Cultured Meat and Seafood

A guide to Regulations, HACCP, Culture Media and Quality Control

- Regulatory Overview
- Application of HACCP
- Cell Culture Media
- Quality Control

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Contents

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1. Cultured Meat – An Introduction

The global population is consuming more meat than ever. Whether it is a chicken sandwich or a juicy burger, most of us find it an irresistible part of our diet.

The meat produced by current farming methods is unsustainable and has a vast environmental footprint. It contributes to the degradation of natural resources such as land and water, biodiversity loss, and greenhouse gas emissions causing climate crises. We need a radical shift in our approach to producing and consuming meat to mitigate the adverse effects of climate change. A recent United Nations report calls on us to substantially cut down our meat consumption to help save the planet.[4] But what if there was a way to continue eating our burgers and bacon, guilt-free?

Cultured meat—grown in a bioreactor, instead of a farm—could offer that sustainable alternative to conventional meat production.

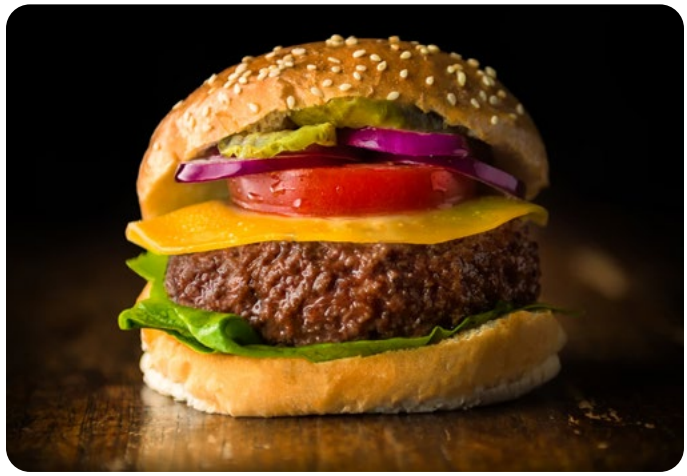
Using technologies already familiar to cell biologists around the world, this approach involves growing meat from real animal cells in a process known as cell-based agriculture. The future vision is to grow meat products such as chicken, beef, pork, or fish—commercially at a large scale in factories that resemble a brewery.

Eliminating the need to breed, raise and slaughter animals for food, cultured meat—which is also known as cultivated or cell-based meat – offers a way to reduce the environmental impact of traditional meat production.

Studies suggest that cultured meat produced using renewable energy reduces carbon footprint by 17%, 52%, and 85–92% compared to conventional chicken, pork, and beef production respectively while also using 63–95% less land.[6]

Cultured meat, is generated from a few animal cells at the start of the process, which yields meat products that avoids animal welfare concerns caused by farming methods.

Since cultured meat is produced in a clean, sterile environment it is less likely to be exposed to environmental contamination caused by viruses and bacteria. However, like any large-scale manufacturing process, rigorous safety tests will be demanded from start to finish—from the validation of everything that enters the bioreactor, to determining the nutritional content and safety of the final product. These stringent regulations will ensure safety and quality assurance before it reaches our plates.



Regulatory Overview for Cultured Meat

United States • USDA -FSIS & FDA - CFSAN • GRAS	**Japan** • MAFF, JACA & MHLW • Expert group formed
European Union • EC & EFSA • Regulation (EU) No 2015/2283	**South Korea** • MFDS • Draft by July 2023
Singapore • SFA • Approved Cultivated Meat	**Thailand** • MOPH • Notification No. 376 B.E. 2559 Novel Food
Australia • FSANZ • Food Standards Code subsection 1.1.1-13(4)	**Israel** • NFS under Minisitry of Health, IIA & FSA • New Food Registration
China • NHC & CFSA • New Food Raw Materials (Novel Foods)	**Canada** • Food Directorate, CFIA Health Canada • Novel Food Regulation
India • FSSAI • FSS (Approval for Non-Specified Food & Food Ingredients)	**Brazil** • ANVISA • Discussion Stage

Abbreviations:

USDA-FSIS: United States Department of Agriculture - Food Safety & Inspection Service

FDA-CFSAN: Food & Drug Administration – Center for Food Safety & Applied Nutrition

GRAS: Generally Recognized As Safe

EC: European Commission

EFSA: European Food Safety Authority

SFA: Singapore Food Authority

FSANZ: Food Standards Australia New Zealand

NHC: National Health Commission of the People's Republic of China

CFSA: China National Center for Food Safety Risk Assessment

FSSAI: Food Safety Standards Authority of India

FSS: Food Safety Standard

MAFF: Ministry of Agriculture, Forestry, and Fisheries

JACA: Japan Association for Cellular Agriculture

MHLW: Ministry of Health, Labour, and Welfare

MFDS: Ministry of Food and Drug Safety

MOPH: Ministry of Public Health

NFS: National Food Service

IIA: Israeli Innovation Authority

FSA: Food Safety Authority

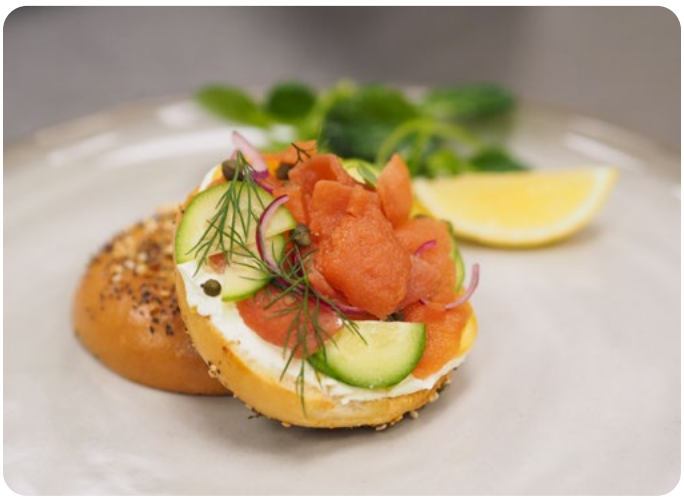
CFIA: Canadian Food Inspection Agency

ANVISA: Brazilian Health Regulatory Agency

Novel Food Regulations

As stated by European Commission, novel food is defined as food that had not been consumed to a significant degree by humans in the EU before 15 May 1997, when the first Regulation on novel food came into force. According to the regulation, 'Novel Food' can be described as newly developed, innovative food or food produced by new technologies and production processes, as well as food which is or has been traditionally eaten outside of the EU (**Novel Food (europa.eu)**). Whereas Singapore Food Authority (SFA) defines novel foods as food and food ingredients that do not have a history of safe use, i.e., consumed by a significant population as part of the diet over 20 years with no adverse health effects reported.

If we look at the various definitions of novel food stated by different global food safety authorities, categorize novel foods as food which either is not consumed in that geography, lacks a consumption history, or the adverse effect of that food and its ingredient is unknown. Novel foods may include cultivated or cultured meat, plant proteins or broadly alternative proteins such as insect proteins, microalgae proteins, genetically modified organisms (GMO) or proteins produced by GMO, pure chemicals, whole foods, etc.



2. Current Regulatory Overview

Singapore:

History was made in December 2020 when Singapore became the first country to approve cultured Meat. The Singapore Food Authority (SFA) introduced a novel food regulatory framework in 2019, which requires manufacturers to seek approval from the SFA by submitting an application. SFA is responsible for conducting a pre-market assessment of novel foods. The applicant or manufacturer of a novel food is required to submit details including the materials used in the manufacturing process along with the control measures for food safety hazards. The applicant or manufacturer must conduct and submit safety assessments of the novel food covering potential food safety risks, including toxicity, allergenicity, safety of its production method, and dietary exposure from consumption. To support cultured meat applicants, SFA created a detailed self-assessment checklist for cell-based food companies or importers of cultured meat. Below are a few key requirements stated by SFA for cultured meat manufacturers/applicants:

- Description of the overall manufacturing process including the Food Safety Management System
- Characterization of the cultured meat product
- Information related to the cell lines and culture media used
- Information related to the scaffolding materials, if used, such as identities and purity
- Information on how the purity and genetic stability of cell culture during the manufacturing process is ensured
- Safety assessments covering possible hazards caused by the manufacturing process
- Other relevant studies to support safety such as digestibility assays, allergen profiling, genetic sequencing, etc.
- Studies referenced in safety assessments should either be published in reputable scientific journals or conducted according to Good Laboratory Practice (GLP)

SFA also created a novel food safety expert working group, wherein the members are specialized in different fields of novel food assessments like toxicology, food science, informatics, microbiology, public health, etc.

The United States:

Regulatory Agencies:

Two federal agencies are charged with regulating human food products in the United States. The United States Department of Agriculture (USDA) with its responsibility over farming and livestock oversees meat products such as beef, pork, lamb, or poultry under its Food Safety and Inspection Service (FSIS) program. The Food and Drug Administration (FDA) regulates food production not covered under the USDA such as processed and packaged foods. The delineation is not always straightforward, however. For example, the FDA oversees seafood, with the exception of catfish since the latter are farmed and therefore fall under USDA. For cultured meat a solution was needed since production of cultured meat is most like food production (FDA) but some elements such as labeling of the product are very similar to USDA expertise.

Regulatory Authority and Jurisdiction:

In the United States, regulatory authority to oversee cultured meat is uniquely shared between FDA and USDA-FSIS. As oversight depends upon the animal species of origin for source cells, unique food safety, development, and labeling challenges of cultured meat, leading the two agencies to establish a joint regulatory framework in 2019.[15] The agreement clarifies how each regulatory agency will provide appropriate oversight and industry stakeholders with a better understanding of the regulatory criteria for the production, distribution, and marketing of cell-cultured products in the United States.

Regulatory oversight for human foods made with cultured cells will also be influenced by the original source of cultured cells. Animal species such as cattle, sheep, swine, and Siluriformes (catfish) are regulated under the current Federal Meat Inspection Act (FMIA), and poultry products such as chicken and turkey are regulated under the Poultry Products Inspection Act (PPIA). As such, cultured meat produced from these species will fall under USDA-FSIS jurisdiction for harvesting, processing, and labeling.

Under the joint regulatory framework, the FDA will provide oversight for the initial stages such as the collection, proliferation, and differentiation of cell types (e.g., fats, proteins, etc.). Upon harvest, USDA-FSIS jurisdiction will cover processing, labelling, and packaging.

For all other foods, including those intended for animal consumption, FDA would have federal authority to oversee all the stages, from collection through distribution.

It is important to note that in the United States, State legislative bodies may also enact their own regulations regarding food and agricultural products including meat. This may impact the labeling of certain products. Over 30 states have enacted bills restricting the legal definition of meat. In the past few years, several states enacted bills stating that food containing cultured animal tissue produced from animal cells may not be labeled as either meat or a meat food product. Most restrict the term "meat" to products derived or harvested from animals by traditional means. There is currently no federal definition of meat, however, the US government has defined other alternative food types. For example a recent FDA determined plant-based milk could use the term „milk“ (e.g., soy milk). It is likely that once cultured meat enters the marketplace the labeling/definition will be determined at the federal level to prevent confusion at the state level.

Oversight and Safety Review Process:

FDA’s current process to assess cell-cultured products consists of a pre-market consultation along with inspections to ensure compliance with current safe food regulations including facility registration, Current Good Manufacturing Practices (cGMP), and preventative controls per the US Federal Food, Drug, and Cosmetic Act (FFDCA).[16]

The pre-market consultation and inspections include the evaluation of all biological material including source tissue, cell lines, cell banks, and any other components expected in the final food product. All risks must be appropriately addressed and managed per the FFDCA.

Establishments that harvest or process cell-cultured meat or poultry will also need to apply for a USDA grant of inspection and should meet all existing USDA-FSIS regulatory requirements to ensure sanitation and an appropriate Hazard Analysis and Critical Control Point (HACCP) system is in place.

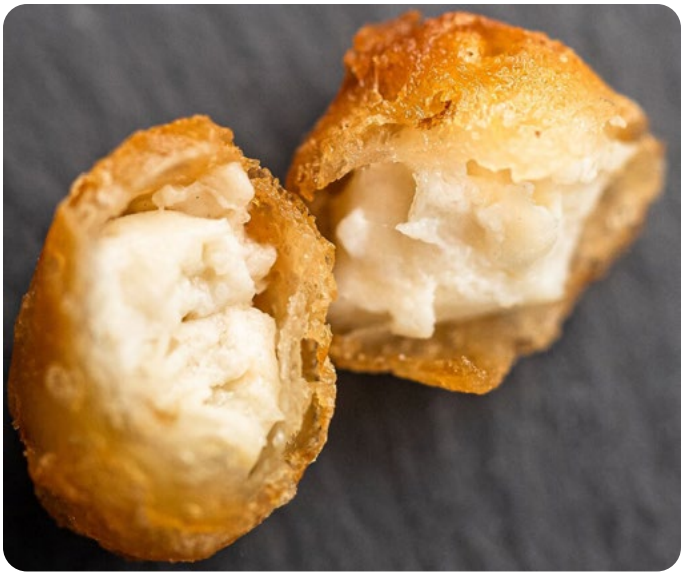
Testing Guidance:

Currently, preventative plans from consulting industries involve adherence to 21 CFR 117, hazard analysis and cGMP, which requires food safety plans to test for any reasonable risk including potential adventitious microbial agents, chemical contaminants, heavy metals, and any other environmental contaminants. Specific test methods or amended methods for food safety analytes of concern used by regulatory authorities will be published in each regulatory agency’s respective foods program compendial laboratory guides such as FDA-Bacteriological Analytical Manual (BAM) or USDA-Microbiology Laboratory Guidebook (MLG).[17, 18] As regulations develop, safety testing requirements for cultured meat will fall under existing preventative control requirements and guidances outlined under cGMP, HACCP, or other guidances will be issued.

Commercial Availability:

Although, cultured meat products in the United States are, as of August 2023, not commonly found in grocery stores or other venues, two firms have fully approved cultured chicken. The FDA accepted UPSIDE Foods’ submission in November 2022. (5) The submission to the FDA by GOOD Meat Inc., which already offers cultured chicken in Singapore, was accepted by the FDA in March 2023. For full approval in the US, however, cultured meat products like cultured chicken also require USDA approval. GOOD Meat Inc., and UPSIDE Foods products both received USDA approval in June 2023. As of August 2023, both firms launched their products for limited tastings at select restaurants and plan to expand their offerings and availability. With these precedents for cultured meat approval, it is likely that more firms and more products will achieve full US approval.

The regulatory framework for cultured meat is still evolving. As the industry continues to grow both the FDA and USDA anticipate further compliance guidance and proposed rulemaking on cultured meat.



European Union:

To ensure that food entering EU markets is safe and regulated properly, it is mandatory to have a food safety monitoring system e.g., HACCP in place throughout the food chain.[20] The data requirements for novel food applications are provided in the European Food Safety Authority (EFSA), “Guidance on the preparation and presentation of an application for authorization of a novel food in the context of Regulation (EU) 2015/2283." It is currently a lengthy process for Novel Food (NF) approval, and risk assessment is needed by EFSA, Inclusion of Union list of NF. Currently there is no Cultured Meat on the EU market.

The main consideration for Safety Assessments for Cultured Meat is mentioned in ‘Insights on Novel food risk assessment - Nutrition Unit of EFSA’[21], which is directly related to the required HACCP assessment.

Identity: Foods consisting of, isolated from, or produced from cell culture or tissue

- Biological source (International Codes of Nomenclature)
- Organ, tissue, or part of the organism
- Information on the identity of cells
- Type of culture
- Stem cells, laboratory, and culture collection
- Cell or tissue substrate used as a novel food

Characterization:

- Identities and quantities of impurities, by-products or residues, and antimicrobial residues
- Nutritionally relevant constituents
- Biological hazards: BSE/TSE, viruses (source, zoonotic), and microbiological contaminants
- Type and spectrum of target analytes depending on sources and production process

Production Process: Detailed description includes:

- Treatment, modification, and immortalization of cells
- Raw materials, starting substances, medium/ substrate, growth factors/hormones culture conditions, antimicrobials, hygiene measures, and equipment.

Generic issues related to manufacturing processes using cultured cells:

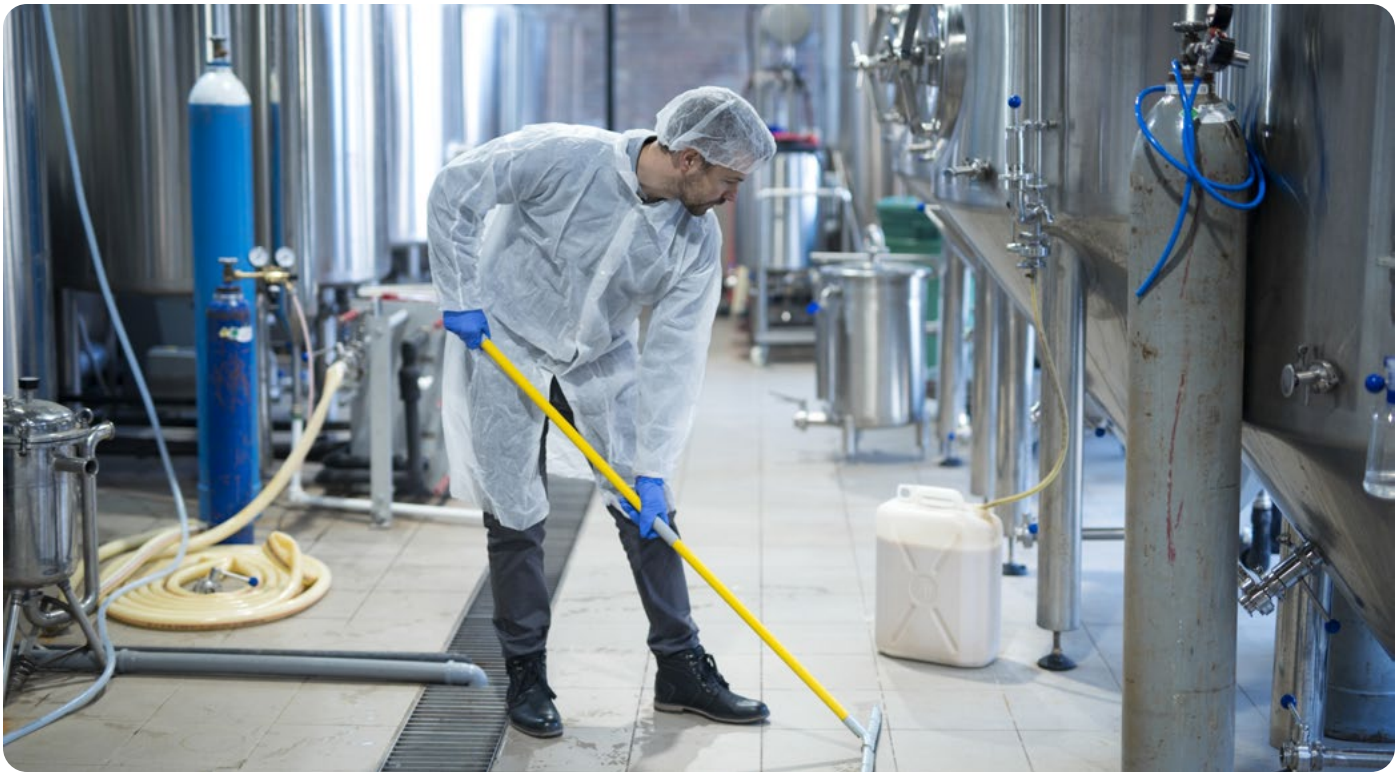
- Potential by-products, impurities, contamination, stability of cells, and consistency of the production process
- Operational limits and key parameters of the production process

Nutritional Information:

- Role of the NF in the diet (based on the intended uses)
- Comparative approach to conventional meat
- Quality and quantity of macro and micronutrients

Allergenicity:

- Basis: comprehensive compositional data
- Potential use of “omics” tools (genomics, transcriptomics, proteomics, and metabolomics)



3. Application of HACCP to the Cultured Meat Process

An Introduction to HACCP

Hazard Analysis Critical Control Point (HACCP) is a system designed for use across all segments of the food and beverage industries. It originates from the 1960s when NASA and the Pillsbury Dough Company collaborated to create a quality monitoring system that could help provide safe food for upcoming space missions. HACCP is a management control system that monitors food safety by analysing and controlling the biological, chemical, and physical hazards throughout the process – from growing and harvesting to the manufacturing process and distribution. Currently, HACCP is typically an integral part of each food regulatory safety system. US regulatory requirements (21 CFR part 117) require hazard analysis and risk-based process controls, most US food manufactures choose a HACCP program to meet this requirement. Similarly, the EU requirements (Regulation (EC) No 852/2004) require a food safety management program based on HACCP. Although there are many models for HACCP implementation it is recommended to confirm any HACCP program is aligned with Codex Alimentarius standard.

Principle 1: Conduct a hazard analysis

During the hazard analysis, safety concerns must be differentiated from quality concerns. A hazard is defined as a biological, chemical, or physical contaminant that could potentially cause illness or injury in the absence of its control.

Principle 2: Determine critical control points (CCPs)

A critical control point is a step in the process at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. Examples of CCPs are thermal processing, chilling, testing ingredients for chemical residues, product formulation control, testing product for metal contaminants, etc.

Principle 3: Establish critical limits

A critical limit is a maximum and/or minimum value to which a biological, chemical, or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce the occurrence of a food safety hazard to an acceptable level.

Principle 4: Establish monitoring procedures

Monitoring is a planned sequence of observations or measurements to assess whether a CCP is under control and to produce an accurate record for future use in verification. Monitoring serves three main purposes. First, monitoring is essential to food safety management as it facilitates tracking of the operation. Secondly, monitoring is used to determine when there is a loss of control, and a deviation occurs at a CCP. Thirdly, it provides written documentation for use in verification.

Principle 5: Establish corrective actions

Corrective actions prevent foods that may be hazardous from reaching consumers. Specific corrective actions should be developed in advance for each CCP and included in the HACCP plan. When a deviation from a critical limit occurs, the HACCP plan should specify the corrective action.

Principle 6: Establish verification procedures

One aspect of verification is evaluating whether the facility's HACCP system is functioning according to the HACCP plan. An effective HACCP system requires little end-product testing since sufficient validated safeguards are built in early in the process.

Principle 7: Compilation of records and record-keeping

The various documentation demonstrate the HACCP program is valid and the CCP are monitored correctly and controlled. These documents must be archived and can be audited by customers or authorities.

In the context of food quality, risk assessments are important tools used to identify potential hazards and assess the likelihood of their occurrence. Such assessments involve a comprehensive examination of the various elements that contribute to food safety and quality. Recent examples of hazards and risk assessments in the food industry include the premarket notice for integral tissue cultured poultry meat, food safety aspects for cell cultured food, and hazard identification in meat products manufactured from cultured animal cells. Recent examples of the elements to consider as part of the hazard identification and risk assessment include the "Premarket Notice for Integral Tissue Cultured Poultry Meat submitted on behalf of Upside Foods and Good Meat" released as part of the "FDA pre-market authorization (2022, 2023)", "Food Safety Aspects for Cell Cultured Food (FAO, 2022) and "Hazard identification: Identification of hazards in meat products manufactured from cultured animal cells. Risk Assessment Unit Science, Evidence and Research Division, FSA (2023)"

Upside Foods & Good Meat references:
https://www.fda.gov/media/166346/download
https://www.fda.gov/media/163262/download
https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=AnimalCellCultureFoods

Adventitious agents are the contaminating microorganisms, such as bacteria, fungi, mycoplasma/spiroplasma, mycobacteria, Rickettsia, protozoa, parasites, transmissible spongiform encephalopathy (TSE) agents, and viruses, that have been unintentionally introduced into the biological manufacturing process. The source of these contaminants may be the legacy of the cell line, raw materials used in the culture medium, external environment, personnel equipment, or elsewhere (WHO, 2013). With the product to be safe for the intended use and the process eventually varying from standard cell culture workflows by including different food processing steps – traditional food manufacturing organisms such as foodborne pathogens or spoilage and indicator organisms need to be part of the risk assessment. Due to the missing slaughter process of naturally raised animals, the occurence and spread of pathogenic microorganisms is expected to be lower, potentially allowing to lower test frequencies for known food pathogens. The premarket notices recently released by the FDA for Upside Foods and Good Meat provide examples of how the industry applied the associated risk assessment.

https://www.fda.gov/media/163262/download

Table 1: Overview of potential hazards*

Identified Hazards	Cell Banking (inc. cell line isolation, generation and storage	Production		Harvest	Food Processing
		Media Development & Preparation	Bioreactor & Scaffold		
Identity	Cell species, Cell Type	Media components and origin	Cell Type. Cell Differentiation	Cell species, cell type	Authenticity
Viral contaminants	Cell line species specific virus incl. considering zoonotic potential	Cell line species specific virus incl. considering zoonotic potential	Cell line species specific virus incl. considering zoonotic potential		
Bacteria, yeast and fungi	Legacy of the cell line, the raw materials used in the culture medium to propagate the cells (in banking, in production, or in their legacy), the environment, personnel, equipment or elsewhere		Legacy of the cell line, the raw materials used in the culture medium to propagate the cells (in banking, in production, or in their legacy), the environment, personnel, equipment or elsewhere	Legacy of the cell line, the raw materials used in the culture medium to propagate the cells (in banking, in production, or in their legacy), the environment, personnel, equipment or elsewhere	
Food borne pathogenic bacteria	Food borne pathogenic bacteria				Food borne pathogenic bacteria
Spoilage / indicator organisms as subject to normal food products				Spoilage / indicator organisms as subject to normal food products	Spoilage / indicator organisms as subject to normal food products
Mycoplasma	Mycoplasma	Mycoplasma	Mycoplasma		
Endotoxin		Endotoxin	Endotoxin		
Allergenicity		Raw material or cross-contamination	Cross-contamination		
Toxicity/ADM	Chemical contaminants (e.g. pesticides, heavy metals, dioxins, PCBS, adulterants, mycotoxoins	Chemical contaminants (e.g. pesticides, heavy metals, dioxins, PCBS, adulterants, mycotoxoins	Chemical contaminants (e.g. pesticides, heavy metals, dioxins, PCBS, adulterants, mycotoxoins		Chemical contaminants (e.g. pesticides, heavy metals, dioxins, PCBS, adulterants, mycotoxoins
Nutritional profile**			Nutritional profile (e.g. Fats, Protein, Vitamins, Minerals, Sugars, Fiber, Moisture, Water)		Nutritional profile (e.g. Fats, Vitamins, Minerals, Sugars, Fiber, Moisture, Water)
Shelf life/ Final product stability:				Nutrional profile, sensoric properties, total count for bacteria, yeast and mold	Nutrional profile, sensoric properties, total count for bacteria, yeast and mold

*Focusing on biological hazards

**Nutritional profile is not seen as a hazard itself, but controlled at different stages.

Hazard Origin: Environment, Raw materials

Table 2: Identified Hazards Overview (appendix*,)**

Type of hazard	Description / Potential sources	Potential testing points	Risk potentially associated with the production process (P), food quality (FQ), food safety (FS)	Preventive options (focusing on testing)
Identity	Identify the cell line (no cross contamination), the expected cell type, and species ID at the final meat product.	Cell banking, manufacturing, and the end product	FQ, FS	
Adventitious agents /biological hazards (Headline)				
Viral contaminants	The source of these contaminants may be the legacy of the cell line, raw materials, environment, personnel, equipment, or elsewhere (WHO, 2013). The requirement to define species-specific virus panels incl. zoonotic potential considering virus tropism and potential for exposure.	Cell banking process (Master and working cell bank)	P, FS	CoA / Ensured quality control of cell lines, culture media, sterile media preparation
Bacteria, yeast, and fungi	Microorganism sources to consider may include traditional food sources, cell lines and cell banking, raw materials, environment incl. personnel, and final food production environment.	Cell banking, scale-up, manufacturing, end product control	P, FP, FS	Environmental monitoring plan (e.g., based on the Food Zone concept) and identified biological materials, sterile media preparation.
Foodborne pathogenic bacteria	Bacteria identified as pathogens in today´s food production such as Salmonella, Listeria, and *E. coli* O157. Those may originate from cell lines, raw materials, ingredients, operators, production environment. The contamination risk of the latter one may increase if the cultured cells are further processed into final food products.	Cell banking, end product, and production environment	P, FP, FS	Microbiological cell line and raw material quality control.
Spoilage/indicator organisms	Bacteria are not harmful to the final product, which may be indicative of unhygienic conditions	Raw material, production process, and the end product	FP	Quality control of raw materials, sterility/ bioburden testing during production, quantitative microbiological testing (e.g. total count, yeast, mold, Enterobacteriaceae) at the end product level.
Mycoplasma	Small, gram-negative bacteria capable of escaping filtration. Cell lines and raw materials, environment are potential sources.	Cell banking, scale-up, manufacturing?	P, FS	Microbiological cell line, raw material quality control, and testing.
Endotoxin	Endotoxins are small, hydrophobic molecules that are part of the lipopolysaccharide complex that forms most of the outer membrane of Gram-negative bacteria (1). They are released when the bacteria die and their outer membranes disintegrate, triggering the toxicity mechanism of the fractionated lipopolysaccharides.	Raw material, production process.	P, FS	Endotoxins could produce variability in cell culture results (Allan et al., 2019). As of today, it needs to be determined if endotoxin represent a consumer risk for food product subject to be eaten.
Allergenicity /Toxicity	Allergenic reactions may occur as a subject of the protein structure or carry over products from the new production process. Toxicity may result from novel compounds and their concentration in the final product.		FQ, FS	Typically clarified as part of the novel food authorization process.
Nutritional profile	Nutritional composition of the meat and final food product required for food labelling. Including food additives and food flavours		P, FQ	Established during product development and subject to routine production analysis
Shelf life/ Final product stability:	The food product may have a different microbial flora composition compared to current products, resulting in potentially different growth kinetics for microbial contaminants. A detailed assessment will be required.	During product development as shelf-life assessment, a periodical review will be required	P, FQ, FS	Stability assessments as part of the product development and production process considering good manufacturing practices.

*Focusing on biological hazards

**Referring to Premarket Notice for Integral Tissue Cultured Poultry Meat submitted on behalf of Upside Foods and Good Meat" released as part of the "FDA pre-market authorization (2022, 2023)", "Food Safety Aspects for Cell Cultured Food (FAO, 2022) and "Hazard identification: Identification of hazards in meat products manufactured from cultured animal cells. Risk Assessment Unit Science, Evidence and Research Division, FSA (2023)"

4. Role of Culture Media

Which risks could occur (raw materials, other components), and how are they controlled?

The cell culture medium is a crucial component in the production of cultured meat. The medium is used to provide the necessary nutrients and growth factors that allow the cells to proliferate and differentiate in the production phase. In addition, a cell culture medium is used during the cell selection phase as well when cells are sourced and isolated from animal tissues[1]. The media composition might vary depending on the specific type of cells being cultured and the phase in the process. The cell culture basal medium typically contains a mixture of amino acids, vitamins, salts and sugars, and other compounds that are essential for cell growth and metabolism. The basal medium is supplemented with Fetal Bovine Serum (FBS) or other protein mixes. The protein mixes often contain recombinant proteins. Other additives such as antibiotics to prevent contamination by microorganisms and shear protectants to protect the cells from shear stress can be used in the medium. The potential risks associated with the different cell culture medium components, supplements, and additives are described below. Different steps to reduce or control the risks are also discussed.

The seed and production phases of cultured meat rely on basal medium to support cellular proliferation and differentiation. The medium is standardly comprised of amino acids, vitamins, salts, carbohydrates, and other components, which vary depending on the metabolic needs of the cells used for the meat production. The composition of the basal cell culture medium exhibits nutritional commonalities with animal feed used in conventional livestock agriculture. In addition, many of the materials are naturally occurring and have long been used in food being manufactured for human consumption under explicit regulatory guidelines. Nonetheless, there is a potential risk related to the basal medium components and the final cultured meat product intended for human consumption. One such risk is the carryover of basal media components into the final product. The possibility of media remnants can be mitigated by a downstream washing step during the scale-up process. Also, a nutritional profile analysis of the cultured meat will help determine if levels are within an acceptable limit of detection. The profile of the cultured meat product can be compared to the relevant industry standard for reference. There might be a potential reduction in activity for some compounds following the cooking process and during digestion. The use of food-grade raw materials sourced from reputable suppliers with GRAS (Generally Regarded as Safe) status, or other relevant quality specification standards, in the basal medium will further mitigate this risk.

Fetal bovine serum (FBS) is a biological material harvested from the blood of cattle used in the meat-packing industry. A complex mixture of proteins, electrolytes, growth factors, carbohydrates, lipids, hormones, and other components – FBS is a broadly adopted supplement of the basal medium used to support a wide variety of cell culture applications. Within the cultured meat industry, the basal medium used during the seed and production phases is often supplemented with FBS to support cellular proliferation and differentiation. Despite its rich composition and success as a cell culture additive, there is significant momentum within the cultured meat industry to eliminate (or largely reduce) FBS from media formulations for both ethical and financial reasons. However, due to the challenges associated with achieving a serum-free media (e.g., growth, performance, yield, etc.), it is anticipated that cells used in the manufacturing of cultured meat will be supplemented with FBS during the process. Since FBS is a biological product, the composition is undefined and there is an inherent risk for adventitious agents that could be harmful to the consumer. In an effort to mitigate this risk, FBS should be sourced from reputable suppliers with documentation certifying that biological material was collected from healthy cattle. In addition, sources of FBS could be screened for possible infectious agents by the cultured meat manufacturer, as outlined in the U.S. Code of Federal Regulations 9 CFR 113.53(c). In addition to the risk of exposure to infectious agents, there is also the threat of potential carryover of FBS in the final cultured meat product. Nonetheless, there is a possibility of human exposure to bovine serum proteins through the consumption of cow's milk and other blood-based food products. As demonstrated in Dossier in Support of the Safety of Good Meat Cultured Chicken as a Human Food Ingredient[23], consumer exposure can be quantified by measuring the level of bovine serum albumin, a major component of FBS, in the cultured meat product. Finally, it is presumed that through the cooking process and during digestion, any residual FBS components will be rendered inactive, posing little to no risk to the consumer.

To avoid some of the risks associated with FBS, supplements containing recombinant growth proteins can be used as an alternative. Recombinant proteins are proteins that are produced through genetic engineering techniques in a host organism such as bacteria, yeast, or even plants. The use of recombinant proteins could potentially introduce new allergenic or toxic properties to the final product. Cultured meat companies have implemented rigorous testing and analysis of the recombinant proteins used in the production of cultured meat as well as analysis of the end product itself. As outlined in "Premarket Notice for Integral Tissue Cultured Poultry Meat (Upside Foods, 2021)", the safety of using recombinant proteins in food is substantiated by the natural presence of similar levels of those proteins in food. UPSIDE Foods only uses growth factors with protein sequences that are 100% homologous to those from agriculturally important animals with a history of safe consumption (e.g., bovine, porcine, chicken).

Other alternative additives to the cell culture medium are antibiotics and sheer protectants. Antibiotics or antimycotics can be used during the cell selection or isolation and cell banking step. Since the biopsy step is not conducted in a controlled sterile environment, antibiotics or antifungal reagents are added to the cell culture medium to prevent contamination by microorganisms. Once the cell bank is established, antibiotics or antifungal reagents will not be added during the production phase so the risk of residuals in the final product is very low. In addition, downstream processes such as multiple washing will reduce the risk even more.

Sheer protectants such as Poloxamer 188 (P188) or Pluronic F-68 are added in the production phase to protect cells from shear forces in agitated suspension culture. As outlined in the Dossier in Support of the Safety of Good Meat Cultured Chicken as a Human Food Ingredient[2] Poloxamer 188 is listed on 21 CFR 310.545(a)(12)(iii) as a stool softener. P188 is also listed as an inactive ingredient 30 in 23 and an approved drug product administered via intramuscular, intravenous, ophthalmic, oral, periodontal, and topical routes. The applications of P188 indicate its low risk and safe consumption for humans. In addition, downstream wash processes will further reduce the amount of residual P188 to almost undetectable as shown by Good Meat.

5. Supporting quality control along the production chain

Depending on the regulatory requirements in the production process, Merck provides a broad range of products developed both for the pharma and Food and Beverage quality control to support this newly developing industry.

Those include core applications for Environmental and Hygiene Monitoring, which are of increasing importance due to expansion of regulations and standards for pharmaceutical, food, and beverage products. In these industries the quality of the production environments is directly linked to the finished products' quality. As an example, air quality plays a critical role in aseptic environments, cleanrooms, and production areas, where microorganisms in the air are a potential risk for cross-contamination of raw materials and final products. Personnel and surface monitoring represent the other two core applications for a complete control of the environment. Easy to use Hygiene Monitoring, such as ATP-Testing, enables rapid surface monitoring and line release.

Production and product safety is ensured by sterility testing. This is a GMP microbiology testing requirement used to confirm sterile products do not contain viable microorganisms before release. Sterility testing methods must be as accurate as possible, and other products that claim to be sterile or free from viable microorganisms. In the presence of mammalian samples, a process of "Direct Inoculation Sterility Testing" is likely to be used.

Microbial food safety testing requires in-process testing for specific microorganisms. Requirements vary from industry to industry, depending on the spoilage parameters for each food type. Traditional methods usually include plating and culturing samples for microbial identification. Rapid methods use molecular probes to detect microbial DNA signatures and are considered alternative methods if not specifically described in USDA, FDA -BAM, ISO, or other standards.

Rapid alternative methods are often preferred when a shorter analysis is needed to release food samples, especially for meat and other food products with a short shelf life. Merck's real-time PCR based GDS pathogen detection system offers rapid pathogen test kits for e.g. Salmonella, Listeria and pathogenic *E. coli*, representing the group of the most important classical meat related pathogens.

For a complete overview of our food safety testing solutions, please refer to **www.SigmaAldrich.com/FoodTestingHero**.

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