well below the relevant DV and will not pose material safety concerns.

The difference in sodium levels between Batches 1-3 and Batch 4 reflects a minor, controlled change in the wash cycle used in Believer's production process. This adjustment is anticipated in our response to Question 2b of the FDA's August 1, 2024 request for information that Believer will need to modify certain wash cycle operational parameters when transitioning to large-scale operations to account for a larger size centrifuge used in the Wilson, North Carolina facility. These operational parameters include rotations per minute (RPM), the NaCl buffer volume used for each wash, and the number of washes completed.

Specifically, the wash cycle for Batches 1-3 consisted of two cycles using a NaCl wash buffer followed by one cycle using distilled water as the wash buffer. Unlike Batches 1-3, the Batch 4 wash cycle included three wash cycles using the NaCl wash buffer and did not include a cycle with distilled water as the wash buffer. We also expressed in our response to Question 2b that the second difference between the operation in Rehovot, Israel and Wilson, North Carolina is more automation in transferring biomass between process steps at large scale. To support efficiency in the larger scale, more automated production process, the wash buffer will remain the same in each wash step (NaCl) rather than incorporating a change between two different wash buffers (NaCl and distilled water). This, in turn, resulted in higher sodium levels in Batch 4, as reflected in Table 5.4-5. Believer Meats tested the above-described updated wash parameters ahead of commissioning the Wilson, North Carolina Facility to confirm that the only analytical difference for the harvested biomass that results from the use of an additional NaCl wash cycle and the removal of the distilled water wash cycle is the increase in sodium levels reflected in Table 5.4-5. To note, there is also an increase in ash levels due to the increase in sodium that discussed below in section b.

While the levels of sodium in the biomass from Batch 4 are higher than the sodium levels in Batches 1-3, EDI levels for sodium based on consumption of 159g of biomass are 586.71 mg/day which is only 25.5% of FDA's 2,300 mg/day recommended for sodium and the 2,300 mg/day RDA in the most recent Dietary Guidelines for Americans (DGA, 2023). Believer further notes that salt (sodium chloride) is generally recognized as safe as outlined in 21 CFR 182.1 (U.S. FDA, 2024), that sodium content will be declared in the nutrition labeling of the cultivated chicken biomass and all finished products that contain Believer's cultivated poultry, and that "salt" will be declared in the ingredient declaration of the cultivated chicken biomass.

We would like to emphasize that while the sodium levels show a difference in Batch 4 compared to Batches 1-3, the levels in Batch 4 were expected, can be controlled to ensure consistency, and have no impact on our conclusion that there is no safety concern for the washed biomass. The sodium levels observed in Batch 4 align with expected levels based on calculations using the concentration of NaCl and the larger scale wash parameters, which leads us to conclude that future batches using these wash parameters will contain comparable sodium levels.

b. Increased Ash Levels in the Batch 4 Washed Biomass

Believer also notes that while the ash levels observed in Batch 4 are higher than the ash levels in Batches 1-3, the levels align with expected levels based on calculations around the increase in sodium. The increase in ash is in line with the increase in NaCl levels as both sodium and chloride ions contribute to the ash levels. Future batches using the same wash parameters will contain comparable ash levels, which we believe does not pose a safety or regulatory concern.

c. Increased Vitamin B6 Levels in Production Media

As noted above, Believer has increased the level of vitamin B6 used in the LiCl-free production media by 12.5%. We note, however, that we use Vitamin B6 in our production media solely for the purpose of supporting cell growth and proliferation and *not* for the purpose of modifying the food's nutritional profile. Thus, consistent with our response to Question 39 in the SCM, our use of Vitamin B6 in the production media falls outside the uses contemplated by FDA's fortification policy and is therefore not precluded under that policy. Believer further notes that the levels of Vitamin B6 in the washed biomass, as reflected in the analytical data in Table 5.4-5 are in line with the levels of vitamin B6 observed in the washed biomass from Batches 1-3. Since Vitamin B6 is water soluble, we expect the proportion of the Vitamin B12 removed to be directly correlated with the amount of wash solution used. Thus, consistent with our response to Question 39 in the SCM, our use of vitamin B12 does not result in the "indiscriminate addition" of nutrients to our product, nor does it affect the safety conclusions presented in the DSN.

Amended Table 5.4-5 – Excerpt of Vitamin B6 Results (mg/kg)

Parameter	**Cultured Chicken Cells – Wet Matter Basis**			
	Batch 1	**Batch 2**	**Batch 3**	**Batch 4**
Vitamin B6 (mg/kg)	1.86	0.58	0.43	0.71

References:

DGA (2023). U.S. Department of Agriculture and U.S. Department of Health and Human Services, Dietary Guidelines for Americans, 2020-2025. 9th Edition. Available at: https://www.dietaryguidelines.gov/resources/2020-2025-dietary-guidelines-online-materials.

U.S. Department of Health and Human Services. (2023, June 16). *Vitamin B6 - Fact Sheet for Health Professionals*. NIH Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/VitaminB6-HealthProfessional/

U.S. FDA (2024). U.S. Code of Federal Regulations (CFR). 21 CFR 182.1 Substances that are generally recognized as safe. Available at: https://www.ecfr.gov/current/title-21/section-182.1

RESPONSE TO FDA FOR CLARIFICATION OF QUESTIONS RELATING TO BELIEVER MEATS CULTIVATED CHICKEN SUBMISSION CCC 000039

Requests for Information to be added to the DSN

Cell Culture Process

Hazard Analysis and Process Controls

Question 1. On Page 18 of the DSN submitted on May 21, 2024, you briefly discuss the measures for controlling physical hazards, stating "Believer Meats identified and evaluated physical hazards ... monitoring the final product with either a metal detector or X-ray." We note that this statement does not provide a stepwise analysis of physical hazards and preventive controls throughout the full production process, which are identified in the SCM (Appendix A). For addition to the DSN, please expand this section to include a discussion of the stepwise hazard analysis for physical hazards and preventive controls in your production process. In addition, please confirm whether the metal detector or x-ray will be used to detect hazards in the harvested cell material or in the final food product. If the metal detector or x-ray will be used only on the final food product, and not on the harvested cell material, please explain what monitoring activities for physical hazards will be applied prior to cell harvest.

Three process steps were identified as introducing a potential physical food safety hazard. First, during media preparation there is a potential for plastic, paper, nylon, and other foreign objects from material packaging. To control this hazard, materials are visually inspected when they are weighed and added to the process. Employees are trained to add ingredients according to standard operating procedures. In addition to employing good manufacturing practices, all prepared nutrients are filtered to 0.22µm before being added to the production process.

The second process step with an identified physical hazard is the mixing step during nutrient preparation. Media and additives are mixed in a tank with a metal impeller to ensure all components are properly dissolved. Foreign objects from the environment and metal from the impeller are identified as food safety hazards. The preventive maintenance program ensures the impeller shaft and blades, and the impeller gasket are maintained in good condition. The mixer is also operated according to standard operating procedures. In addition to these good manufacturing practices, all prepared nutrients are subsequently filtered to 0.22µm before being added to the production process.

The last process step with an identified physical hazard is expansion of cells in bioreactors. Like the mixing step for nutrient preparation, the (n) bioreactor in Rehovot as well as the (n-2), (n-1), and (n) bioreactors in Wilson also have an impeller and gasket that are identified as physical hazards. The same preventive maintenance program is in place to ensure the impeller and gasket in the bioreactors are in good condition.

To clarify Believer's statement from the May 21, 2024 submission, the production process to make harvested cell material does not include metal detection or x-ray. The intended use for harvested cell material is to be combined with non-cell cultured ingredients traditionally used in food production and further processed into a final food product. As there are additional physical hazards during further processing that will be addressed in the FSIS HACCP Plan, the final food

product will be screened using a metal detector that is calibrated and verified in accordance to 9 CFR 417 for HACCP systems (USDA 2024).

Question 2. On Page 8 of the October 4, 2024, amendment to the DSN, you state, "… the two differences between the Rehovot production and Wilson production process are (1) the use of larger sized bioreactors and wash centrifuges at the Wilson, NC facility and (2) the use of more automation in transferring biomass between process steps at large scale at the Wilson, NC facility." We note that the difference in bioreactor size may require different employee operation procedures, sanitation procedures, and extra preventive controls during steps including inoculum addition, media addition, machinery handling, and sample collection. For addition to the DSN, with supporting details provided in the SCM as necessary:

a) Please identify the size difference between bioreactors used in the Rehovot, Israel facility and the Wilson, NC facility.

The (n) production bioreactors used in the Wilson, NC facility are 100 times larger than the (n) production bioreactor used in Rehovot, Israel. Additional details on the differences in the seed-train used in Wilson, NC and used in Rehovot, Israel are included in the SCM response for question 2a).

b) Please provide a summary of the differences in operational procedures, sanitation control, environmental monitoring, and other controls as applicable between the two facilities

Below, we outline the modest differences in operational procedures, sanitation controls, and environmental monitoring procedures between the Rehovot, Israel and Wilson, NC facilities. As noted below, these differences are appropriate given the increased size and scale of operations at the Wilson facility, and the limited nature of the differences will ensure consistency in the safety, identity, and regulatory status of harvested material produced at both facilities.

<u>Operational Procedures:</u>
Operationally, the Rehovot, Israel and Wilson, NC facilities follow similar principles with minor differences to account for the larger size and scale of operations at the Wilson facility. For media preparation, the process flow is the same between Rehovot and Wilson, as presented on page 3 of Appendix A. In Rehovot and Wilson, media is prepared using the same media components and formulation by weighing and mixing ingredients until they are fully dissolved in water treated by reverse osmosis. In both facilities, media is tested and adjusted for osmolarity and pH to meet the same acceptance criteria.

There are three differences in media preparation between the facilities: 1) the size of media batches, 2) storage conditions after media preparation and 3) automated controls of the mixing process. None of these operational differences has any impact on the identity, safety, or regulatory status of the media.

Media batches in Wilson will be up to 40 times larger than the batch size in Rehovot, but the media will be sterile filtered and used more immediately in Wilson due to higher throughput. Because throughput is lower in Rehovot, the holding time for media in Rehovot can be much longer for each batch of prepared media. To control for microbiological growth in prepared media in Rehovot, it is stored in single-use sterile bags after it is prepared. By contrast, prepared media in Wilson will be stored in stainless-steel, bulk holding tanks where media remains agitated and held under refrigerated temperatures to prevent microbial growth. These tanks allow for the safe storage of larger media batches while avoiding the need to rely on single-use bags to store these larger batches. These tanks will be regularly cleaned via an automated clean-in-place process that is described in more detail in the next section titled "Sanitation Controls." These modified storage conditions effectively mitigate any risks associated with increased media batch sizes and thus ensure that use of increased batch sizes at the Wilson facility do not affect the safety, identity, or regulatory status of the product.

During media preparation and mixing, there will be additional automation controls for weighing media components and mixing the components in Wilson. Examples of the automation controls in the Wilson facility include controls to verify weight accuracy of each media component added to the mixer, totalizing of raw materials added to the mixer, and automated settings for agitator speed and mixing time. These automation controls are appropriate given the increased scale of operations at the Wilson facility and they mitigate any potential risk that would otherwise be associated with scaling up the use of certain processes used at the Rehovot facility that rely on human intervention. In both facilities, media is sterile filtered prior to entry to bioreactors to 0.22µm to remove adventitious agents. Thus, use of these automation controls helps further ensure that there is no change in the identity, safety, or regulatory status of the production media or harvested material between facilities.

For WCB storage, thawing, and flask expansion, there are no operational differences between the Rehovot, Israel and Wilson, NC facilities. Qualified working cell banks are thawed using aseptic practices in a biosafety cabinet and expanded in flasks until cells reach a target density to seed the bioreactor seed-train. The acceptance criteria for cells in flask expansion is identical between the two facilities.

All bioreactors in Rehovot, Israel and Wilson, NC, both including those used in the seed train and the (n) production vessels, are operated using the same general procedures: Media, solutions of glucose and glutamine, and sodium bicarbonate to maintain the pH are connected to bioreactors using controlled pumps. All inputs are sterile filtered to 0.22µm prior to entry to the bioreactor. Following inoculation of the bioreactors, feed pumps are set to run in response to defined automated cascades where sodium bicarbonate maintains pH, and media, glucose, and glutamine are cascaded based on predicted cell growth. All bioreactors are operated as closed, sterile systems where the growth conditions are automatically controlled and monitored to exclude contamination.

Antifoam is also added to the (n-1) and (n) production vessels in both Rehovot, IL and Wilson, NC on an as-needed basis to control foam buildup. One operational difference between Rehovot and Wilson is the sterilization method for antifoam. In Rehovot, antifoam is gamma-irradiated by the supplier to be sterile while in Wilson, NC, antifoam will be heat sterilized to at least the highest reported D-value of 110° for 12.42 minutes to control for spores from *Clostridium botulinum* (FDA, 2000) prior to being added to bioreactors. Because both sterilization methods are effective at controlling adventitious agents in antifoam, this does not impact the identity, safety, or regulatory status of cell material produced at each facility.

Another difference in operations between Rehovot, Israel and Wilson, NC occurs during cell expansion in bioreactors and involves the number of bioreactors in the seed-train ahead of the (n) bioreactor and the size of the bioreactors used. In Rehovot, there is one bioreactor expansion step in the seed train before the (n) bioreactor whereas in Wilson, NC there will be four bioreactor expansion steps in the seed train before the (n) bioreactor. In addition to more bioreactors in the seed train in Wilson, the size of the bioreactors will also be larger which requires higher throughput volume of media and inputs to the bioreactor. As previously mentioned, the (n) production bioreactor in Wilson, NC will be 100 times larger than the volume of the (n) production bioreactor in Rehovot. As discussed in the next section, appropriate sanitation controls have been implemented at Wilson to account for these differences in scale. Moreover, regardless of the cell expansion operational differences, the acceptance criteria of cells throughout the bioreactor seed-train and in the (n) production vessel is the same between the two facilities. The operational differences in cell expansion in bioreactors therefore do not change the identity, safety, or regulatory status of cell material produced at each facility.

Lastly, the separation step in Rehovot and Wilson where cells are removed from the bioreactor and separated from residual media also follows the same principles. Material is removed from the bioreactor, washed with an NaCl buffer solution, and separated into 1) cell cultured chicken and 2) residual media . Both facilities use the same concentration of wash buffer and number of washes to remove residual media from cell material. In Wilson, the size of the separation equipment is larger, the amount of cell material that is removed from bioreactors is larger, and the frequency in which cell material is removed from the bioreactors is higher compared to Rehovot, Israel. Cell material produced in Rehovot and Wilson must meet the same nutritional, chemical, and microbiological specification parameters outlined in Table 5.2-1 of the May 21, 2024 DSN, and thus these differences should not affect the safety, identity, or regulatory status of the harvested material.

Sanitation Controls:

Many of the sanitation controls are the same between the two facilities with a few enhanced processes to highlight in Wilson, NC to account for the size and scale of operations at the facility. Both facilities have CIP and SIP processes that are controlled using a SCADA system and use approved food sanitation chemical suppliers. The sanitation controls in Wilson, NC include a CIP skid that automatically dilutes chemicals to a target concentration and monitors wash solution concentration via an in-line conductivity meter. This higher degree of automation and control for chemical concentrations in Wilson, NC is a process enhancement compared to the more manual chemical dilution and concentration monitoring processes in Rehovot and is appropriate for the size and scale of operations at the Wilson facility.

Additionally, both facilities have a Master Sanitation Schedule to control environmental contamination and maintain high hygienic conditions in the facility. In the media preparation area, equipment is dry-cleaned before each use. In the inoculation laboratory where the WCB vials are thawed and expanded in flasks, Sanitation Preventive Control #1 ensures the biosafety cabinet is sanitized prior to use.

As mentioned in the response to question 11 in the SCM, the (n-2), (n-1), and (n) bioreactors (note: there is no (n-2) bioreactor in Rehovot) are sterilized to 121°C for a minimum of 20C as outlined in Sanitation Preventive Control #2. In the event where sterilization temperature and holding time for a piece of equipment is not met, automated process controls in both facilities prevent the use of that equipment until the root cause is corrected and equipment is re-sterilized. While there will be more pieces of equipment in Wilson, NC that require sterilization-in-place, the acceptance criteria and monitoring procedures are the same between the two facilities.

The separation equipment in both facilities goes through regular clean-in-place cycles per Sanitation Preventive Control #3. In the event where clean-in-place criteria such as conductivity, flow rate, and contact time for each step of the CIP cycle are not met, the automated process controls in the Wilson facility will prevent the use of that equipment until the equipment is re-cleaned. This is an additional enhancement of the CIP system in Wilson compared to the more manual monitoring processes for chemical concentrations used in Rehovot.

In addition to automation controls for cleaning and sanitization processes in Wilson, NC that are not present in Rehovot, the Wilson, NC equipment is hygienically designed to be easily cleaned surfaces that prevent organic material accumulation. There are also aseptic sampling valves installed on the antifoam storage tank, (n-2), (n-1), and (n) bioreactors in Wilson, NC to allow for adequate in-process sampling without posing a contamination risk.

To summarize, the differences in sanitation controls is minimal between the two facilities. The increased level of automation of the cleaning and sanitization process, hygienic design, and aseptic sampling valves all support the larger-scale process and mitigate contamination risks in Wilson, but will not affect the identity, safety, or regulatory status of the harvested cell material.

Environmental Monitoring:

In Rehovot, Israel, the environmental monitoring plan targets total microbiological load while the plan in Wilson, NC will target total microbiological load and species-specific swabbing for *Listeria spp.* and *Salmonella spp.* A comparison of the two plans is outlined in Table 1. below. Again, these differences are appropriate given the increase in size and scale of operations at the Wilson facility, but will not affect the identity, safety, or regulatory status of the harvested cell material.

Table 1. Environmental Plan Comparison - Rehovot, Israel and Wilson, NC

Environmental Monitoring Plan Element	Rehovot, Israel	Wilson, NC	Comparison
Microorganisms of Concern	Total bacterial load	Total bacterial load *Listeria spp.* *Salmonella spp.*	Species-specific detection of environmental pathogens in Wilson, NC.
Test methods used	ATP Total Plate Count	ATP Total Plate Count Sponge swabs to be tested for *Listeria spp.* and *Salmonella spp.*	Species-specific detection of environmental pathogens in Wilson, NC.
Areas Sampled	• Air in bioreactor room and biosafety cabinets • Floors and drain locations in bioreactor room • Walls and ceilings in bioreactor room • Transition zones between high-care and low-care areas	• Air in bioreactor room and biosafety cabinets • Floors and drain locations in bioreactor room • Walls and ceilings in bioreactor room • Transition zones between high-care and low-care areas	No Difference
Sampling Frequency	ATP and Total Plate Count locations and swab frequency detailed on pages 14 and 15 from the October 4, 2024 addition to the DSN for Question10.	ATP and Total Plate Count locations and swab frequency detailed on pages 14 and 15 from the October 4, 2024 addition to the DSN for Question10. Minimum one location weekly for *Salmonella* and *Listeria spp,* each.	Increased number of swabs in Wilson, NC due to larger facility size.

c) Please provide details about the transferring of biomass between process steps at the Wilson, NC facility (e.g., briefly describe any automation used, the process steps that occur between the transfer of the biomass).

Figure 1. from question 2a) of the SCM shows the successive steps in the seed-train in Wilson, NC that is a helpful reference to supplement the following explanation for transferring biomass between process steps. First, frozen cells of chicken fibroblasts are thawed from the WCB vial to room temperature, the vial is opened in an aseptic manner, and used to inoculate a shaker flask to begin the seed-train. This operation is performed using aseptic handling practices and relies on Sanitation Preventive Control #1, Biosafety Cabinet Sanitation to prevent contamination. Once cells are expanded in shaker flasks to the target cell density, they are used to inoculate the bioreactor seed train.

The (n-4) bioreactor is single-use and sterilized from the supplier. Sterile filtered media is added to the bioreactor and the growth conditions in the (n-4) bioreactor such as the pH, dissolved oxygen, and temperature are controlled to match the requirements for the cells it will receive. Transferring biomass from the shaker flask to inoculate to the (n-4) bioreactor seed-train relies on a single-use sterile flask that is welded on to the (n-4) bioreactor receiving line using an

automated tube welder (i.e. Biowelder®) to prevent contamination during the transfer step. A peristaltic pump moves biomass from the sterile flask through sterile tubing into the bioreactor. Once cells reach a target cell density in the (n-4) bioreactor, biomass is extracted from the bioreactor and collected in a single-use, sterile bag using gravitational force.

Like the (n-4) bioreactor, the (n-3) bioreactor is also single-used and sterilized from the supplier. Sterile filtered media is added to the bioreactor and the growth conditions in the (n-3) bioreactor such as the pH, dissolved oxygen, and temperature are controlled to match the requirements for the cells they will receive. Biomass in a sterile bag from the (n-4) bioreactor is connected to the (n-3) bioreactor using an automated tube welder (i.e. Biowelder®) to prevent contamination during the transfer step. A peristaltic pump moves biomass from the sterile bag through sterile tubing into the bioreactor. Biomass is extracted from the (n-3) bioreactor using the same process as previously described for the (n-4) bioreactor and transferred to the (n-2) bioreactor using the same process as transferring into the (n-3) bioreactor. The shaker flask to (n-4) and (n-3) to (n-4) transfer process is the same procedure followed in Rehovot, Israel and has been demonstrated to control contamination when proper procedures are followed.

Transferring between stainless steel (n-2), (n-1), and (n) bioreactors involves more automation than the previously described transferring process. Sterile filtered media is added to the bioreactors and the growth conditions in the (n-2), (n-1), and (n) bioreactors such as pH, dissolved oxygen, and temperature are controlled to match the requirements of the cells they will receive. Each bioreactor, associated transfer lines, and perfusion centrifuges for the (n-1) and (n) bioreactors also must be cleaned-in-place and sterilized-in-place (Sanitation Preventive Control #2) before receiving biomass. To transfer biomass from the (n-2) or (n-1) bioreactors, sterile filtered air is added to the bioreactor to create overpressure that moves biomass to the next bioreactor in the seed-train. Removing the biomass from the (n) bioreactor is performed using a centrifugal hygienic pump that automatically transfers biomass within a closed system to the wash centrifuge to remove residual media components. Once the cultured cell material has been washed to the required criteria, washed biomass is automatically transferred within the closed system to holding tanks, where it held until it is harvested to be used in downstream further food processing operations in the FSIS regulated process.

For transfers within the (n-2), (n-1), and (n) bioreactor section of the seed train, automation software loads pre-determined production recipes from the SCADA system depending on which bioreactors biomass is being transferred from and to. To execute the recipe, interlocks are verified and signed off before the transfer begins. This transfer process will be unique to the Wilson, NC facility and was designed in conjunction with industry experts to control contamination using sanitation controls, sterile valves with steam barriers to maintain aseptic conditions inside the bioreactors, and sterile filtration on all bioreactor inputs.

Question 3. On Page 15 of the October 4, 2024, amendment to the DSN, you provide a brief description of risks and controls at the "cell bleeding and dilution (optional)" and "perfusion culture in bioreactor and rejuvenation" steps. We note there was no detailed explanation of these processes in the May 21, 2024, submission. For addition to the DSN, please list what specific biological, chemical, or physical hazards could occur at these steps, and describe how the preventive controls would mitigate the risks of these hazards.

Page 15 of the October 4, 2024 amendment to the DSN outlines the microbiological controls and testing summary that is used in the Rehovot, IL facility. Details on cell bleeding and dilution, perfusion culture, and rejuvenation were included in Appendix A from the May 21, 2024, SCM submission. As requested in Question 10 of the August 1, 2024 Request for Information, we moved to the DSN the following explanation of each process step:

> Cell bleeding and dilution - Partial removal of cell suspension from bioreactor to reduce cell density, followed by a refill with fresh media **NOTE**: Cell bleeding and dilution step is only performed in Rehovot at smaller scale operation and is not included in the process flow for Wilson (see response for Question 10 in the SCM).
>
> Perfusion Culture: Continuously perfusing culture media out of the bioreactor vessel, retaining cells via cell retention filter. The bioreactor is continuously refilled with a combination of fresh media and optionally refilled with rejuvenated media.
>
> Rejuvenation of Media: An optional water conservation step that uses a series of filtration steps to remove ammonia, lactate, and trace nutrients from used media.
>
> **NOTE:** Rejuvenation is an optional process at scale that improves efficiency by reducing water usage while maintaining consistent levels of media for cell growth. Because the media and product specifications are unchanged if rejuvenation is used, and the process remains a closed-system with the same cleaning protocols, then whether rejuvenation is activated is not expected to impact the identity, safety, or regulatory status of the process. Notwithstanding, the cell cultured material produced as part of Believer's submissions to date did not use rejuvenation and we are not using this feature during the initial launch at Wilson, NC. Before using the rejuvenation step in any commercial production at Wilson, we will revisit with the review team.

The following hazard analysis for cell bleeding and dilution and perfusion is pulled from the May 21, 2024 version of Appendix A of the SCM to show biological, chemical, and physical hazards and the associated preventive controls to mitigate risks:

Table 2. Excerpt from Table 1. Hazard Analysis Plan from Appendix A

(1) Ingredient/ Processing Step	(2) Identify potential food safety hazards introduced, controlled or enhanced at this step		(3) Do any potential food safety hazards require a preventive control?	(4) Justify your decision for column 3	(5) What preventive control measure(s) can be applied to significantly minimize or prevent the food safety hazard?	(6) Is the preventive control applied at this step?
Cell bleeding and dilution (Optional step in Rehovot, only)	B	Microbial contamination from employee hands (*S. aureus*, coliforms, *E. coli*)	No	Bioreactor system is closed and maintained aseptic. The process is performed through aseptic welding. Manual work according to hygiene SOP – using appropriate PPE. Employees are trained and their hands routinely sampled.		
	C	None				
	P	None				
Perfusion culture in bioreactor	B	Increased microbial contamination risk due to perfusion cycle.	Yes	Perfusion of media increases the risk of environmental contamination.	Process preventive control #1 - 0.22µm filtration – filtration of media. Filter integrity maintained.	Yes
	C	None				
	P	None				
Rejuvenation (Future Optional Step)	B	To be determined				
	C	To be determined				
	P	To be determined				

Question 4. For addition to the DSN, with supporting information in the SCM, as needed, please provide a hazard analysis and accompanying preventive controls for preparation of initial media, "media nutrient feed," and "media rejuvenation," depicted in Figure 4.1-1 in the May 21, 2024 DSN.

A hazard analysis and preventive controls summary was included for media preparation and media rejuvenation in Table 1. on Page 9 of the May 21, 2024 version of Appendix A in the SCM. "Media nutrient feed" preparation is described in Steps 1.01 through Steps 1.05 of Appendix A and "media rejuvenation" is described in the response to Question 3, above.

For media nutrient feed, bacterial hazards such as pathogen contamination from *Salmonella* and *E. coli* are identified from raw materials such as media components. In addition, aerobic plate count and yeasts and molds are identified as biological hazards that can accumulate in prepared media. To mitigate these biological hazards, all prepared media is filtered to 0.22µm before being used to support any cell growth. This filtration step is a process preventive control.

A chemical hazard identified from raw materials and media components is the presence of non-food grade solvents used by our suppliers. To mitigate this risk, Believer Meats' procurement only purchases media components from approved suppliers who meet internal thresholds for solvents. Materials are inspected upon receipt to ensure they are approved materials.

Physical hazards are identified and discussed in the response to Question 1. There is a potential for plastic, paper, nylon, and other foreign objects from raw material packaging to be introduced during media preparation. In addition, metal and plastic from the mixer impeller and

gasket on the mixing tank are potential physical hazards. All prepared media is filtered to 0.22µm before use which prevents these physical hazards from being introduced to bioreactors where cell material is produced.

Environmental Sampling

Question 5. On Page 17 of the October 4, 2024, amendment to the DSN, you state "Cell proliferation in large-scale bioreactors happens under aseptic conditions until biomass is removed from the reactors. Biomass exits the bioreactor and is washed in a closed system centrifuge before the harvest step." For addition to the DSN:

a) Please consider and briefly discuss other possible sources of environmental contamination caused by bioreactor vault malfunction, sterilization failure, media addition by personnel, and materials transportation.

c) Please list and describe the preventive controls you would apply to control for these hazards, or explain whether your current preventive control strategies could mitigate these risks.

We will use the agency's prompts in Question 5 to discuss the potential sources of contamination during cell expansion (5a) and discuss how the preventive control strategy addresses these hazards (5c). Table 3, below, is intended to address Question 5a) and 5c) with an additional discussion following to address question 5b). Possible causes of environmental contamination during the cell culture process fall into one of two categories: 1) human error and 2) equipment malfunction.

Table 3. Sources of Environmental Contamination and Control Strategies

Sources of Contamination Response to Question 5a)	Control Strategies and Preventive Controls Response to Question 5c)	Control Strategy	
		Pre-Requisite Program	Preventive Control
Human Error			
Contamination of inoculum during vial thaw due to unsanitary materials used or improper handling procedures followed.	- Employees are trained to use aseptic handling procedures. - All materials used to handle inoculum are sourced from approved suppliers and are sanitized prior to use.	X	
Contamination of inoculum due to Improper sanitation practices of biosafety cabinet where vial thaw and initial inoculation of shaker flasks occurs.	- Sanitation Preventive Control – Sanitization of Biosafety Cabinet before use		X
Contamination of media through employee handling during media weighing and preparation.	- Good manufacturing practices such as handwashing and wearing of personal protective equipment minimize contamination risk of media. - Media is sterile filtered to 0.22µm prior to being added to bioreactors. Sterile filtration is managed as a Process Preventive Control.	X	X
Contamination of bioreactors due to a manifold breach.	- Employees are trained to use aseptic sampling procedures - Manifold assemblies used for sampling are visually inspected for any possible welding errors	X	

Sources of Contamination Response to Question 5a)	Control Strategies and Preventive Controls Response to Question 5c)	Control Strategy	
		Pre-Requisite Program	Preventive Control
Equipment Malfunction			
Contamination of the bioreactors can occur if there is a malfunction with any valve that does not maintain the bioreactor expansion area a closed, sterile system.	- Employees are trained to use aseptic sampling procedures - Preventive Maintenance Program in place for all valves to inspect, repair, and replace on a specific frequency based on the original equipment manufacturer recommendations. - Automated alarms and notifications are in place for critical valves on stainless steel bioreactors (n-2), (n-1), and (n) in Wilson and for the (n) bioreactor in Rehovot	X	
O-rings in the supply lines, gaskets, and diaphragms in valve assemblies can become deformed, have grooves, or become damaged after extended use or from incorrect installation. These defects can lead to breaches in sterility that can cause contamination.	- Preventive Maintenance Program is in place for all O-rings, gaskets and valves to inspect and replace on a specific frequency based on the original equipment manufacturer recommendations. - Maintenance personnel are also trained on proper installation procedures.	X	
Contamination could also be caused by ineffective sterilization cycles due to time and temperature criteria not being met. - Assemblies used in (n-4) and (n-3) bioreactors are autoclaved. - Cold spots in (n-2), (n-1), and (n) bioreactors, supply lines, or perfusion centrifuges. - Loss of steam from boilers that interrupts sterilization cycle	- Each sterilization cycle for assemblies connected to bioreactors, stainless-steel bioreactors, supply lines, and perfusion centrifuges are monitored as a Sanitation Preventive Control in both facilities. - Sterilization-in-place of stainless-steel bioreactors, supply lines, and perfusion centrifuges are controlled by a SCADA system. Sterilization criteria at all temperature probes in the equipment being sterilized must meet minimum criteria of 121°C for at least 20 minutes to progress the process. - The design of bioreactors, supply lines, and perfusion centrifuges equipment that are sterilized using automated SIP programs places temperature probes in areas where cold spots are most likely to occur. - Boilers are appropriately sized to support the steam pressure needed for sterilization-in-place cycles. Additionally, steam pressure is continuously monitored to detect drops. - Aseptic sampling valves present on (n-2), (n-1), and (n) bioreactors in Wilson to verify effective sterilization	X	X
Contamination could occur if there are any breaches to in-line filters for inputs to bioreactors.	- Media, additives, and air are all sterile filtered to 0.22µm prior to being added to bioreactors. Sterile filtration is managed as a Process Preventive Control. - Preventive Maintenance Program is in place for all in-line filters to inspect and replace filters on a set frequency. - In-line pressure in either side of in-line filters is monitored as a Process Preventive Control to detect any breaches in filtration.	X	X
Breaches in sterile-welds when transferring cultured cell materials between bioreactors in the seed train can be causes of contamination (more details regarding this process are included in response for Question 2c).	- Employees are trained to follow the standard operating procedure for using the automated sterile bio welder. - Welds to bioreactors are visually inspected to detect any possible welding errors	X	

Believer Meats' Hazard Analysis appropriately identifies and mitigates sources of environmental contamination using pre-requisite programs and preventive controls as discussed in Appendix A of the SCM and as summarized in Table 3, above. In the event where a sanitation preventive control is not met, a process preventive control is not met, or a contamination event occurs, Believer Meats Quality Assurance Team initiates a corrective action and preventive action investigation (CAPA). Root cause analysis for any contamination event is an element of the CAPA investigation process. As part of the root cause analysis investigation, production records, environmental monitoring records, and sample analysis results associated with any contamination event are reviewed to identify potential equipment failures or contamination associated with human error. Based on the identified root cause for the contamination failure, elements of the food safety plan such as the hazard analysis plan, pre-requisite programs such as employee training, preventive maintenance, or sanitation, and preventive controls are reassessed and updated to prevent the failure.

b) Please consider whether contamination by other microorganisms from environmental sources (e.g., *Bacillus cereus, Staphylococcus aureus, Ralstonia insidiosa*) which could accumulate around this area, may occur and discuss how you control for the presence of these microorganisms.

Contamination from microorganisms ubiquitous to the environment such as *Bacillus cereus* and *Ralstonia insidiosa* could occur in the production environment that could contaminate food contact surfaces. In addition, *Staphylococcus aureus* contamination from human handling could occur at process steps where product is manually handled. As discussed in part a) of this request for information, the only points of the process where product is manually handled are during vial thawing, expansion in flasks, and weighing of media components.

Both the Rehovot, Israel and Wilson, NC facilities have a master sanitation schedule that maintains processing areas to a high sanitary standard and control the presence of environmental microorganisms. In addition, there is a captive shoe policy which prevents employee work boots from being worn outside the facility. Only authorized personnel are permitted in the upstream production areas where bioreactors are to minimize contamination caused by foot traffic. Employee hygiene stations including handwashing stations and personal protective gear such as hair and beard nets are present at all entrances to production areas. These good manufacturing practices are in place to prevent environmental contaminants like *Bacillus cereus* and *Ralstonia insidiosa* from being introduced to the production environment.

To control for employee-introduced contaminants like *Staphylococcus aureus*, aseptic handling practices and use of a biosafety cabinet are used for vial thaw and flask expansion to prevent exposing cells to employee-introduced adventitious agents.

Question 6. On pages 16-17 of the October 4, 2024, amendment to the DSN, you describe testing for *Listeria monocytogenes* in the Master Cell Bank (MCB). We note that the addition of a *L. monocytogenes* monitoring step for the MCB is not sufficient to address concerns related to introduction of this adventitious agent from the processing environment, as testing for *L. monocytogenes* would occur before a large portion of the production process. For addition to the DSN, please provide a robust strategy for controlling and mitigating the risk of *L. monocytogenes* contamination in the harvested cell material. We recommend that you either consider including *L. monocytogenes* in your environmental monitoring plan, with an accompanying discussion regarding how your environmental monitoring plan sufficiently controls and mitigates the risk of contamination of *L. monocytogenes* throughout the production process; or include testing of the harvested cell material and batch release specifications for *L. monocytogenes*, with accompanying citations to the analytical method employed (or a brief description of the method, if it is an internal method) and a statement that the method is validated for its intended purpose.

Our approach to controlling and mitigating the risk of *L. monocytogenes* contamination in the harvested cell material relies on three principles: exclude from production environment, maintain aseptic conditions during cell-expansion bioreactors, and maintain sanitary conditions after removal from the bioreactor during the biomass wash and harvest steps. *L. monocytogenes* is widespread in the environment and can be found in soil, water, sewage and silage (U.S. FDA, 2017). The good manufacturing programs in Wilson, NC support exclusion of all environmental pathogens, including *L. monocytogenes* from the facility. For each identified vector of contamination, mitigation strategies are in place:

Table 4. Listeria Control and Mitigation Strategy for Wilson, NC

Contamination Vector	Mitigation Strategies
General	- Each lot of WCB tested negative for *L. monocytogenes.* - *Listeria* is included in the environmental monitoring program using species specific testing.
Foot Traffic, Footwear	- Captive boot policy - Boot covers for inoculation laboratory where vial thaw and flask expansion (n-5) and expansion in the (n-4) and (n-3) bioreactors take place. - Controlled access to bioreactor room to minimize foot traffic in the area.
Water	- Source water is from City of Wilson with annual potability report. - Process water used for media preparation is treated using reverse osmosis.
Sewage	- Bathroom facilities drainage is separate from food processing waste system. - Handwashing policy in place.

In response to the review team's request for information, we have revised the DSN to reflect that *L. monocytogenes* is now included in the facility environmental monitoring plan. Environmental samples will be tested for *Listeria* spp. using Lis spp-IQ Check AOAC PTM #090701 or other validated test method for environmental samples. In the event of a positive *Listeria spp.* result, species confirmation testing for *Listeria monocytogenes* is tested using Lis Mono-IQ Check AOAC PTM #010802 or other validated test method for environmental samples.

The Lis spp-IQ Check and Lis Mono-IQ Check methods from Bio Rad are AOAC approved PCR-based methods and validated for detection of *Listeria spp* and *Listeria monocytogenes,* respectively, in environmental samples. Method validation certificates for the Bio Rad test methods are appended to the SCM.

Question 7. On page 22 of the October 4, 2024, amendment to the DSN, you state, "Because *Salmonella* and *Listeria monocytogenes* have an extensive history of being 'resident environmental pathogens' in processing facilities, they will be initially monitored as part of the environmental monitoring program for the commercial production facility in Wilson NC (FDA, 2024a)" [emphasis added]. You also state that "For test methods, we use 3MTM Clean-TraceTM ATP Surface Tests and for total plate count, we use method SI 885 part 20." For addition to the DSN:

a) **Please clarify the discrepancy in these two statements (i.e., testing for the presence of two specific adventitious agents, and the use of non-specific analytical methods) and confirm whether your environmental monitoring program includes specific analytical methods intended to test for the presence of *Salmonella* serovars and *L. monocytogenes* or whether it only includes the non-specific ATP surface test and total plate count analyses.**

   The environmental monitoring program includes testing for species specific microorganisms (including *Salmonella* serovars and *Listeria* spp) AND non-specific ATP surface test and total plate count analysis. Species-specific environmental samples will be taken from locations including drains, floors, walls, and surfaces non-adjacent to food contact surfaces in the production area. *Listeria* spp. will be first tested using Lis spp-IQ Check AOAC PTM #090701 or other validated method for environmental samples. In the event of a positive *Listeria spp.* result, species confirmation testing for *Listeria monocytogenes* is tested using Lis Mono-IQ Check AOAC PTM #010802 or other validated method for environmental samples. *Salmonella* spp. will be tested using Sal-IQ Check AOAC OMA 2017.06 or other validated method for environmental samples.

b) **We acknowledge that you indicate that the harvested cell material is tested for *Salmonella* serovars.**

c) **For *L. monocytogenes*, we note that the literature is unclear on the ability of ATP monitoring to serve as an effective indicator for this adventitious agent in a food processing environment1. If you intend to use ATP monitoring as the only monitoring method for environmental adventitious agents, including *L. monocytogenes*, please provide additional discussion, including citations to the publicly available literature, that addresses these comments. Alternatively, we recommend that you consider specifically testing for the presence of *L. monocytogenes* in the environment using an analytical method intended to test for the presence of this adventitious agent.**

   As indicated in the response to part a) of this request, *L. monocytogenes* in the environment will be monitored using a species-specific environmental test method for *Listeria* spp.

d) **Please clarify what "initially" means in this context, and whether you intend to continue environmental monitoring for *Salmonella* serovars and *Listeria monocytogenes*, and state the frequency analyses will be performed; or if you intend to discontinue environmental monitoring for these adventitious agents. Please provide justification as to whether your**

testing regime for *L. monocytogenes* sufficiently controls and mitigates its risk in your production process.

As there is no facility history for the Wilson NC, we intend to reevaluate the environmental monitoring plan after an initial baseline for the facility is established. We plan to evaluate swab locations, frequencies, and ensure we have identified the appropriate microorganisms of concern. As *Salmonella*'s growth environment is primarily warm, dry areas that are characteristic of the environment in the production phase of cell-cultivation, we do not anticipate discontinuing monitoring for *Salmonella* (U.S. FDA, 2024). *L. monocytogenes* growth environment is primarily cool, wet areas which are more common to our downstream operation where cell-material is further processed into finished food product (U.S. FDA, 2024). That said, and as reflected in our response to Question 6, we plan to include *L. monocytogenes* in our environmental monitoring plan and do not anticipate discontinuing monitoring for *L. monocytogenes* even following the reevaluation described above.

We have evaluated common contamination vectors and mitigation strategies for *Listeria* as shown in Table 4. for our response to Question 6, we have evaluated potential contamination points for each process step as part of our food safety plan, and we have included *Listeria* spp. into the facility environmental monitoring plan as discussed in our response to Question 6. Including *Listeria* spp. sampling in the facility environmental monitoring plan will allow Believer Meats to verify the effectiveness of our mitigation strategies for *Listeria* in the environment, detect *Listeria* and harborage sites in the facility, and ensure that the facility takes appropriate corrective actions to eliminate *Listeria* and harborage sites when found through environmental monitoring. This updated testing regime to include *Listeria* spp. as part of the environmental monitoring plan sufficiently controls and mitigates the risk for *Listeria* spp. in the production process.

Substances Used During Cell Culture

Safety Assessment of Media Inputs

Question 8. On page 25 of the October 4, 2024, amendment to the DSN, you provide a safety argument for media inputs used in cell line initiation. For completeness of the administrative record, we request that you also provide a list of the media inputs used for cell line establishment of the FMT-SCF-4 production cell line as described in the reference you provided, Pasitka et al. 2023.

The "Materials" subsection beginning on page 44 of the Pastika, et al. 2023 study lists both materials used in establishing the cell line and materials (e.g., analytical test kits) used to analyze the cell line that were not inputs to the cell line. All steps required to establish our Manufacturer's Working Cell Bank (MWCB) are described in section 4.3.3 and in Figure 4.3-1 in the DSN. The media inputs described in this response were used only during the initiation of the FMT-SCF-4 cell line (step 1 in Figure 4.3-1), well before the cell culture production process. Once the cell line was established, many of these inputs were subsequently removed as part of the cell line's adaptation to grow in suspension without any animal-derived material in the growth media.

The media inputs used during initiation of the FMT-SCF-4 cell line were metabolized by cells during the cell line initiation stage. Once removed, these are not carried over to the subsequent cell line nor do they affect the biology of subsequent cell lines or the eventual MWCB used during the production process. Therefore, they have no impact on the identity, safety, or regulatory status of the cultured cell material created in our manufacturing process.

During the cell line initiation step, the following media inputs were used to support cell health and provide nutrients: Dulbecco's Modified Eagle's Medium (DMEM), F-12, Oleic Acid, L-alanine, L-glutamine, Sodium Selenite, Canola Oil, Fetal Bovine Serum (FBS, Heat Inactivated). Other media inputs were used to help mitigate contamination risks during the transition from source animal to cell line (Penicillin-Streptomycin), to protect against shear stress during this time (10% Pluronic F-68), and to cryopreserve the cells (DMSO 5%). Other media inputs were used to drive cell metabolic activities (hydrocortisone), stabilize gene expression (Human Fibroblast Growth Factor 2 and Recombinant Human Insulin), and further support cell growth in a laboratory setting (TrypLE Express and Phosphate-buffered saline cell propagation tools).

The steps to remove these inputs are described in section 4.3.3 and in Figure 4.3-1 in the DSN. First, the primary Master Cell Bank (MCB) was adapted to grow in serum-free media (up to step 4 in Figure 4.3-1) and then the secondary MCB was adapted to grow in animal component-free (ACF) media (up to step 5 in Figure 4.3-1). In addition, the secondary MCB was further adapted to produce biomass without the use of recombinant growth factors in the growth media (up to step 6 in Figure 4.3-1). The production phase media formulation used to produce cultured cell material as described in the January 15, 2025 version of Appendix B in the SCM excludes serum,

animal derived components, and recombinant growth factors. Because of the many generations of cell-lines associated with the previously discussed adaptations and high dilution factors of up to 10^{-22} in the production phase, the media inputs used to establish the FMT-SCF-4 cell line are not present in and have no impact on the identity, safety, or regulatory status of cultured cell material.

Characterization of the Harvested Cell Material

Composition

Question 9. On page 27 of the October 4, 2024, amendment to the DSN, you reference a USDA chicken comparator (FDC ID: 171077) and provide data from the USDA comparator in Table 5.4-1. For completeness of the administrative record, please update Tables 5.4-3 and 7 on pages 28-30 to include data from the USDA chicken comparator. Please comment on whether levels of trans fat in the harvested cell material are safe relative to the chosen comparators (i.e., USDA FDC 171077, store-bought ground chicken and chicken breast).

The fatty acid profile for the USDA Chicken Breast Comparator has been included in the amended Table 5.4-3, below. The data is available from the U.S. Department of Agriculture FoodData Central in the SR Legacy Food FDC ID: 171077, Chicken, broiler or fryers, breast, skinless, boneless, meat only, raw (USDA ARS, 2019). The table is accessible at https://fdc.nal.usda.gov/fdc-app.html#/food-details/171077/nutrients.

Amended Table 5.4-3 - Fatty Acid Profile of Cultured Chicken Cells, Store-Bought Chicken Samples, and USDA Comparator Chicken Breast

Fatty Acid	Cultured Chicken Cells (g/100g oil)				Cultured Chicken Cells (g/100g)				Store Bought Chicken (g/100 g oil)		Store Bought Chicken (g/100g)		USDA Comparator (g/100g oil)	USDA Comparator (g/100g)
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken Sample	Chicken Breast Sample	Ground Chicken Sample	Chicken Breast Sample	Boneless, Skinless Chicken Breast	Boneless, Skinless Chicken Breast
C4:0	ND	0.035	ND	ND	ND	0.0004	ND	ND	ND	ND	ND	ND	ND	ND
C6:0	ND	0.046	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C8:0	0.058	0.543	ND	ND	0.0004	0.0056	ND	ND	0.0200	ND	0.0011	ND	ND	ND
C10:0	0.207	0.442	ND	ND	0.0013	0.0046	ND	ND	0.0350	0.0070	0.0020	0.0001	0.4630	0.015
C11:0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C12:0	12.571	5.644	1.149	2.79	0.0817	0.0587	0.0048	0.0100	0.2570	0.1720	0.0147	0.0036	0.1543	0.005
C14:0	3.158	2.655	1.114	1.589	0.0205	0.0276	0.0047	0.0057	0.6840	0.5640	0.0391	0.0117	0.5864	0.019
C14:1 c9	ND	0.053	ND	ND	ND	0.0006	ND	ND	0.1650	0.0910	0.0094	0.0019	0.1543	0.005
C15:0	0.157	0.065	0.117	ND	0.0010	0.0007	0.0005	ND	0.1160	0.1190	0.0066	0.0025	0.1235	0.004
C16:0	20.46	18.902	20.489	28.038	0.1330	0.1966	0.0861	0.1009	24.2050	24.9590	1.3821	0.5191	23.0247	0.746
C16:1 c9	2.657	4.192	3.988	3.242	0.0173	0.0436	0.0167	0.0117	5.6530	4.5410	0.3228	0.0945	4.7840	0.155

Fatty Acid	Cultured Chicken Cells (g/100g oil)				Cultured Chicken Cells (g/100g)				Store Bought Chicken (g/100 g oil)		Store Bought Chicken (g/100g)		USDA Comparator (g/100g oil)	USDA Comparator (g/100g)
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken Sample	Chicken Breast Sample	Ground Chicken Sample	Chicken Breast Sample	Boneless, Skinless Chicken Breast	Boneless, Skinless Chicken Breast
C17:0	0.102	0.055	0.06	ND	0.0007	0.0006	0.0003	ND	0.1780	0.1560	0.0102	0.0032	0.1235	0.004
C18:0	9.158	11.836	10.329	10.017	0.0595	0.1231	0.0434	0.0361	7.5840	10.1220	0.4330	0.2105	6.5123	0.211
C18:1 trans	1.841	1.803	1.946	2.668	0.0120	0.0188	0.0082	0.0096	0.5780	0.7780	0.0330	0.0162	0.3395	0.011
C18:1	36.203	39.521	46.916	42.594	0.2353	0.4110	0.1970	0.1533	36.9300	34.7700	2.1087	0.7232	33.3333	1.08
C18:2 trans	0.66	0.644	0.641	ND	0.0043	0.0067	0.0027	ND	0.2400	0.1390	0.0137	0.0029	0.0617	0.002
C18:2 all cis-9,12	4.505	3.075	0.506	9.003	0.0293	0.0320	0.0021	0.0324	18.5800	14.4400	1.0609	0.3004	18.4568	0.598
C18:3 trans	0.478	0.72	0.77	ND	0.0031	0.0075	0.0032	ND	0.0980	0.2610	0.0056	0.0054	18.4877	0.599
C18:3 all cis 6,9,12 G	ND	ND	ND	ND	ND	ND	ND	ND	0.1970	0.1350	0.0112	0.0028	0.8025	0.026
C18:3 all cis 9,12,15 ALA	0.239	0.335	0.3	0.954	0.0016	0.0035	0.0013	0.0034	0.9310	0.5280	0.0532	0.0110	0.8025	0.026
C20:0	0.308	0.351	ND	ND	0.0020	0.0037	ND	ND	0.9100	0.1820	0.0520	0.0038	0.0617	0.002
C20:1 c11	0.133	1.637	1.743	ND	0.0009	0.0170	0.0073	ND	0.3170	0.3520	0.0181	0.0073	0.5556	0.018
C20:2 all cis-11,14	ND	ND	ND	ND	ND	ND	ND	ND	0.2600	0.4300	0.0148	0.0089	0.3395	0.011
C20:3 all cis-8,11,14	0.051	0.274	0.732	ND	0.0003	0.0028	0.0031	ND	0.2450	0.6430	0.0140	0.0134	0.6790	0.022
C20:3 all cis-11,14,17	0.19	ND	ND	ND	0.0012	ND	ND	ND	0.0320	0.0500	0.0018	0.0010	0.6481	0.021
C20:4 all cis-5,8,11,14	0.306	0.226	0.163	ND	0.0020	0.0024	0.0007	ND	1.0030	2.7390	0.0573	0.0570	2.6543	0.086
C20:5 all cis-5,8,11,14,17 EPA	0.336	0.493	0.385	ND	0.0022	0.0051	0.0016	ND	0.0390	0.0700	0.0022	0.0015	0.1235	0.004
C21:0	ND	0.058	ND	ND	ND	0.0006	ND	ND	0.6260	1.5690	0.0357	0.0326	Not Reported	Not Reported
C22:0	1.683	2.088	2.728	0.425	0.0109	0.0217	0.0115	0.0015	0.0370	0.0930	0.0021	0.0019	0.1543	0.005
C22:1 n11	0.472	0.155	0.191	ND	0.0031	0.0016	0.0008	ND	0.0400	0.1170	0.0023	0.0024	ND	ND
C22:1 c11	0.154	0.446	0.349	ND	0.0010	0.0046	0.0015	ND	0.0220	0.0520	0.0013	0.0011	ND	ND
C22:1 c13	0.41	0.127	ND	ND	0.0027	0.0013	ND	ND	0.0050	ND	0.0003	ND	ND	ND
C22:2 c-13,16	0.288	0.154	0.07	ND	0.0019	0.0016	0.0003	ND	0.0210	0.0420	0.0012	0.0009	ND	ND
C22:4 all cis-7,10,13,16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.7099	0.023

Fatty Acid	Cultured Chicken Cells (g/100g oil)				Cultured Chicken Cells (g/100g)				Store Bought Chicken (g/100 g oil)		Store Bought Chicken (g/100g)		USDA Comparator (g/100g oil)	USDA Comparator (g/100g)
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken Sample	Chicken Breast Sample	Ground Chicken Sample	Chicken Breast Sample	Boneless, Skinless Chicken Breast	Boneless, Skinless Chicken Breast
C22:3 all cis-13,16,19	0.456	0.245	0.78	ND	0.0030	0.0025	0.0033	ND	0.0300	0.0940	0.0017	0.0020	Not Reported	Not Reported
C22:4 n6	ND	0.033	ND	ND	ND	0.0003	ND	ND	0.3480	0.8920	0.0199	0.0186	0.7099	0.023
C22:5 all cis-4,7,10,13,16	ND	ND	ND	ND	ND	ND	ND	ND	0.0350	0.0890	0.0020	0.0019	0.3086	0.01
C22:4 n3	ND	0.126	ND	ND	ND	0.0013	ND	ND	0.0530	0.1460	0.0030	0.0030	0.7099	0.023
C22:5 n3	0.179	0.172	0.152	ND	0.0012	0.0018	0.0006	ND	0.1460	0.4000	0.0083	0.0083	0.3086	0.01
C22:6 n3 DHAC 22:6 all cis-4,7,10,13,16,19 DHA	ND	ND	0.639	ND	ND	ND	0.0027	ND	0.0740	0.2650	0.0042	0.0055	0.2160	0.007
C23:0	0.099	0.077	0.048	ND	0.0006	0.0008	0.0002	ND	NA	ND	ND	ND	Not Reported	Not Reported
C24:0	0.348	0.314	0.511	0.509	0.0023	0.0033	0.0021	0.0018	0.0300	0.0710	0.0017	0.0015	ND	ND
C24:1 c15	2.009	1.961	2.761	1.17	0.0131	0.0204	0.0116	0.0042	0.0870	0.2840	0.0050	0.0059	ND	ND

Amended Table 7, below, shows the levels of trans fat for cultured chicken fibroblasts, store both samples, and the USDA chicken breast comparator product on a wet matter basis (g/100g). As wet matter basis is more representative of how the cultured chicken material will be used, these results are accurate comparators. The levels of trans-fat from cultured chicken fibroblasts are in-line with trans-fat levels in the store-bought samples and the USDA chicken breast comparator product and can be considered safe.

Amended Table 7. Trans Fat Acid Comparison

Parameter	Method	Cultured Chicken Fibroblasts				Comparator Chicken Products		USDA Comparator
		Batch 1	Batch 2	Batch 3	Batch 4	Ground Chicken	Chicken Breast	Chicken Breast
Trans Fat Acids (g/100g)	AOAC 996.06	0.02	0.034	0.014	0.01	0.053	0.025	0.01
Trans Fat Acids (g/100g oil)	AOAC 996.06	3.08	3.27	3.33	2.78	0.93	1.20	0.40

References

U.S. Food and Drug Administration (FDA). (29, March 2000). "Kinetics of microbial inactivation for alternative food processing technologies - overarching principles: Kinetics and pathogens of concern for all technologies." Available at: https://www.fda.gov/files/food/published/Kinetics-of-Microbial-Inactivation-for-AlternativeFood-Processing-Technologies.pdf

U.S. Food and Drug Administration (FDA). (2017, January). Control of Listeria monocytogenes in Ready-To-Eat Foods: Guidance for Industry Draft Guidance. Available at: https://www.fda.gov/media/102633/download

U.S. Food and Drug Administration (FDA). (2024, May 30). Draft guidance on Hazard Analysis and risk-based preventive controls. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-hazard-analysis-and-risk-based-preventive-controls-human-food

USDA ARS (2019). Chicken, broiler or fryers, breast, skinless, boneless, meat only, raw [food category: Poultry Products; FDC ID: 171077; NDB No: 5062]. In: FoodData Central. U.S. Department of Agriculture, Agricultural Research Service (USDA ARS). Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/171077/nutrients.

USDA (2024). *U.S. Code of Federal Regulations (CFR). Title 9 —Animal and Animal Products*. (Food Safety and Inspection Service, Department of Agriculture)). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/2021/title21.

Table of CFR Sections Referenced (Title 9—Animals and Animal Products)

Part	Section §	Section Title
417 – Hazard Analysis and Critical Control Point (HACCP) Systems	417.2	Hazard Analysis and HACCP Plan

RESPONSE TO FDA FOR CLARIFICATION OF QUESTIONS RELATING TO BELIEVER MEATS CULTIVATED CHICKEN SUBMISSION CCC 000039

Requests for Information to be added to the DSN

Believer Meats
7 March 2025

Substances Used During Cell Culture

Safety Assessment of Media Inputs

Question 1. In the January 15, 2025, amendment to the DSN, you state, "Other media inputs were used to … stabilize gene expression (Human Fibroblast Growth Factor 2 and Recombinant Human Insulin), and further support cell growth in a laboratory setting (TrypLE Express and Phosphate-buffered saline cell propagation tools)."

a) For addition to the DSN, please state whether the human Fibroblast Growth Factor 2 used upstream is recombinant or if it is extracted from human tissues. For addition to the SCM, please provide a certificate of analysis (COA) for this growth factor.

The Human Fibroblast Growth Factor 2 used upstream is a recombinant growth factor expressed in *E. coli*; it is not extracted from human tissues. An example certificate of analysis for this growth factor is appended in the SCM.

b) We note that TrypLE Express contains an enzyme. Please state whether the sequence of this enzyme is derived from humans or an agriculturally relevant species. Please note that all recombinant human proteins used at any stage of the production process, including upstream (i.e., cell isolation and cell banking) should be disclosed in the DSN.

The TrypLE Express enzyme sequence was isolated from a species of fungus and is then used in a production strain of fungus, *Pichia pastrois,* where the enzyme is expressed. While the recombinant enzyme sequence is proprietary, and therefore not disclosed by the manufacturer, the supplier provided an origin statement showing that the enzyme sequence was not derived from human or animal species. This statement and an example certificate of analysis for TrypLE Express are appended to the SCM for completeness of the administrative record.

As previously shared in the DSN submission dated 15 January 2025, recombinant growth factors and enzymes used during initiation of the cell line were metabolized by cells during the cell line initiation stage. Once removed, these are not carried over to the subsequent cell line nor do they affect the biology of subsequent cell lines, or the eventual cell bank used during the production process. Because of the many generations of cell-lines associated with the previously discussed adaptations and high dilution factors of up to 10^{-22} in the production phase, the media inputs used to establish the FMT-SCF-4 cell line are not present in and have no impact on the identity, safety, or regulatory status of cultured cell material. Furthermore, the media recipe used by Believer Meats in the production phase is free of serum, animal derived components, and recombinant growth factors.

2. On page 33 of the January 15, 2025, revised Appendix B, you classify hydroxypropyl-beta cyclodextrin as a "class 2" substance. Please note that we consider this substance to be Type 4, as it is not naturally present in food and has not been previously evaluated by FDA for use in the human food supply in the United States. For addition to the DSN, please provide an estimated daily intake (EDI) based on analytical data and full safety assessment for hydroxypropyl-beta-cyclodextrin.

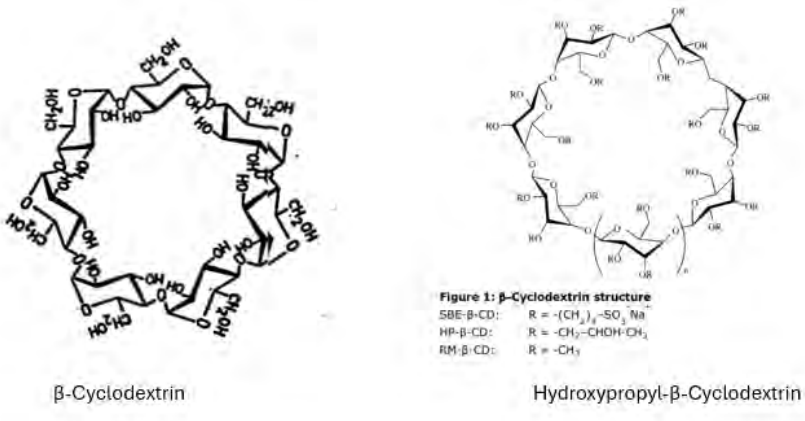
The safe use of HP-βCD in the chicken cell culture product was based on the 12-month study in which HP-βCD was fed as a dietary component to male and female Wistar rats at 500, 2000 and 5000 mg/kg bw/day (Gould and Scott, 2005). The publication indicated that a full battery of toxicological parameters was evaluated in the study. Gould and Scott (2005) stated that "at 500 mg/kg/day, there were no toxicological effects". Statements made on toxicological analyses of the higher doses (2,000 and 5,000 mg/kg bw/day) indicate that organ weights, histopathological analyses, hematology and clinical chemistry parameters were evaluated in this 12-month dietary study. Gould and Scott (2005) suggested that administration via diet may have reduced the bioavailability and maximum systemic exposure compared with oral gavage dosing. As food ingredients are consumed as part of the diet and not as a bolus dose that bypasses the salivary enzymes and other processes, administration of HP-βCD via the diet in nonclinical toxicology studies is most appropriate for evaluation of this ingredient when used as indicated. Taken together, the 12-month study is the most appropriate study to evaluate the safety of HP-βCD and 500 mg/kg bw/day is concluded as the NOAEL.

β-Cyclodextrin (βCD) is an appropriate read-across substance for HP-βCD, based on the structural, absorption, metabolism, and excretion similarities. βCD is a ring-shaped molecule made up of seven glucose units linked by α-1,4- bonds. HP-βCD structure adds hydroxypropyl groups to the D-glucopyranose units (see Figure 1) but does not alter the basic ring structure. The addition of the hydroxypropyl units does not substantially alter the absorption, metabolism, or excretion of the βCD molecule, as discussed below. The cyclic structure limits metabolism but may be hydrolyzed to maltose and glucose by gut microflora and endogenous amylase enzymes in the gut (EFSA, 2016).

A study by Gerloczy et al. (1990) was discussed in the Gould and Scott (2005) review, in which oral administration of HP-βCD to rats resulted in only 3% of the dose eliminated by the kidney in the urine and 70% in the feces within 72 hours. Similarly, Gerloczy et al. (1986) found that the radioactivity from oral administration of 14C-βCD reached maximum between the 4th and 10th hours after administration, at approximately 5% of the total administered radioactivity. Gerloczy et al. (1986) concluded that the majority of the radioactivity in the rat was from βCD metabolites in stating that "therefore, most of the blood radioactivity does not origin from 14C-βCD itself, though the absorption of a very small amount of intact βCD cannot be excluded." Recent studies by Mu et al. (2022) confirmed that "oral [cylodextrin] was mostly metabolized in the intestine, and a small part was metabolized through the kidney." The major metabolite was maltodextrin, eventually metabolized to exhaled carbon dioxide. These studies confirm that HP-βCD and βCD are absorbed, metabolized, and excreted similarly and as such the acceptable daily intake (ADI) for βCD is applicable to HP-βCD. JECFA (1995) evaluated the safety of βCD, concluding that the ADI of 0-5 mg/kg bw/day for βCD was based on a no observed effect level (NOEL) of 1.25% in the diet (equal to 470 mg/kg bw/day from a 1-year study in dogs and a safety factor of 100). A NOAEL of 1.25% in the diet was also concluded for rats that consumed βCD for 1 year, with an estimated intake at 650 mg/kg bw/day and 860 mg/kg bw/day in male and

female rats, respectively. The lower NOAEL was used for the basis of the ADI. The NOAEL cited by JECFA is similar to the NOAEL cited by Gould and Scott (2005) at 500 mg/kg bw/day from a 1-year toxicity study in rats. Overall, the data indicate that βCD is an appropriate read-across for HP-βCD.

Figure 1.



Analytical testing of three noncontinuous batches of cell cultured chicken production shows that βCD is not present above the limit of quantitation, <0.009% w/w. To calculate the estimated daily intake of βCD, two conservative assumptions were made for risk assessment purposes, only. The two assumptions are 1) Using the level of βCD present at the limit of quantitation, 0.009% w/w and 2) conservatively assuming that cultured chicken cells 100% replace all consumption of unprocessed and processed chicken products using the highest intake population group from the National Health and Nutrition Examination Survey (NHANES) survey for an estimated daily intake of cultured chicken cells as 159 g/person/day. Using the limit of quantitation results, the estimated daily intake (EDI) of βCD based on analytical testing is 14.31 mg/day which equates to 0.24 mg/kg body weight per day in a 60kg adult. Comparing this intake against the previously discussed safety studies, the EDI is over 2,000-fold below the NOAEL reported in the chronic rodent toxicity study. In addition, the daily intake of βCD has been estimated to be 2 mg/kg bw/day at the 90th percentile consumers-only use level (GRN 74 – US FDA, 2001). This EDI is over 8 times lower than the estimated daily intake of total dietary consumption of βCD.

Adventitious Agent Hazard Assessment

Hazard Analysis and Process Controls

Question 3. In the January 15, 2025, amendment to the DSN, you state "… the cell cultured material produced as part of Believer's submissions to date did not use rejuvenation and we are not using this feature during the initial launch at Wilson, NC. Before using the rejuvenation step in any commercial production at Wilson, we will revisit with the review team." Therefore, we did not consider this production step during our evaluation of your final submission. Please note, we are not requesting a response to our statement.

References

EFSA (2016) European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, *et al*. Scientific opinion on the re-evaluation of β-cyclodextrin (E 459) as a food additive. *EFSA Journal*;14(12):4628, 44 pp. doi:10.2903/j.efsa.2016.4628.

Gerloczy A, Antal S, Szatmari I, Muller-Horvath R, Szejtli J. (1990). Absorption, distribution and excretion of 14-C; labelled HP-β-CD in rats following oral administration. In: Duchene, D. (Ed.), Minutes of the 5th International Symposium on Cyclodextrins. Editions de Sante. Paris. pp. 507–513.

Gould S and Scott RC (2005). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): A Toxicology Review. Food Chem Tox; 43:1451-1459.

JECFA (1995). Beta-Cyclodextrin. Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives. FAS 35-JECFA 44/257.

Mu K, Jiang K, Wang Y, Zhao Z, Cang S, Bi K *et al*. (2022). The Biological Fate of Pharmaceutical Excipient β-Cyclodextrin: Pharmacokinetics, Tissue Distribution, Excretion, and Metabolism of β-Cyclodextrin in Rats. *Molecules*; 27:1138. https://doi.org/10.3390/molecules27031138

U.S. FDA (2001) GRAS Notice No. GRN 74 [Beta-Cyclodextrin, Adrian (MI): Wacker Biochem Corp.]: U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of food Additive Safety. Available at: https://wayback.archive-it.org/7993/20171031060203/https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM261320.pdf [Oct 25, 2001 – FDA response – no questions].

RESPONSE TO FDA FOR CLARIFICATION OF QUESTIONS RELATING TO BELIEVER MEATS CULTIVATED CHICKEN SUBMISSION CCC 000039

Requests for Information to be added to the DSN

Believer Meats
20 March 2025

Substances Used During Cell Culture

Safety Assessment of Media Inputs

Question 1. In Question 2 of the December 20, 2024, RFI, we requested that you provide an estimated daily intake (EDI) for hydroxypropyl-β-cyclodextrin (HP-βCD), a compound used in the production media that, for the purposes of our program, we classify as a "Type 4" substance. We requested that the EDI be based on analytical measurements in the harvested cell material. In the March 7, 2025, amendment, your response relied on analytical data for β-cyclodextrin (β-CD), reporting levels in the harvested cell material as "<LOQ." However, measurements for β-CD do not substitute for measurements of HP-βCD. Therefore, please confirm, for inclusion in the DSN, whether HP-βCD was quantitatively measured in three nonconsecutive batches of the harvested cell material and provide the resulting EDI for HP-βCD based on these measurements.

For addition to the SCM, please provide corresponding certificates of analysis (COAs) for each batch.

We have confirmed with our testing laboratory that their analytical testing method quantifies hydroxypropyl-beta-cyclodextrin (HP-βCD), *not β-CD*. This method was described in the method validation report for HP-βCD analysis on Pages 214-231 of the SCM file dated October 4, 2024. After further review with the testing laboratory, we confirmed that the analysis for Batch 4 (Run 421) was not correctly labeled on the certificate of analysis. Specifically, the analysis should have indicated that the results were for HP-βCD, not β-CD. This COA for Batch 4 has been corrected and provided in the SCM. This is solely a typographical oversight and does not affect the underlying data or our safety analysis. Analytical results from batches 5, and 6 (Runs 426 and 430, respectively) were properly labeled to indicate results were for HP-βCD and are also attached to the SCM. As a result of the oversight on the COA for Batch 4, the EDI and safety narrative included from our response to Question 2 in the March 7, 2025 DSN amendment states that the analytical results from three non-consecutive batches were for β-CD when in actuality the results were for HP-βCD. Again, this was a typographical issue and does not affect the underlying data or our safety analysis.

The analytical test method validation report and updated certificate of analysis reports are included in the SCM amendment dated March 20, 2025 showing results for HP-βCD for three nonconsecutive batches of harvested cell material. Please see Table 1, below, with analytical results for HP-βCD:

Table 1. HP-βCD Results Summary

	Batch 4 (Run 421)	**Batch 5 (Run 426)**	**Batch 6 (Run 430)**
COA Report Number	P24-08493	P24-10241 (Page 1 of 2)	P24-10241 Page (2 of 2)
HP-βCD Results	<0.009 (% w/w) (equivalent to <90ppm)	<90 ppm (equivalent to <0.009 (% w/w))	<90 ppm (equivalent to <0.009 (% w/w))

For completeness of the administrative record, we have amended the EDI calculation and safety conclusion from our response to Question 2 from the March 7, 2025, DSN reflecting the analytical results of HP- βCD, below:

> Analytical testing of three noncontinuous batches of cell cultured chicken production shows that HP-βCD is not present above the limit of quantitation, <0.009% w/w. To calculate the estimated daily intake of HP-βCD, two conservative assumptions were made for risk assessment purposes, only. The two assumptions are 1) Using the level of HP-βCD present as the limit of quantitation, 0.009% w/w and 2) conservatively assuming that cultured chicken cells 100% replace all consumption of unprocessed and processed chicken products using the highest intake population group from the National Health and Nutrition Examination Survey (NHANES) survey for an estimated daily intake of 159 g/person/day. Using the limit of quantitation results, the estimated daily intake (EDI) of HP-βCD based on analytical testing is 14.31 mg/day which equates to 0.24 mg/kg body weight per day in a 60kg adult. Comparing this intake against the previously discussed safety studies, the EDI is over 2,000-fold below the NOAEL reported in the chronic rodent toxicity study. In addition, the daily intake of βCD has been estimated to be 2 mg/kg bw/day at the 90th percentile consumer–only use level (GRN 74 – US FDA, 2001). This EDI for HP-βCD is over 8 times lower than the estimated daily intake of total dietary consumption of βCD.

RESPONSE TO FDA FOR CLARIFICATION OF QUESTIONS RELATING TO BELIEVER MEATS CULTIVATED CHICKEN SUBMISSION CCC 000039

Requests for Information to be added to the DSN

Believer Meats
28 May 2025

Overview

1. **We recognize that you aim to scale-up your production process to include the use of a 2,000 L and 20,000 L bioreactor, but are not yet operational at this capacity. Considering that it is possible that the control and mitigation strategies for the identified hazards may not be the same as you scale up from smaller-stage expansion steps to large scale production, we did not consider large scale production in the context of our evaluation. As such, we encourage you to submit a supplement for your large-scale production process once operational to ensure that all appropriate hazards have been identified, and the appropriate control and mitigation strategies are in place.**

   As part of this consultation, we submitted amendments dated January 15, 2025, March 7, 2025, and March 20, 2025, that discussed the ways in which the control and mitigation strategies for the hazards identified in our submission apply to our large-scale production process. As reflected in these amendments, we do not expect an increase in scale to affect the safety, identity, or regulatory status of the material produced according to the process outlined in our submission.

   The information that we've provided as part of this consultation reflects the known hazards and mitigation strategies from the Rehovot, IL site, as well as foreseeable hazards and mitigation strategies for the large-scale process in our Wilson facility. Cell material produced in either Rehovot or Wilson must meet the same nutritional, chemical, and microbiological specification parameters outlined in Table 5.2-1 of the May 21, 2024 DSN. Accordingly, at this time, we are not aware of any unidentified hazards that would necessitate the filing of a supplement for our large-scale operations.

   As the Wilson facility is commissioned, we expect to generate additional data from the 2,000L and 20,000L bioreactors that we will assess to further ensure the product meets the safety criteria outlined in the CCC submission. If any new hazards are discovered during the commissioning stage that are not adequately covered by our food safety plan or elsewhere in our submission, or if there are significant changes to the manufacturing process that impact the safety, identity, or regulatory status of the product, we commit to notifying the FDA, and, if necessary, filing a supplement that would address those hazards and changes as part of our overall commitment to food safety and quality.

Requests for Clarification

2. **In Table 4.3.3-1 on page 22 of the DSN, you list "RT-PCR (in-house)" as the methodology for detecting tropomyosin expression in the MWCB. Then, in Table 5.2-1, on page 35 of the DSN, you list "qRT-PCR" as the methodology for detecting tropomyosin in the harvested cell material. We note that "RT-PCR" is commonly used as an abbreviation for "reverse transcriptase PCR," but you define "RT-PCR" as "real-time PCR." Further, "qRT-PCR" is commonly used as an abbreviation for "real-time quantitative reverse-transcription PCR," but you define it as "quantitative real-time PCR." As real-time PCR is typically conducted on a DNA (or cDNA) template, and the assay for detecting tropomyosin expression would begin with an RNA template, please clarify whether real-time PCR, reverse transcription PCR, or real-time quantitative reverse-transcription PCR was used to detect tropomyosin expression in the master working cell bank (MWCB).**

   For the detection and quantitation of tropomyosin, Believer Meats utilizes an in-house method based on Real-time quantitative RT-PCR (RT-qPCR). RNA is purified from cells and converted into cDNA by a reverse-transcription reaction. cDNA is then quantitively measured in a real time PCR reaction.

3. **On page 43 of your May 21, 2024, DSN, you set the specification for total plate count in the harvested cell material at <5000 CFU/g. Please consider lowering your specification as low as reasonably possible, in line with the batch data presented which demonstrates <10 CFU/g.**

   The specification for cultured chicken fibroblasts from Table 5.2-1 from the May 21, 2024, DSN is for material that has undergone the wash step to remove residual media components, not for pre-wash cultured chicken fibroblasts directly from the aseptic part of the process. The equipment used for the wash process is not part of the aseptic system, but it is a closed system that is cleaned via an automated CIP system controlled via SCADA to maintain high-hygienic conditions. Details related to the Sanitation Controls for the harvest centrifuge were shared on Page 13 of the October 4, 2024, amendment to the DSN. The CIP cycles are documented and monitored by chemical concentration, time, and flow rate. CIP effectiveness is verified through testing of CIP rinse water for total plate count and testing of the biomass for total plate count CIP process for the harvest centrifuge.

   The microbiological specification for Total Plate Count of <5,000 CFU/g for material that has been washed using an NaCl wash buffer to remove residual media components is based on commercial food safety standards and is an appropriate indicator that hygienic conditions were maintained during non-aseptic wash steps. We determined that it was appropriate to review the limits for pasteurized fluid milk products as an appropriate reference point for safety. The

Pasteurized Milk Ordinance for Grade A pasteurized milk products sets a bacterial limit of <20,000 CFU/g for standard plate count (NCIMS, 2023). Our total plate count specification of <5,000 CFU/g falls far below this <20,000 CFU/g limit and is therefore a highly conservative specification that will ensure the safety of cell cultured chicken. As we commission the large-scale process and wash step in Wilson, we will review microbiological testing data and, if warranted, make adjustments to the specifications for total plate count.

4. **On page 43 of the May 21, 2024, DSN, you list "*Staphylococcus coagulase*" under "specification parameter." We note that "*Staphylococcus coagulase*" is not a genus-species designation. For addition to the DSN, please clarify whether this refers to coagulase-positive staphylococci, or something else.**

   The test method referenced in Table 5.5.1-1 from the May 21, 2024, DSN for Staphylococcus coagulase, SI 885 Part 6, measures coagulase-positive staphylococcus aureus (Standards Institution of Israel, n.d.).

5. **On page 7 of the October 4, 2024, amendment to the DSN, you list "Candida" as an adventitious agent that is tested for in the primary isolated cells. For addition to the DSN, please clarify whether this refers to the genus, generally (i.e., *Candida* spp.), or whether it refers to a particular species.**

   "Candida" listed on Page 7 of the October 4, 2024 amendment to the DSN refers to the genus *Candida* spp., generally. The Certificate of Analysis for this test showing *Candida* spp. Is included on page 7 from the October 4, 2024 amendment to the SCM.

6. **For addition to the DSN, please clarify whether antifungal agents are used during any stage of the production process.**

   Antifungal agents are not used in any stage of the production process.

7. **On page 22 of the October 4, 2024, amendment to the DSN, you write "Historical swab data from the Rehovot, IL facility is a valuable tool to use as a baseline for the environmental monitoring plan in Wilson, NC. As the large-scale operation in Wilson, NC is commercialized, we will continue to assess the frequency and exact swab locations and make appropriate modifications." You then list four "general environmental sampling locations from Rehovot that will be adapted as appropriate to the operation in Wilson, NC." On the Table 1 of Page 7 of the January 15, 2025, amendment, you indicate that there is no difference between these two facilities in terms of testing locations, and also state that the ATP and total plate count locations as well as swab frequency were detailed on pages 14 and 15 in the October 4, 2024, amendment. However, it is not clear from the table what frequency is applied for the four**

locations you identified for environmental monitoring (Air in bioreactor room and biosafety cabinets; Floors and drain locations in bioreactor room; Walls and ceilings in bioreactor room; and Transition zones between high-care and low-care areas). For addition to the DSN, please identify what the testing frequency of these environmental locations were at the Rehovot, Israel facility and describe your approach for modifying the testing frequency for the Wilson, NC facility other than increasing the number of swabs due to larger size of the production facility.

At the Rehovot site, monitoring of air in biosafety cabinets and bioreactor rooms is performed immediately prior to designated sensitive activities such as vial thaw or passaging cells in flasks. Walls, ceilings, floors, drains, and transition zones between high-care and low-care areas are sampled on a monthly basis. Monitoring at the Wilson site will be conducted in the same functional areas, i.e. biosafety cabinets, bioreactor rooms, and walls, ceilings, floors, drains, and transition areas between high-care and low-care areas. The primary difference will be the frequency with which these functional areas are tested, which will be adjusted based on environmental trends, observed contamination levels, and process-specific risk factors.

During the early stages of production, environmental sampling for air in the biosafety cabinets and in the bioreactor room will still be conducted immediately prior to sensitive activities being performed with the frequency adjusted over time based on trend analysis and risk evaluation. Operational characteristics will also inform sampling intervals: for example, flask expansion in biosafety cabinets, which involves significant manual handling, will require more frequent monitoring relative to the number of production days, whereas bioreactor cultivation, being more automated and maintained as a closed system, may justify a lower sampling frequency. Environmental sampling for walls, floors, ceilings, drains, and transition areas between low-care and high-care areas will be conducted on a weekly basis to establish an environmental baseline for the Wilson site.

We apply a risk-based approach to defining environmental monitoring frequencies. While the difference in facility size is an important consideration, we also evaluate additional risk factors, including equipment usage patterns, personnel and material traffic, and unique aspects of each site's layout. These parameters guide the assignment of appropriate EM frequencies across critical monitoring locations.

Across all areas, monitoring data will be continuously trended to detect deviations from alert or action limits and shifts in microbial flora. EM activities are conducted by trained personnel using calibrated instruments, with microbiological testing performed by a qualified third-party laboratory for incubation, enumeration, and identification. The response to question 7 from the January 15, 2025, amendment to the DSN includes additional details related to analytical methods for the environmental monitoring plan for the Wilson, NC facility.

8. **On page 9 of the January 15, 2025, amendment to the DSN, you write, "Notwithstanding, the cell cultured material produced as part of Believer's submissions to date did not use rejuvenation and we are not using this feature during the initial launch at Wilson, NC." However, on page 35 of the May 21, 2024, DSN, you write, "The production runs were performed in bioreactors supported by media rejuvenation." For the administrative record, please confirm whether batch data was collected on batches produced using the media rejuvenation step.**

   Media rejuvenation was not used for any batches produced. This reference to rejuvenation in the May 21, 2024, DSN was an error and we confirm the statement from the January 15, 2025, DSN is accurate - cell cultured material produced as part of Believer's submissions to date did not use rejuvenation and we are not using this feature during the initial launch at Wilson, NC.

9. **For addition to the DSN, please provide a statement confirming that authorized food contact materials are used throughout your production process.**

   Food contact materials used throughout our production process are authorized to be used for the food type and conditions of use from Appendix V of the FDA Guidance for Industry: Preparation of Premarket Submissions for Food Contact Substances (FDA, 2018).

10. **On page 2 of your March 20, 2025, amendment to the SCM, you confirmed that recombinant proteins were produced in organisms that are non-pathogenic and non-toxigenic. Please confirm whether this statement on non-pathogenicity and non-toxigenicity may be disclosed.**

    Yes, this statement may be disclosed.

11. **For addition to the DSN, please provide a statement about the similarity of the sequences of recombinant human insulin and FGF2 to their homologues in agriculturally relevant species.**

    Mature process insulin of human, bovine and porcine origins consists of a mature two-chain insulin of identical overall length; differences lie only at the substituted residues shown below, which are all present in the final processed hormone (Sanger, 1951) (Sanger, 1953). Porcine insulin differs from human insulin by a single amino acid: alanine replaces threonine at B30. Bovine insulin differs from human insulin by three amino acids: A8 Thr → Ala, A10 Ile → Val, and B30 Thr → Ala (Bell, 1980), (Chance, 1968).

    Bovine and porcine FGF2 amino acid sequences are 100% identical. Bovine, porcine, and human FGF2 amino acid sequences are 155 amino acids long. T121S and S137P are the only amino acid

differences between human FGF2 and bovine or porcine FGF2 making them 99% identical (Katsahambas, 1996).

12. **For addition to the DSN, please provide a certificate of analysis (COA) for the recombinant human insulin used during cell line initiation. Please also move the COA for recombinant human FGF-2 to the DSN.**

    The Certificate of Analysis for recombinant human recombinant insulin is appended as pages 12 and 13 of this amendment to the DSN. Note that the production phase media formulation used to produce cultured cell material as described in the January 15, 2025, version of Appendix B in the SCM excludes serum, animal derived components, and recombinant growth factors. Because of the many generations of cell-lines associated with the previously discussed adaptations and high dilution factors of up to 10^{-22} in the production phase, the media inputs used to establish the FMT-SCF-4 cell line are not present in and have no impact on the identity, safety, or regulatory status of cultured cell material.

    The COA for recombinant FGF-2 has been moved to the DSN and is appended as page 14 of this amendment.

13. **For addition to the DSN, please clarify whether the serum-containing medium used during cell isolation and establishment of the primary master cell bank contained recombinant proteins derived from the human genome (rHP, i.e., insulin and FGF2), or if rHPs were only used in the serum-free medium.**

    Serum-containing medium used during cell isolating and establishment of the primary master cell bank did not contain recombinant proteins derived from the human genome. rHPs were used in serum-free medium for the secondary master cell banks. Tertiary cell banks from which the MWCB are derived do not contain rHPs, serum, or animal components.

14. **On page 28 of the May 21, 2024, DSN, you state, "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." On page 18 of the January 15, 2025, amendment to the DSN, you do not list bovine catalase, porcine trypsin, or bovine serum albumin as media components used in cell line initiation. For addition to the DSN, please clarify whether you used bovine catalase, porcine trypsin, or bovine serum albumin to establish the cell line. If not, please explain what animal components were removed in the "animal-component free" media used to establish the MWCB.**

    To clarify the statement from page 28 of the May 21, 2024, DSN, porcine trypsin was not used during the manufacturing process and is also not a media component used in cell line initiation. The trypsin used in cell line initiation is TrypLE Express enzyme sequence originated from a species of fungus and then the enzyme is expressed using a strain of fungus, *Pichia pastrois.* A safety assessment and representative certificate of analysis for TrypLE Express enzyme were included in the March 7, 2025 amendment to the DSN for Question 1(b).

    The three animal-derived media components that were removed in the "animal-component free" media are bovine catalase, bovine serum albumin (BSA), and fetal bovine serum (FBS). FBS was previously discussed on page 18 of the January 15, 2025 amendment to the DSN as it was used in step one from Figure 4.3-1 from the May 14, 2024 DSN. Bovine catalase and BSA were used in steps three and four from Figure 4.3-1 for the primary and secondary master cell banks. Step five from Figure 4.3-1 shows the point in cell line establishment where cells are adapted to the "animal-component free" media where bovine catalase, BSA, and FBS are no longer used.

15. **We note that Figure 4.3-1 on page 19 of the May 21, 2024, DSN appears to depict the primary master cell bank (MCB) being formed from cells that are adapted to suspension culture, and the secondary MCB being formed out of cells that are adapted to growth in a serum-free medium. On page 26 of the October 4, 2024, amendment to the DSN, you state, "First, the primary MCB was adapted to grow in serum-free media (up to step 4 in Figure 4.3-1), eliminating the need for FBS. Next, the secondary MCB was adapted to grow in animal component-free (ACF) media (up to step 5 in Figure 4.3-1). In addition, the secondary MCB was further adapted to produce biomass without the use of recombinant growth factors in the growth media (up to step 6 in Figure 4.3-1)." We have interpreted this to mean that the primary MCB is created and stored using a serum-containing medium, and then a subset of primary MCB cells are adapted to grow in a serum-free medium to create the secondary MCB. It is our understanding that the secondary MCB is stored in a serum-free medium that contains animal components and recombinant growth factors, but that a subset of cells from the secondary MCB are adapted to grow without these components and then used to create**

the MWCB. Please confirm whether our understanding of the figure and your statement is correct.

You are correct in stating that the primary MCB is created and stored using a serum-containing medium, and then a subset of primary MCB cells are adapted to grow in a serum-free medium to create the secondary MCB. The secondary MCB is stored in a serum-free medium that contains animal components and recombinant growth factors.

Additionally, a subset of cells from the secondary MCB are adapted to grow without animal components and without recombinant growth factors. This tertiary subset of cells is stored in serum-free, animal-component-free, and recombinant growth factor-free media. MWCB is created from this subset of cells and tested to meet the specifications in Table 4.3.3-1 of the May 21, 2024 version of the DSN.

16. **For addition to the DSN, please provide a discussion on the allergenicity of albumin, estimate exposure to albumin in the harvested cell material originating from the use of fetal bovine serum and/or bovine serum albumin (BSA) upstream, and compare the theoretical estimated daily intake (EDI) to potential allergenicity thresholds to ensure the safe use of these substances. You may use peer-reviewed publications, such as Zhu, J., Pouillot, R., Kwegyir-Afful, E. K., Luccioli, S., & Gendel, S. M. (2015). A retrospective analysis of allergic reaction severities and minimal eliciting doses for peanut, milk, egg, and soy oral food challenges. Food and Chemical Toxicology, 80, 92-100, for identifying possible thresholds. Alternatively, if BSA was not used at any stage, please clearly state this.**

Egg protein, or albumin, the estimated ED_{10} value, or the lowest dose which induces allergenic response with an incidence or magnitude of 10% effect level above background data, for egg allergic individuals was 3.7mg protein (Zhu, 2015).

Bovine Serum Albumin and Fetal Bovine Serum were used in early stages of cell-line establishment but are not included in the media used to establish the Master Cell Bank (MCB) or Manufacturer Working Cell Bank (MWCB) used to produce cell-cultured chicken. As previously shared in responses to questions related to media components used in cell-line establishment, the dilution factor between materials presents in the MWCB and cell-cultured chicken is 10^{-22}. To evaluate the allergenicity of albumin from BSA and FBS, a few very conservative assumptions are made to calculate the Estimated Daily Intake of albumin.

Albumin Calculation from FBS:

- Albumin levels are 22.5mg/mL
- Maximum usage of FBS is 15% in media
- Maximum Estimated Albumin from FBS – 3.375mg/mL

Albumin Calculation from BSA:

- Albumin levels are assumed to be 100% of BSA
- Maximum usage of BSA in media is 2.5mg/mL
- Maximum Estimated Albumin from BSA – 2.5mg/mL

Total Albumin from FBS and BSA = 3.375mg/mL + 2.5mg/mL = 5.875mg/mL

As previously stated throughout the safety assessment, all animal-derived media components were removed from the media recipe beginning at the secondary master cell bank. As previously shared in our response to Question 8 from the January 15, 2025 amendment to the DSN, any reagent used in the cell line development process would be diluted to at least 10^{-22} in the production phase of cultured chicken cells. For FBS and BSA, this is a very conservative estimate since it does not consider additional dilution factor from the secondary master cell bank to the working cell bank.

Using 10^{-22} as a conservative dilution factor, the total albumin estimated to be present in cultured chicken cells is 5.875^{-22} mg/ml in the final biomass. To estimate the daily intake of albumin from cultured chicken cells, a daily intake of 159g/person/day, which is based on eaters-only data from the National Health and Nutrition Examination Survey (NHANES), is used. The estimated daily intake of albumin from cell cultured chicken is 9.34^{-20} mg per day which is well over 100,000-fold lower than the ED_{10} value of 3.7mg for egg protein.

References

Bell, G. I., Pictet, R. L., Rutter, W. J., Cordell, B., Tischer, E., & Goodman, H. M. (1980). *Sequence of the Human Insulin Gene*. Nature News. https://www.nature.com/articles/284026a0

Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). 2024. National Health and Nutrition Examination Survey (NHANES): 2017-2018. Hyattsville (MD): Available at: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2017.

Chance, R. E., Ellis, R. M., & Bromer, W. M. (1968, July 12). *Porcine Proinsulin: Characterization and Amino Acid Sequence*. Science. https://www.science.org/doi/10.1126/science.161.3837.165

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U.S. Food and Drug Administration (FDA). (2018, September 20). *Chemistry recommendations for submissions of Food Contact substances*. . https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-preparation-premarket-submissions-food-contact-substances-chemistry#avSanger, F., & Tuppy, H. (1951, September). *The amino-acid sequence in the phenylalanyl chain of insulin. i. the identification of lower peptides from partial hydrolysates*. The Biochemical Journal. https://pubmed.ncbi.nlm.nih.gov/14886310/

Sanger, F., & Thompson, E. (1953). *The amino-acid sequence in the glycyl chain of insulin. i. the identification of lower peptides from partial hydrolysates*. The Biochemical Journal. https://pubmed.ncbi.nlm.nih.gov/13032078/

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BIOTON

KONTROLA JAKOŚCI
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ŚWIADECTWO ANALIZY
CERTIFICATE OF ANALYSIS

Nr świadectwa *Certificate No.*	**1728-2021-C**	Data wydania *Date of issue*	**2021-12-10**

Produkt *Product:* **Recombinant human insulin**

Nr serii/ szarży *Batch No.* **21-07-068G**

TEST	WYMAGANIA wg *REQUIREMENTS of* SP/23 wyd. E	WYNIKI *RESULTS*
Postać *Characters*	Biały lub prawie biały proszek *White or almost white powder*	Biały proszek *White powder*
Tożsamość *Identification*		
Test A	Na chromatogramie występuje pik o czasie retencji substancji referencyjnej insuliny ludzkiej *The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution*	Na chromatogramie występuje pik o czasie retencji substancji referencyjnej insuliny ludzkiej *The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution*
Test B	Profil chromatogramu jest zgodny z profilem chromatogramu substancji referencyjnej insuliny ludzkiej *The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution*	Profil chromatogramu jest zgodny z profilem chromatogramu substancji referencyjnej insuliny ludzkiej *The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution*
Zanieczyszczenia o masie cząsteczkowej większej niż insulina *Impurities with molecular masses greater than that of insulin*	≤ 1.0%	0.1%
Zawartość cynku *Zinc*	≤ 1.0% w przeliczeniu na substancję suchą */ calculated with reference to the dried substance*	0.4%
Proteiny pokrewne *Related proteins*		
– A21 dezamido insulina *A21 desamido insulin*	≤ 2.0%	0.3%
– Suma (bez A21 dezamido insuliny) *Total (without A21 desamido insulin)*	≤ 2.0%	0.7%
Zawartość insuliny ludzkiej *Assay*	95.0% - 105.0% w przeliczeniu na substancję suchą */ calculated with reference to the dried substance*	101.4%
Strata masy po suszeniu *Loss on drying*	≤ 10.0%	3.6%
Popiół siarczanowy *Sulphated ash*	≤ 2.5% w przeliczeniu na substancję suchą */ calculated with reference to the dried substance*	0.7%
Jednołańcuchowy prekursor *Single chain precursor*	≤ 0.05%	< 0.05%
Zawartość DNA *Residual DNA*	≤ 10 pg/mg w przeliczeniu na substancję suchą */ calculated with reference to the dried substance*	< 1 pg/mg (< LOQ)
Zawartość białek E. coli (HCP) *Residual HCP*	≤ 10 ppm w przeliczeniu na substancję suchą */ calculated with reference to the dried substance*	< 5 ppm (< LOQ)
Zawartość karboksypeptydazy B *Residual CPB*	≤ 5 ppm w przeliczeniu na substancję suchą/ *calculated with reference to the dried substance*	< 2.5 ppm (< LOQ)

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ŚWIADECTWO ANALIZY
CERTIFICATE OF ANALYSIS

Nr świadectwa *Certificate No.*	**1728-2021-C**	Data wydania *Date of issue*	**2021-12-10**

Produkt *Product:* **Recombinant human insulin**

Nr serii/ szarży *Batch No.* **21-07-068G**

TEST	WYMAGANIA wg *REQUIREMENTS of* SP/23 wyd. E	WYNIKI *RESULTS*
Endotoksyny bakteryjne *Bacterial endotoxins*	< 10 IU/mg	< 10 IU/mg
Czystość mikrobiologiczna *Microbial purity*		
- TAMC	≤ 100 CFU/g	< 1 CFU/g
- TYMC	≤ 10 CFU/g	< 1 CFU/g

Orzeczenie: Próba/Substancja spełnia wymagania specyfikacji SP/23 wyd. E.
Statement: The sample/ active pharmaceutical ingredient meets requirements of SP/23 ed. E.

W trakcie wykonywania badań nie stwierdzono wyników poza specyfikacją. / *No OOS results were found during analytical tests.*

Sporządził: *Prepared by:*

Zatwierdził: *Approved by:*

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Part of Thermo Fisher Scientific

Certificate of Analysis (CoA)

Recombinant Human FGF-basic (154 a.a.)

Catalog# 100-18B
Lot# 090908-1
Source: *E.coli*

Expiration Date: December 2027
Manufacturing Site: Rocky Hill, NJ, USA

Synonyms: Fibroblast Growth Factor-basic, FGF-2, HBGF-2, Prostatropin

Description: FGF-basic is one of 23 known members of the FGF family. Proteins of this family play a central role during prenatal development, postnatal growth and regeneration of a variety of tissues, by promoting cellular proliferation and differentiation. FGF-basic is a non-glycosylated, heparin-binding growth factor that is expressed in the brain, pituitary, kidney, retina, bone, testis, adrenal gland, liver, monocytes, epithelial cells and endothelial cells. FGF-basic signals through FGFR 1b, 1c, 2c, 3c and 4. Recombinant Human FGF-basic is a 17.2 kDa protein consisting of 154 amino acid residues.

Sequence:
AAGSITTLPA LPEDGGSGAF PPGHFKDPKR LYCKNGGFFL RIHPDGRVDG VREKSDPHIK LQLQAEERGV VSIKGVCANR
YLAMKEDGRL LASKCVTDEC FFFERLESNN YNTYRSRKYT SWYVALKRTG QYKLGSKTGP GQKAILFLPM SAKS

Storage & Handling:

Handling: **Centrifuge vial prior to opening. Do not vortex after reconstitution. Avoid repeated freeze-thaw cycles.**

Reconstitution: **Initially reconstitute in 5mM Tris, pH 7.6, to 0.1-1.0 mg/ml. Store at 2°C to 8°C for up to 1 week or prepare for extended storage.**

Extended Storage: **After initial reconstitution, further dilute in a buffer containing a carrier protein or stabilizer (e.g. 0.1% BSA). Store working aliquots at -20˚C to -80˚C.**

Storage/Stability:

Product Form	Temperature	Storage Time
Lyophilized	-20˚C to -80˚C	December 2027
Lyophilized	4°C	6 months
Lyophilized	Room Temperature	1 month
Reconstituted	2°C to 8°C	1 week
Extended Storage	-20˚C to -80˚C	12 months

Specifications:

Formulation: Sterile filtered through a 0.2-micron filter. Lyophilized from 5mM Tris, pH 7.6 + 150mM NaCl.

Authenticity: Verified by N-terminal and Mass Spectrometry analyses (when applicable).

Purity: ≥ 95% by SDS-PAGE gel and HPLC analyses.

Endotoxin: Endotoxin level is < 0.1 ng/µg of protein (< 1 EU/µg).

Protein Content: Verified by UV Spectroscopy and/or SDS-PAGE gel.

Biological Activity: **Assay# 1:** Determined by the dose-dependent stimulation of thymidine uptake by BaF3 cells expressing FGF receptors. The expected **ED_{50}** is ≤ 0.5 ng/ml corresponding to a specific activity of ≥ 2 x 10^6 units/mg.
Assay# 2: Determined by a cell proliferation assay using Balb/c 3T3 cells. The expected **ED_{50}** is ≤ 0.1 ng/ml, corresponding to a specific activity of ≥ 1 x 10^7 units/mg.

Usage: Not for human use.
Country of Origin: USA

Quality Assurance Representative

RESPONSE TO FDA FOR CLARIFICATION OF QUESTIONS RELATING TO BELIEVER MEATS CULTIVATED CHICKEN SUBMISSION CCC 000039

Requests for Clarification to the DSN

Requests for Clarification

1. **You report the specification for Enterobacteriaceae as <100 colony forming units (CFU)/g, while the results from the analytical testing reported in Table 5.3-1 on page 35 of the May 21, 2024 DSN are <10 CFU/g. As Enterobacteriaceae is a large family of microorganisms that includes notable foodborne pathogens, and the fact that you have reported levels much lower than your proposed specification, we request that you lower your specification for Enterobacteriaceae to as low as can be reasonably obtained.**

   The *Enterobacteriaceae* specification can reasonably be lowered to ≤50 CFU/g. As previously stated in our response to Question 3 of FDA's May 20, 2025, request for clarification, the specifications for cultured chicken fibroblasts in Table 5.2-1 and 5.3-1 from the May 21, 2024, DSN are for material that has undergone the wash step to remove media components, not for pre-washed cultured chicken fibroblasts directly from the aseptic part of the process. As noted in that response, the wash process is not part of the aseptic system but is still a closed system that is cleaned via an automated CIP system controlled via SCADA to maintain high-hygienic conditions. Based on these facts, coupled with the analytical testing results reflected in the DSN, we believe that a lower ≤50 CFU/g *Enterobacteriaceae* specification is appropriate. We would also like to clarify that the microbiological specifications from Table 5.2-1 of the May 21, 2024, DSN, include testing specifications for specific *Enterobacteriaceae*, i.e. *Salmonella* spp. and *Escherichia coliform*, providing further assurance that these microorganisms are not present on post-wash product at levels that are expected to pose a safety risk.

2. **For addition to the administrative record, please state whether the production strain of *Pichia pastoris* used to produce the TrypLE Express enzyme employed in your production process is non-pathogenic and non-toxigenic.**

   Pichia pastoris is a yeast used in recombinant protein production that is also non-pathogenic and non-toxigenic and is approved for use in enzyme preparation in GRAS Notice No. GRN 001025 (FDA, 2023).

References

U.S. Food and Drug Administration (FDA). (2023, October 17). *GRAS Notice No. GRN 001104* . FDA.gov. https://www.fda.gov/media/175248/download

From:	Megan Lesch
To:	HFP-OFCSDSI-Animal Cell Culture; Marc Shelley; Mills, Jessica
Cc:	Hice, Stephanie
Subject:	RE: [EXTERNAL] Re: CCC 000039: FDA"s Scientific Memorandum (Draft) & proposed narrative for release
Date:	Wednesday, June 25, 2025 9:47:48 AM
Attachments:	image002.png image003.png image004.png image005.png image006.png image007.png image008.png image009.png image010.png

Hello Jessi,

Yes, we confirm that your understanding is correct for when FBS, BSA, and bovine catalase were used in our process, and the other statements in our May 28, 2025, amendment are consistent.

Our intent with Point 3 from the June 13, 2025, email was only to clarify that we do not perform RNA sequencing for bovine and porcine viruses during the Establishment of MWCB phase of the production process (Page 12 of the draft scientific memo) as all animal-derived components were removed in previous stages of cell line establishment. The statement regarding RNA sequencing for bovine and porcine viruses is accurate for the other phases of the production process referenced in the draft scientific memorandum (i.e. Cell Isolation (page 7), Establishment of Cell Lines (Page 8), Establishment of Primary MCB (Page 9).

We trust this confirmation is helpful. In light of this, do you also have an update to the timing of the letter and scientific memo?

Thank you,

Megan Lesch
Sr. Director of Regulatory
megan.lesch@believermeats.com
+1 (716) 472-7492
believermeats.com

From: HFP-OFCSDSI-Animal Cell Culture <HFP-OFCSDSI-AnimalCellCultureFoods@fda.hhs.gov>
Sent: Wednesday, June 25, 2025 7:16 AM
To: HFP-OFCSDSI-Animal Cell Culture <HFP-OFCSDSI-AnimalCellCultureFoods@fda.hhs.gov>; Marc

Shelley <marc.shelley@believermeats.com>; Mills, Jessica <Jessica.Mills@fda.hhs.gov>; Megan Lesch <megan.lesch@believermeats.com>
Cc: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Subject: RE: [EXTERNAL] Re: CCC 000039: FDA's Scientific Memorandum (Draft) & proposed narrative for release

Good morning, Marc –

We would like to clarify one point raised in your June 13, 2025, email.

In your email, you note that the May 28, 2025, amendment to the disclosable safety narrative (DSN), response to question 16, includes the statement, "... Bovine Serum Albumin and Fetal Bovine Serum were used in early stages of cell-line establishment but are not included in the media used to establish the Master Cell Bank (MCB) ..." (emphasis added).

We note that this statement appears to contradict two other statements made in the same amendment. In the response to question 15, Believer writes, "You are correct in stating that the primary MCB is created and stored using a serum-containing medium, and then a subset of primary MCB cells are adapted to grow in a serum-free medium to create the secondary MCB. The secondary MCB is stored in a serum-free medium that contains animal components and recombinant growth factors" (emphasis added).

Furthermore, in the response to question 14, Believer writes, "The three animal-derived media components that were removed in the 'animal-component free' media are bovine catalase, bovine serum albumin (BSA), and fetal bovine serum (FBS). FBS was previously discussed on page 18 of the January 15, 2025 amendment to the DSN as it was used in step one from Figure 4.3-1 from the May 14, 2024 DSN. Bovine catalase and BSA were used in steps three and four from Figure 4.3-1 for the primary and secondary master cell banks" (emphasis added).

It is our understanding that "serum" refers solely to FBS, and that FBS, BSA, and bovine catalase are used during cell isolation through establishment of the primary MCB. Furthermore, it is our understanding that BSA and bovine catalase are used in establishment of the secondary MCB. Please confirm that our understanding is accurate, and if it is not accurate, please clearly state the phases in the production process where each of these substances are used. In doing so, please differentiate between the primary and secondary MCBs.

Best,

Jessi

Jessica Mills, Ph.D.
Biologist
Innovative Foods Staff