JACA
Japan Association
for Cellular Agriculture

Vireo Advisors, LLC

# INTERNATIONAL TRENDS IN SAFETY AND REGULATORY ASSESSMENT OF CULTIVATED FOOD

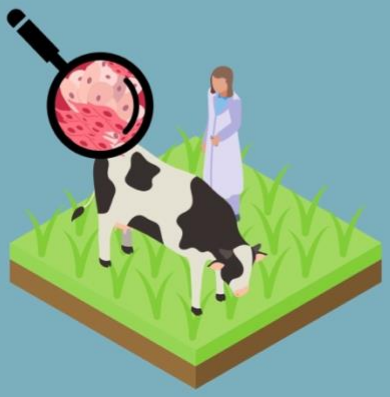
## PUBLIC REPORT


Project led by

**Japan Association for Cellular Agriculture (JACA)**

Prepared by Kimberly Ong and Jo Anne Shatkin

**Vireo Advisors**

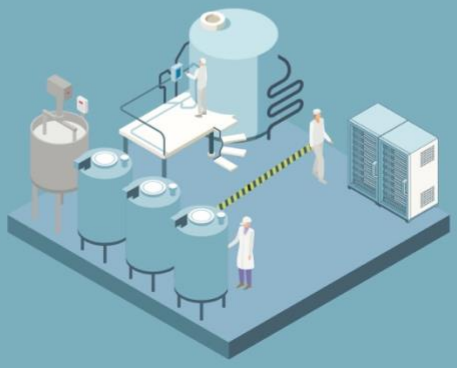



1

Cell collection

2

Preparation and quality assurance for mass culture



3

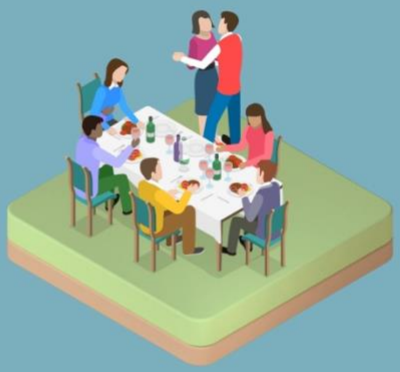
Culturing & monitoring the culture

4

Growing cells in culture medium

5

Cooking various foods from cells



6

Safe, healthy & tasty consumption experience

# International Trends in Safety and Regulatory Assessment of Cultivated Food

**Public report**

Project led by
**Japan Association for Cellular Agriculture (JACA)**

Prepared by Kimberly Ong and Jo Anne Shatkin
**Vireo Advisors**

Current to 24 May 2024
Date published 13 July 2024

For any information, please contact admin@jaca.jp or visit JACA website https://jaca.jp/en/

## EXECUTIVE SUMMARY

The Japan Association for Cellular Agriculture (JACA) is an organization where academia and industry stakeholders collaborate to make policy and industrial guideline recommendations for the appropriate social implementation of cellular agriculture. Even though the industry is emerging, JACA recognizes the importance of ensuring that relevant stakeholders are on the same page regarding understanding general and international trends in the risk assessment and management practices for the cultivated food, as much as possible, to build a consensus among them on appropriate domestic measures to be taken. Additionally, Japan lacks a framework for Novel Foods, and cell agriculture is a field where many technologies are still being established, making information gathering challenging. Therefore, JACA believes that the academic and industrial communities should actively organize the "general information" on safety in this field, so that the government can focus on "individual discussions" with each company. Thus, JACA appointed Vireo Advisors to report on the current state of regulatory and safety evaluation of cultivated meat and seafood products.

The information gathered is based on a literature review complemented by surveys and one-on-one interviews with Japanese and international cultivated meat and seafood companies. JACA members and external collaborators, such as The Good Food Institute reviewed the report. JACA has attempted to prioritize the issues that should be discussed in the domestic regulatory response among the various types of cultivated food products. For this purpose, it is important to note that all the companies selected for the interviews aim to sell in the Japanese market, and their perspectives may be different compared to the overall international development trends. An effective aspect of creating this "customized" report is that it aims to provide startups targeting Japan with an opportunity to preemptively inform domestic regulatory authorities about safety measures based on the individual characteristics of each cultivated food through this document.

This report consists of four sections: a comparison of data required by different regulatory authorities for the safety demonstration of cultivated food (**Section 1**), a description of the manufacturing methods and substances used in cultivated food production (**Section 2**), an analysis of the hazards and control measures for cultivated food products (**Section 3**), and a summary of recommended information requirements to be included in a regulatory submission for approval of cultivated meat and seafood products (**Section 4**). Sections 2 and 3 are supplemented with case studies from cultivated food companies during interviews. Figure 1 summarizes the structure of the report.

**Section 1** compares safety requirements for cultivated food outlined by regulatory stakeholders and key safety experts. Regulatory stakeholders include the Singapore Food Agency (SFA), European Food Safety Authority (EFSA), United Kingdom (UK) Food Standards Agency (FSA), United States (US) Food and Drug Administration (FDA) and Department of Agriculture Food Safety Inspection Service (FSIS), South Korea Ministry of Food and Drug Safety (MFDS), and the Food Standards Australia New Zealand (FSANZ). This section also considers publications by experts on cultivated meat and seafood safety, including the two current FDA pre-market consultations for cell-cultured food (GOOD Meat 2022, UPSIDE 2022); the report of the FAO/WHO Expert Consultation on cell-based food safety (FAO 2023); reports from the Good

Food Institute (GFI 2020, 2021a-c, 2022a-b, 2023a-d, 2024); publications from the Vireo Advisors/New Harvest Cultured Meat Safety Initiative (Ong *et al.,* 2021, Ong *et al.,* 2023); and presentations by Dr. Kitajima from the National Institute of Health Science and Professor Igimi from the Tokyo University of Agriculture.

Many jurisdictions require companies applying for authorization to submit a comprehensive manufacturing process description, which includes identifying potential food safety risks associated with each step. Additionally, in those countries, companies must provide information on the substances used during the manufacturing process, details on safety testing, and how they plan to mitigate any food safety risks. These include extrinsic risks such as contamination (with adventitious agents, allergens, and chemicals), or intrinsic risks such as genetic stability of cells and potentially hazardous residues. Some jurisdictions, but not all, require or recommend an assessment of genetic stability, standardized toxicity testing, and shelf-life studies. The information requirements commonly recommended or required by various regulatory agencies or experts are compared in Table 1.

**Section 2** provides background information describing the methods and inputs used to produce cultivated food. Each stage of manufacture is described, including cell sourcing, cell isolation, cell line establishment, cell banking, mass cultivation, cell harvest, food processing, and product packaging and distribution. Key differences in the manufacture of cultivated foods include cell source (species and cell type), method of cell line establishment (cell immortalization, cell suspension), types of inputs (culture media, scaffold), and mass cultivation method (bioreactor type/size and cultivation parameters). Various cell lines may have distinct requirements for media additives and growth conditions, even though basal components may be similar across different cell types. Safety demonstration may be more challenging for cultivated food produced using manufacturing processes and inputs that are less well-characterized and/or lack a history of safe use in food.

**Section 3** examines food safety hazards and control measures for each stage of cultivated food manufacture. This section builds on the known manufacturing methods and inputs described in Section 2.

The primary hazards associated with cultivated meat and seafood are adventitious agents from source cells, the environment, and inputs such as culture media; changes in cell characteristics due to genetic drift or genetic engineering that lead to the production of proteins/metabolites that may pose a food safety risk; allergenicity, particularly if cells are sourced from allergenic species or that can result from inputs or cross-contamination; use of substances or inputs without a safe history of use in food; and heavy metals contamination.

Control measures companies use to address these hazards include: certification of source animal health, which reduces the risk of adventitious agent contamination in cells and animal-derived culture media components; use of quality-controlled cell banks; and testing for hazardous substances, including foodborne pathogens, chemicals, and heavy metals. Cell line characterization and monitoring throughout the manufacturing process using growth parameters, as well as whole genome sequencing, karyotyping, and transcriptomics, along with related methods, can identify genetic and phenotypic changes of potential concern for food safety and/or quality. An established food safety management program (*e.g.,* Good

Manufacturing Practice [GMP], Hazard and Critical Control Point [HACCP]) helps systematically identify and control food safety hazards.

**Section 4** provides a summary of recommendations suggested by industry interviewees in combination with recommended information requirements to be included in a regulatory submission for approval of cultivated meat and seafood products. This information was developed by analyzing current requirements from regulatory agencies, expert recommendations, literature, and industry interviews.

It would be beneficial for regulatory bodies to establish guidelines pertaining to the submission and approval of dossiers. By providing clear and concise guidance, companies would be able to submit more comprehensive dossiers, thereby streamlining the review process for regulatory agencies. In general, many of the approaches and testing methods used for other types of food or related industries are applicable to the safety assessment of cultivated meat and seafood. The industry has matured to the point where regulatory agencies can expect to receive thorough safety dossiers from companies.

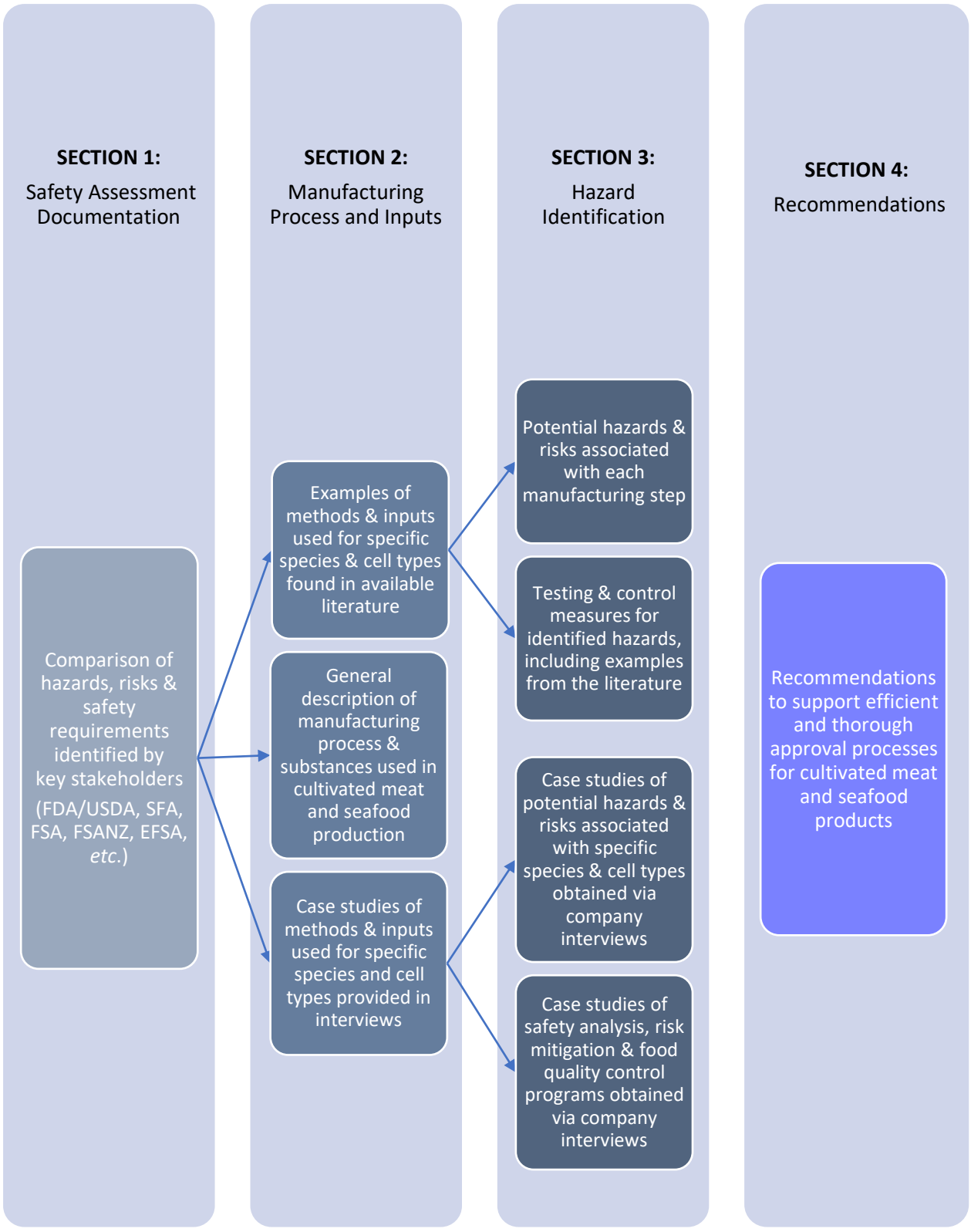
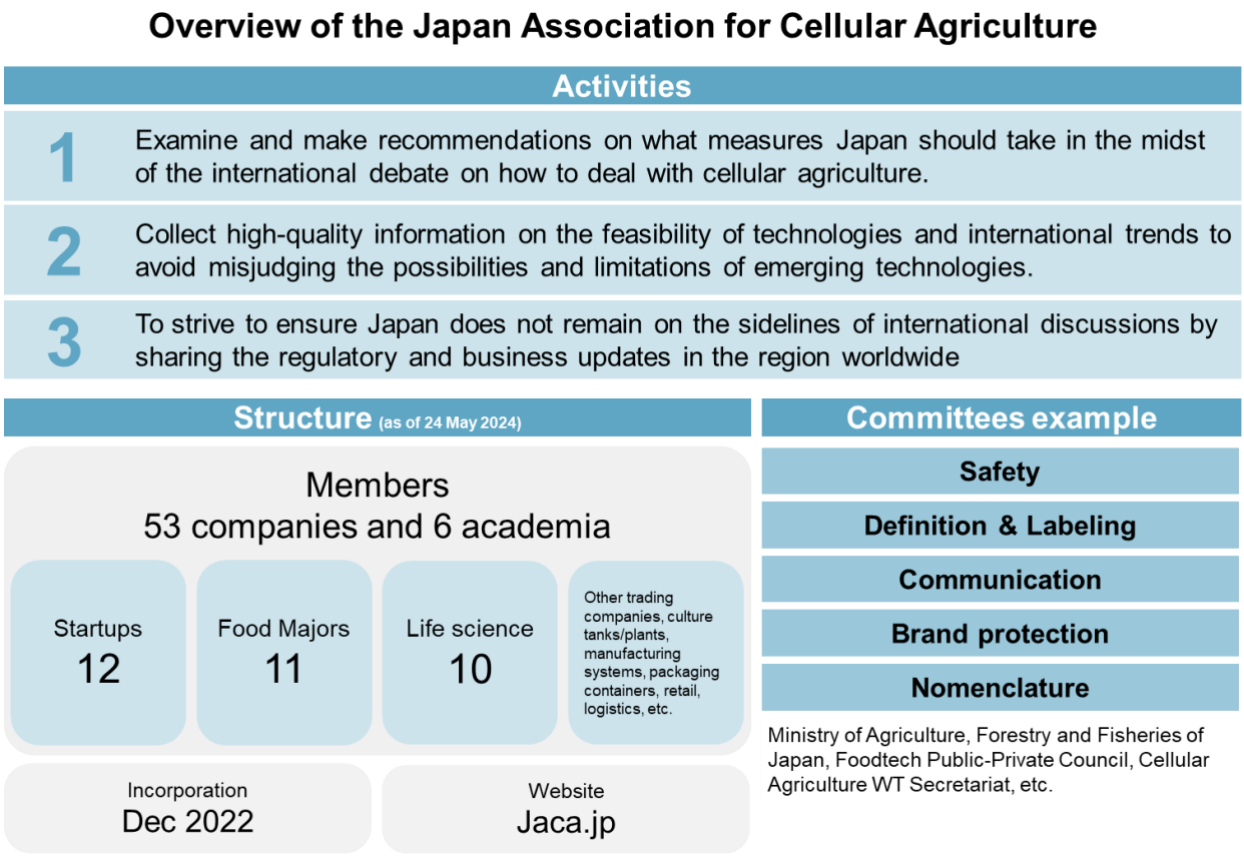



Figure 1. Overview of Report

**ABOUT JAPAN ASSOCIATION FOR CELLULAR AGRICULTURE AND THE BACKGROUND OF THE PROJECT**

JACA is an industrial organization established in December 2022 with around fifty corporate members (as of 24 May 2024). JACA also has academic supporters from cell culture technology, food safety, and social science. In addition, the organization administers the “Cellular Agriculture Working Team” under the Food-tech Public-Private Council, hosted by the Ministry of Agriculture, Forestry, and Fisheries.

JACA was established to make policy and industrial guideline recommendations for the appropriate social implementation of the cellular agriculture field. JACA communicates with various ministries, such as the authorities that oversee health and food, the economy, consumer affairs, and food safety assessments. It also exchanges information and opinions with politicians, other industry organizations, and four of the largest consumer organizations in Japan. JACA internally manages multiple committees, working on important topics for the social implementation of the industry, such as food safety, definition and labeling, nomenclature, food brand protection, and consumer communication.




The background for creating this document is as follows.

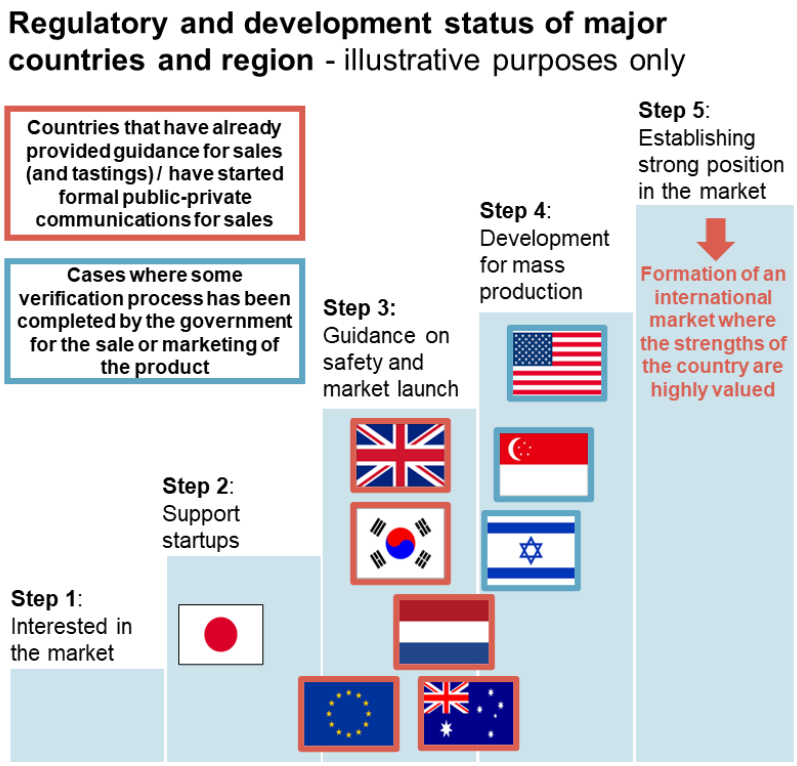
Cellular agriculture, food technology, and biomanufacturing are gaining attention in Japan. This interest may relate to exploring possibilities for more options for sustainable food production 20 or 30 years from now, preparing for international competition under the theme of sustainability, and the fact that this field is seen as one where Japan's technology and soft power could be effectively utilized.

For any information, please contact admin@jaca.jp or visit JACA website https://jaca.jp/en/

While it is important to accelerate the research and development of technology of the area, accurately grasping the technological outlook and creating a foundation for safe, secure, and stable operation of the technology in the country, which JACA refers to as “implementation,” is also crucial. “Implementation” does not mean “promote industrial advancement.” Amid intensifying international competition in critical and emerging fields, it is extremely important to accurately assess the potential of specific technologies to contribute to Japan’s economic development and national security. An accurate assessment of it may lead to a country taking prompt measures to prevent the leakage of critical technologies at early stages while promoting domestic development. “Implementation” involves creating a system to ensure a reliable primary source of information domestically to evaluate and select emerging technologies. Through “implementation,” more concrete discussions in various fields are also expected. For instance, nurturing domestic experts on safety as well as technology, rediscovering the strengths of existing industries after understanding the limitations of emerging technologies, and evaluating the potential for market co-creation between existing and new industries.

When aiming for the implementation of new technological areas, it is crucial to confront information warfare in collaboration with industry, government, and academia due to the non-public nature of competitive information, the need for extensive expertise to understand information, and the rapid changes in information. The Ministry of Agriculture, Forestry, and Fisheries' Public-Private Council for Food Tech, established in October 2020, plays an important role as one of the existing public-private information-sharing frameworks. The Ministry of Economy, Trade, and Industry has also secured 300 billion yen in the revised budget for the fiscal year 2022 to promote the Biomanufacturing Revolution Promotion Project, supporting the accumulation of domestic knowledge through funding the development of cultivated Wagyu beef as one of the projects.

Despite these ongoing efforts, considering the state of the regulatory framework and corporate initiatives regarding sales, it must be acknowledged that Japan's progress lags behind that of other countries. In Japan, the organization of cultivated food safety concepts and the development of quality-control policies are becoming increasingly important. However, there is no clear legal framework for Novel Food in the country, which makes it difficult for the government to allocate reasonable resources to collect information and consider appropriate rules for the social implementation of cellular agriculture.




Considering the above circumstances, this document was created as part of a project to advance the basis for operating technologies further safely, securely, and stably within the country, by smoothening the communication among public, private, and academic stakeholders. Even though the industry is emerging, JACA recognizes the importance of ensuring that relevant stakeholders are on the same page regarding understanding general and international trends in the risk assessment and management practices for the cultivated food, as much as possible, to build a consensus among them on appropriate domestic measures to be taken. Additionally, Japan lacks a framework for Novel Foods, and cell agriculture is a field where many technologies are still being established, making information gathering challenging. Therefore, JACA believes that the academic and industrial communities should actively organize the “general information” on safety in this field, so that the government can focus on “individual discussions” with each company. Thus, JACA appointed Vireo Advisors to report on the current state of regulatory and safety evaluation of cultivated meat and seafood products.

## ABOUT THE AUTHOR

Vireo Advisors is an expert advising firm working internationally to overcome barriers to new technology commercialization through the development and translation of sound science into practice. Our work advances safer and more sustainable innovations to create a healthier economy. Led by Jo Anne Shatkin, a pioneer in risk assessment and safety analysis for emerging substances, Vireo works with private and public organizations internationally to evaluate the impacts of novel bio-based and nanoscale ingredients and related products, reducing commercialization risk through strategic development of safety testing approaches and studies, including engagement of key stakeholders to ensure regulatory success. Vireo develops methods for establishing the safety of new products across the product life cycle and builds collaborative projects, including among competitors, to address common safety issues.

The Vireo team brings significant experience in cultured meat and seafood safety, including working with companies on safety testing strategies and regulatory packages. Vireo provided regulatory, strategy, and dossier support for the approval of Vow cultured quail in Singapore, and has helped culture media companies, cell line developers, and cultured meat and seafood manufacturers conduct food safety assessments of their products.

Vireo also organizes interactive workshops resulting in peer-reviewed publications with diverse participants, including leading the Cultured Meat Safety Initiative (CMSI) on cellular agriculture with New Harvest, as well as efforts with the Japan Association of Cellular Agriculture (JACA), Organization for Economic Cooperation and Development (OECD), the international Society for Risk Analysis (SRA), and the Tappi Nanotechnology Organization.

**Jo Anne Shatkin, Ph.D. Founder and President**

Vireo Advisors founder and president **Jo Anne Shatkin, PhD**, combines her business acumen and technical expertise into strategies for advancing the commercialization of safe and sustainable advanced technologies that meet the requirements of multiple stakeholders. A recognized expert with more than 25 years of experience in health and environmental risk analysis, emerging technologies, bio-based and nano-enabled technologies, life cycle impacts of materials in the environment and stakeholder engagement, Jo Anne collaborates with governments and industry internationally to help guide responsible product development of a wide range of emerging technologies – from cellular agriculture to the use of nano-enabled, syn-bio, and recycled materials as source material and applications of non-animal testing.

Since 2005, Jo Anne has provided leadership on the responsible development of novel technologies. She has served as an expert to several international committees, including the 2022 FAO/WHO Technical Panel on Food Safety Aspects of Cell-based Food, the National Academy of Sciences Quadrennial Review of the National Nanotechnology Initiative, the NanoRelease Project, the joint World Health Organization/Food

and Agriculture Organization Expert Panel on Nanotechnology in Food, the Council of Canadian Academies, and the US/Russia Bilateral Commission for Science and Technology Nanotechnology Environmental Health and Safety Panel.

Jo Anne is a fellow of the international Society for Risk Analysis (SRA), where she founded the Advanced Manufacturing and Materials Specialty Group, served as an SRA councilor and received several SRA awards. Jo Anne also was the 2019 recipient of the TAPPI Nanotechnology Division Award for Leadership and Service. She serves on the advisory board of the Center for Environmental Policy at American University.

Jo Anne received an individually designed PhD in environmental health science and policy and an MA in risk management and technology assessment from Clark University (Worcester, Massachusetts), and a BS in molecular biology and biotechnology from Worcester Polytechnic University. While working on her doctorate, Jo Anne received an environmental fellowship from the Switzer Foundation.

**Kimberly J. Ong, Ph.D. Toxicologist**

Vireo Advisors toxicologist and sustainability expert **Kimberly Ong, PhD**, collaborates with a wide range of industrial, governmental, and academic organizations on the safety, benefits, and risk assessment of bio-based and other advanced materials and technologies, including cultured meat ingredient and product safety. Kim helps organizations develop efficient strategies to achieve regulatory acceptance toward safe and sustainable commercialization, while meeting stakeholder needs for novel food, consumer and related applications. Experienced in protocol development and *in vivo* and *in vitro* methods, she assesses, develops, and modifies safety-testing protocols for emerging technologies – such as nanomaterials, genetically modified foods and bio-based products – to improve reliability for risk and exposure assessment.

With Vireo Advisors since 2014, Kim has worked with a wide range of industrial, governmental, and academic collaborators on the safety, benefits, and risk assessment of biobased and other advanced materials and technologies. Work has included regulatory authorizations, human health and ecological safety, and risk assessments for internal evaluation and toward efficient commercialization of new products. She was lead author on seminal papers on food safety considerations for the cultured meat and seafood industry (Ong *et al*. 2021; Ong *et al.,* 2023), and is supporting the New Harvest/Vireo Safety Initiative to bring together industry, regulators, academia, non-profits and other stakeholders to address safety questions and stimulate research related to cultured meat. She served as a Vice-Chair the 2022 FAO/WHO Expert Consultation on the Scientific Advice on Cell-Based Food Products and Food Safety Considerations. She helps groups develop and execute strategic roadmaps to thoroughly assess safety of new products and ensure efficient commercialization. Kim has presented at numerous international conferences, meetings and events and helped organize workshops, as well as published book chapters and peer-reviewed publications. She also is active in a number of scientific groups, including serving as a councilor on the executive board of the Canadian Society of Zoologists and as a member of the NRC-NSERC-BDC Nanotechnology Initiative (NNBNI).

Kim received a PhD in physiology, cell, and developmental biology from the University of Alberta (Canada). Her PhD work consisted of testing the biological effects of nanoparticles in embryonic zebrafish and the physiological effects on adult trout, and *in vitro* testing of mammalian and fish cells. She received an MSc in environmental management and policy at Lund University (Sweden) – with a thesis that considered sustainability assessments and their relevance for companies producing or using cellulose nanomaterials – and a BSc in marine and freshwater biology from the University of Guelph (Canada). She also has education and training in environmental management within companies and organizations and with international policies regarding sustainability.

## Table of Contents

## INTRODUCTION

An informed consensus on the necessary safety requirements is essential for the safe, timely adoption and commercialization of cultivated meat and seafood products. Developing safety and regulatory requirements guidance supports consistent and efficient dossier generation and subsequent regulatory review. Harmonization with safety requirements from other regulatory agencies can minimize the burden on companies applying for regulatory approval in different regions.

This report aims to develop a list of safety data documentation recommended by international jurisdictions and experts in the industry.

This deliverable is composed of four sections:

**Section 1. Safety assessment documentation -** A comparison of the safety data documentation provided to, or recommended by, food safety authorities in different jurisdictions.
**Section 2. Manufacturing process and inputs -** A description of manufacturing processes and inputs for different species and cell types.
**Section 3. Hazard identification and controls -** A description of hazards at each manufacturing step, mitigation and preventative measures, and safety tests.
**Section 4. Recommendation -** A summary of recommendations suggested by industry interviewees in combination with recommended information requirements to be included in a regulatory submission for approval of cultivated meat and seafood products.

During the interviews Vireo conducted with international cultivated meat and seafood companies, information on hazards, potential exposure, and risk characterization was collected to complement the information in Sections 2 and 3.

## SECTION 1. SAFETY ASSESSMENT DOCUMENTATION

### Introduction

Section 1 provides a comparative analysis of the data requested to be provided to international regulatory agencies for assessing the safety of cultivated food for human consumption. A comparison was made across the safety recommendations detailed in the most recent regulatory guidance from the Singapore Food Agency (SFA), European Food Safety Authority (EFSA), United Kingdom (UK) Food Standards Agency (FSA), and United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS), South Korea Ministry of Food and Drug Safety (MFDS), the Food Standards Australia New Zealand (FSANZ) Application Handbook as well as the public dossiers reviewed by the US Food and Drug Administration (FDA) that received 'no questions' letters, and the 2023 report, 'Food safety aspects of cell-based food' published by the Food and Agriculture Organization and World Health Organization (FAO/WHO) for regulatory guidance in other jurisdictions (*i.e.,* Qatar, Israel). Finally, we examined any additional safety considerations identified by Dr. Kitajima from the National Institute of Health Science and Professor Igimi from the Tokyo University of Agriculture, the Good Food Institute (GFI), and a publication from Vireo Advisors and New Harvest as part of the Cultured Meat Safety Initiative (Ong *et al*., 2021).

Table 1 summarizes the safety data documentation recommended by international jurisdictions and industry experts.

### Resources used to compare information requirements

The hazard and safety information from the following sources is summarized and compared:

1. The final report of the Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Consultation on 'Food safety aspects of cell-based food'.
2. The two public dossiers that have received 'no questions' letters following pre-market consultation with the US Food and Drug Administration (FDA), including GOOD Meat cultivated chicken and UPSIDE Foods cultivated poultry meat.
3. Two programs by the USDA Food Safety Inspection Service (FSIS) titled 'FSIS Responsibilities in Establishments Producing Cell-Cultured Meat and Sampling Program' and 'Updated Cell-Cultured Meat and Poultry Food Products Sampling Program'.
4. The Singapore Food Agency (SFA) 'Requirements for the Safety Assessment of Novel Food and Novel Food Ingredients July 2023'.
5. UK Food Standards Agency (FSA) 'Hazard identification: Identification of hazards in cultured animal cells'. Note that this is not an official guidance document.
6. Guidance from the European Food Safety Authority (EFSA):
    a. Guidance on preparing and presenting an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283, 2016. Note that this guidance is *not* specific to cultivated meat and seafood
    b. Novel foods: alternative proteins and their sources, EFSA webinar, 2021
    c. Draft guidance on the scientific requirements for an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283, 2024

7. The South Korean Ministry of Food and Drug Safety (MFDS) Notification of partial revision of temporary standards and standards for food, etc. (No. 2024-13, February 21, 2024) which includes 'Scope and preparation instructions for cell cultured food raw material submission data' (in MFDS 2024, Appendix 2).
8. Guidance from Food Standards Australia New Zealand (FSANZ):
    a. FSANZ Application Handbook (July 2019). While this Application Handbook is not specifically about cultivated foods, it mentions the main information required to apply for authorization of a novel food. Cultivated meat and seafood are considered novel foods.
    b. FSANZ Supporting Document 1 (SD1) - Hazard and risk assessment related to the Application A1269 Cultured quail as a novel food (2023). This application was submitted by the Australian company Vow to FSANZ to obtain permission to commercialize Vow cultivated quail as a novel food in Australia/New Zealand. SD1 is the publicly available hazard and risk assessment of Vow's application by FSANZ.
9. Presentations by Dr. Kitajima ("Hazard and risk considerations of the food safety of cultivated food July 2023", translated by JACA) and Prof. Igimi ("Progress of research on risk assessment method for cultivated food, July 2023", translated by JACA), as provided by JACA.
10. Reports and webinars published by the Good Food Institute (GFI) on cell sourcing, cell line establishment, and cultured meat production:
    a. Food safety considerations for cultivated meat (Welch and Swartz, 2019).
    b. Deep dive: Cultivated meat bioprocess design (GFI 2021a)
    c. Deep dive: Cultivated meat cell culture media (GFI 2021b)
    d. Incorporating omega-3s into cultivated seafood (GFI 2021c)
    e. Cultivated meat and seafood - 2022 State of the Industry, webinar (GFI 2022a)
    f. Ensuring appropriate food safety controls for cultivated meat (GFI 2022b)
    g. Cultivated meat and seafood - 2022 State of the Industry Report (Bomkamp *et al.,* 2022)
    h. Cultivated meat science and product development webinar (Krieger 2022)
    i. Deep dive: Cultivated meat scaffolding (GFI 2023a)
    j. Promoting stemness and proliferation in fish cell cultures (GFI 2023b)
    k. Reimagining Meat: Pathways for Cell Biologists in the Cultivated Meat Field, webinar (GFI 2023c)
    l. The science of cultivated meat (GFI 2023d)
    m. Assuring the Safety of Cultivated Meat: HACCP plan development an application to a cultivated meat target-product (Sant'Ana *et al.*, 2023)
    n. Cell line development and utilisation trends in the cultivated meat industry (Ravikumar and Powell, 2023)
    o. Manufacturing cultured fish fillet, webinar (Ferreira 2023)
11. The research article by Ong *et al*. (2021), 'Food safety considerations and research priorities for the cultured meat and seafood industry.'

There are differences in the information requested by each jurisdiction and those identified in non-guidance documentation. Notably, some agencies, such as EFSA, have yet to receive any regulatory applications for cultivated meat or seafood and do not have cultivated meat and seafood-specific guidelines. Agencies such as SFA provide regular updates to its Requirements document. It is anticipated that recommendations and requirements may change as the industry matures.

Table 1 is a summary of the documents and information categories common amongst the majority of regulatory jurisdictions.

Table 1. Documents and information categories that are commonly recommended or required

| Manufacturing step | Documentation/ information | Description | Authority/ Expert |
|---|---|---|---|
| **Cell sourcing** | Cell origin | Description of cell origin (species, biopsy, slaughtered animal, cell line provider, *etc*.) | FDA, SFA, FSA, EFSA, FSANZ, MFDS, Ong |
| | Type of cell | Description of type of cell (GMO, immortalized, stem cell, tissue, *etc*.) | FDA, SFA, FSA, EFSA, FSANZ, MFDS, Ong, GFI |
| | Species identity | Verification of species identity (if sourced from a cell line provider) | FDA, SFA, FSANZ, MFDS, FAO, GFI |
| | Source animal health | Demonstration that biopsies/cell sourcing comply with animal health and food safety requirements. Health of the sample animal (if possible) | FDA, SFA, FAO, GFI, Ong, EFSA, FSANZ, MFDS |
| | Prions | Description of prevention/mitigation steps to avoid prion contamination (if applicable – bovine sources) | FDA, FSA, EFSA, FSANZ, FAO |
| | Analysis of inputs | Listing of substances used (antibiotics, substances for sterilization, *etc.*) and safety assessment | FDA, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong, GFI |
| **Establishment of cell lines** | Cell characteristics | Documentation of cell characteristics, e.g., morphology, cell viability, doubling time, cell stability, cell density, protein yield | FDA, EFSA, MFDS, Ong, GFI |
| | Genetic modification | If genetically modified, description of genetic modification process & safety evaluation | FDA, SFA, FSA, EFSA, FSANZ, MFDS, Ong |
| | Analysis of inputs | Listing of substances used and safety assessment | FDA, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong, GFI |
| | Adventitious agents | Microbiological safety assessment - testing for viruses, bacteria, and mycoplasma | FDA, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong |

| Manufacturing step | Documentation/ information | Description | Authority/ Expert |
|---|---|---|---|
| **Cell storage** | Analysis of inputs | Safety assessment of substances (cryoprotectant, antibiotics, substances for sterilization, *etc*.) | FDA, SFA, FSA, EFSA, FSANZ, MFDS, Ong, GFI |
| | Adventitious agents | Microbiological safety assessment - testing for viruses, bacteria, and mycoplasma | FDA, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong |
| **Mass cultivation: Cell proliferation and differentiation** | Analysis of inputs | Safety assessment of media components, scaffold, and other added substances demonstrating that the substance is food-safe<br>Animal derived components: Documentation demonstrating that animal-derived substances do not contain disease-agents or other hazardous substances<br>Biological agents: Documentation demonstrating safe use<br>Components derived from genetically modified organisms: Documentation demonstrating safe use | FDA, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong, GFI |
| | Cell contamination | Monitor for microbiological or chemical contamination | FDA, SFA, FSA, EFSA, FAO, Ong, GFI |
| | Chemical contaminants | Mitigation or measurement of chemical contaminants from equipment, cleaning products, ingredients, *etc*. | FDA, SFA, FSA, EFSA, FAO |
| | Genetic stability | Monitor genetic stability | FDA, SFA, FSA, EFSA, FAO |
| **Cell harvest** | Composition | Analysis of nutritional composition (proximate, amino acid, vitamins, minerals, fatty acids) | FDA, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong |
| | Residue analysis | Measurement of potentially hazardous residues and safety assessment | FDA, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong, GFI |

| Manufacturing step | Documentation/ information | Description | Authority/ Expert |
|---|---|---|---|
| | Washing efficiency | Assessment of the efficacy of the media removal steps | SFA, FSANZ, FDA (Only GOOD Meat), MFDS |
| | Adventitious agents | Measurement of viruses, bacteria, yeast, mold | FDA, FSIS, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong, GFI |
| | Genetic stability | Assessment of genetic stability and assess potential production of unintended toxins or allergens | FDA, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong |
| | Tumorigenicity | Assessment of tumorigenicity | FDA (GOOD Meat – testing; UPSIDE – preventative controls), MFDS |
| | Chemical contaminants (from environment) | Measurement of chemical contaminants from equipment, cleaning products, ingredients, *etc*. | FDA, FSIS, SFA, FSA, EFSA, MFDS, FAO, Ong, GFI |
| | Chemical contaminants (from inputs) | Measurement of chemical contaminants (e.g., heavy metals, etc.) | FDA, FSIS, SFA, FSA, EFSA, MFDS, FAO, Ong, GFI |
| | Food allergens | Assessment for food allergens | FDA, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong, GFI |
| | Toxicity testing | Acute, sub-chronic, and chronic dietary toxicity testing, genotoxicity, carcinogenicity, reproductive and developmental toxicity | SFA, FSA, EFSA, FSANZ, MFDS (if needed) |

| Other information | Documentation/ Information | Description | Authority/ Jurisdiction |
|---|---|---|---|
| **Estimated dietary intake and Intended use** | Use level | Proposed maximum use level/serving size portion, or calculation of potential exposure | FDA, SFA, EFSA, FSANZ, MFDS |
| **History of safe use** | History of safe use | Description of the history of use and safe consumption for food ingredient safety assessment | FDA, SFA, FSA, EFSA, FSANZ, MFDS, FAO, Ong |
| **Shelf-life** | Shelf life | Shelf-life analysis | FDA (partial), FSA, EFSA, FSANZ, MFDS |
| **Food safety programs** | Food safety programs | Description of food safety programs, including Good Manufacturing Practices (GMP), Hazard Analysis Critical Control Points (HACCP), Hazard Analysis and Risk-Based Preventive Controls (HARPC), Quality control measures, Good Cell Culture Practices (GCCP) | FDA, FSIS, SFA, FSA, EFSA, FSANZ, MFDS, Ong, GFI |
| | | Training plans and records of staff members in food safety/food handling/food hygiene courses and aseptic techniques or cleanroom training. | FDA, SFA, FSA, GFI |
| | | Safety documentation for raw materials | FDA, EFSA, FSANZ, Ong |
| | | Production control and quality and safety assurance | FDA, SFA, FSA, EFSA, FSANZ, FAO, Ong, GFI |
| | | Supplier Approval Program | FDA, FSANZ, GFI |
| | | Sanitation controls, and sanitary design of equipment and tools | FDA, FSA, FSANZ, Ong, GFI |

## Description of safety documentation/information

All jurisdictions require the submission of detailed information on the manufacturing process to ensure a consistent and safe product for human consumption.

### Cell sourcing

Regulatory agencies require companies to describe the cell origin (species, whether cells were sourced via biopsy, whether the cells originated from a slaughtered or live animal or cell line provider, *etc.*) and the type of source cells (stem cell, immortalized cells, *etc*.). If sourced from a cell line provider, companies may conduct a species identity analysis. Information may be required to demonstrate that the source animal was healthy and did not harbor any disease or vectors of disease. If cells are from cows, documentation certifying sourcing from prion-free herds may be collected. A risk analysis of inputs used for cell sourcing may be conducted.

### Cell Isolation and establishment of cell lines

Once sourced, cells are developed into the desired starting cell types through isolation, culture, and optimization. Companies may evaluate the cell characteristics (morphology, cell viability, doubling time, cell stability, cell density, protein yield, *etc*.).

Some companies may genetically modify the cells. If this is the case, the companies conduct a safety evaluation of the genetic modification*, e.g.,* whether the modification affects cell morphology, cell phenotype, or cell metabolism, could cause unintended changes in cell proliferation and differentiation, or result in the production of unintended substances. If GM cells are used, then safety information is required (*e.g.,* Codex Guidelines on the Conduct of Food Safety Assessment of Foods Produced Using Recombinant DNA Microorganisms (CAC/GL 46-2003), or Derived from Recombinant DNA Animals (CAC/GL 68-2008), Singapore Guidelines on the Release of Agriculture-Related Genetically Modified Organisms (GMOs) (GMAC 1999)):

i Detailed procedures of the genetic modification process.

ii An evaluation of whether genetic modification would give rise to any significant changes resulting in additional food safety hazards (*e.g.,* the presence of toxins or allergens, or effects on nutritional quality). This includes the genetic stability of the production strain.

iii Risk assessment and risk management measures to address food safety hazards present or introduced due to (i).

iv Safety information of the host/recipient strain (*e.g.,* Whole Genome Sequencing and proteomics data to investigate whether genes are known to produce toxins or allergens).

v Genome characterization to determine the absence of virulence-related genes and antibiotic resistance genes and their potential horizontal transfer, and other potentially adverse metabolic features such as toxin production.

vi Any documented history of use with absence of adverse effects to human health.

A risk analysis of inputs used for cell isolation and cell line establishment may be conducted. Testing or monitoring for adventitious agents may be conducted.

**Cell banking and cell storage**

Cells are stored in Master or Working Cell Banks as the starting cells for future production. Safety assessment of the inputs used during this step (cryoprotectant, antibiotics, *etc*.) is conducted. Companies may conduct an adventitious agent safety assessment of the cell banks, including tests for viruses, bacteria, and mycoplasma.

**Mass cultivation: Cell proliferation and differentiation**

During the mass cultivation stage, cells are proliferated on a large scale to increase the biomass. Some companies may also differentiate their cells.

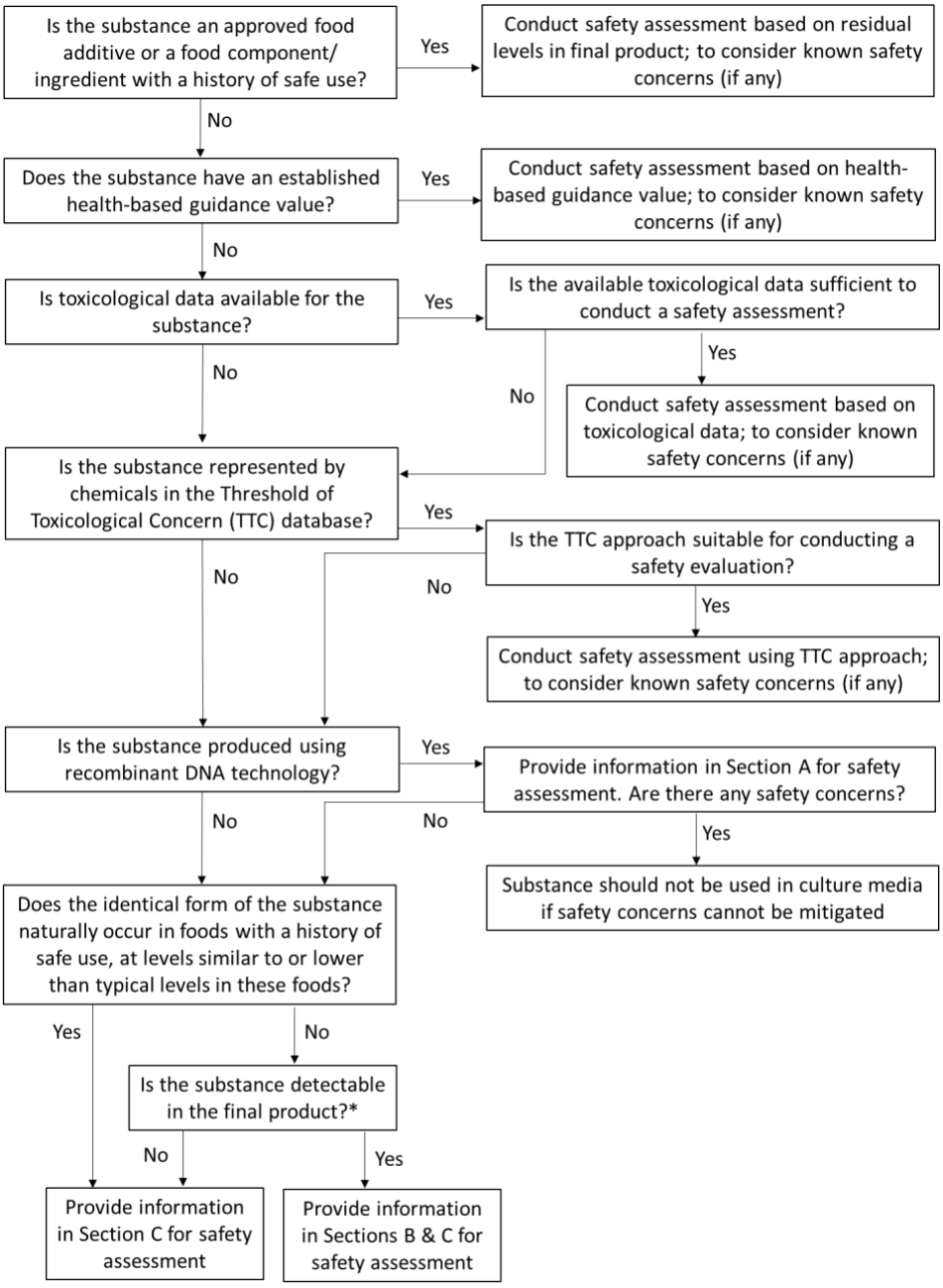
Companies generally conduct a safety assessment of inputs such as media components, scaffold and other added substances (*e.g.*, antibiotics, surfactants) to demonstrate the safe use of these substances under the intended conditions of use.

In general, the safety assessment includes documentation to demonstrate that the substances are safe for use in food, including identity and purity testing and a description of all potential unintended metabolites that could be produced. A risk assessment of inputs may include a combination of:

- Classification of input materials according to regulatory status;
- *in silico* evaluation of safety based on chemical structure or activity;
- Comparison to levels in conventional foods;
- 'Worst-case' theoretical calculation of inputs in the final product; and/or
- Measurement of the substance in the final product.

While many companies plan to produce cultivated meat and seafood products on a large scale in serum- and animal-free media, some currently use animal-derived components, such as fetal bovine serum (FBS) or bovine serum albumin (BSA). Safety information may include documentation demonstrating sourcing from countries with low prion risk, sourcing from healthy herds, and testing for zoonotic viruses, bacterial contamination, and mycoplasma. In addition, companies may sterilize these components before use.

SFA provides a flowchart for evaluating the safety of biological substances (*e.g.,* growth factors) in cultivated meat and seafood (Figure 2).




**The detection methodology and limit of detection must be comparable to scientifically established detection methodologies for the substance*

Figure 2. SFA safety assessment approach for biological substances used in media for cultivated meat or seafood production (SFA 2023).

If companies use inputs derived from genetically modified (GM) organisms, they must evaluate the safety of the GM organism and the resulting substances. The SFA provides guidelines on this process, summarized in Table 2.

Table 2. Summary of SFA recommended safety assessment approach for substances produced using GM organisms

| **Information** | **Details** |
|---|---|
| Characterization of GM organism | Scientific name, genetic modification process, safety and pathogenicity of the recombinant host, history of safe use |
| Purpose | Purpose of addition of substance to media |
| Characterization of protein | Primary sequence of the recombinant protein and comparison to native protein, evaluation of transgenes, tags or sequence modifications (allergenicity or toxicity concerns) |
| Purity | Chemical purity, protein purity, presence of recombinant DNA, specifications, toxicity or allergenicity concerns with any impurities |
| Residue | Level present in cultivated meat and comparison to levels in conventional food |
| Activity | Mode of action of the substance in the human body, impact of food processing (*e.g.*, denaturation during cooking), potential absorbance into the body and impacts on physiology |

Documentation is required demonstrating that the company is monitoring and testing for microbiological and chemical contamination throughout the mass cultivation process.

The cell lines are monitored for consistent growth characteristics, such as morphology, cell viability, doubling time, cell stability, cell density, protein yield, nutrient usage, *etc.* to verify genetic stability.

**Cell harvest**

After cell harvest, companies conduct a thorough safety assessment of the harvested cells.

A composition analysis, such as proximate (protein, fat, carbohydrate, moisture), amino acid, vitamin, mineral, and fatty acid analysis, may be conducted and compared to conventional meats or seafood to identify compositional differences. Any differences may be evaluated to demonstrate that the differences do not pose a food safety risk. In the FDA's Cell Culture Consultation Review Memos for UPSIDE and GOOD Meat, it was noted that the FDA, "…did not consider the establishment of exact equivalence of all nutrients and components relative to a particular conventional comparator as a necessary component of UPSIDE's/GOOD Meat's safety conclusion".

Measurement and documentation of potentially hazardous residues from the media or other input is necessary. A full risk assessment is generally required for any substances in the harvested cells, demonstrating that the substances do not pose a food safety risk. If a company claims that a residue is fully removed, information is required demonstrating the removal of these substances.

If a company washes the cells post-harvest, information on the washing efficiency for specific components may be provided. GOOD Meat submitted a chemical analysis of specific inputs in the final wash solution to evaluate the efficiency of the wash solution. UPSIDE submitted a chemical analysis demonstrating that the PBS wash solution was removed from the cultivated chicken. The SFA Requirements document requests, "information demonstrating the removal of culture media and/or added substances (if these are removed completely)".

Microbiological testing is necessary to demonstrate that the cells are free of harmful adventitious agents.

Testing and documentation may be provided to evaluate the genetic stability of the product. There has yet to be a standard approach to assessing genetic drift. Cells may be monitored for abnormal or inconsistent growth and unintended physicochemical changes. FDA has accepted data demonstrating that cell lines are stable on the basis of consistent growth and viability, cell-cycle checkpoints, response to cues for differentiation, karyotypic stability, or transcriptomics analysis. SFA recommends information to demonstrate that Good Cell Culture Practice (GCCP) has been applied to ensure the reproducibility and consistency of cellular products. This may include karyotyping and close monitoring for variations in growth rates, nutrient usage, and biomass composition in the end product. In addition, SFA provides flexibility in demonstrating that genome instability and genetic drift would not result in the production of undesirable substances through a combination of strategy (1) AND strategy (2) or (3):

(1) By conducting a systematic scientific literature review to identify all known undesirable substances of food safety concern associated with the animal species of the cell culture and establish a list of such substances for subsequent targeted analysis.

(2) By performing an *in-silico* genome screen against relevant databases to establish a list of potential toxins/allergens for subsequent targeted analysis.

(3) By carrying out a quantitative comparison of the end-product cells against the starter cells through methodologies such as transcriptomics, proteomics or metabolomics so that a list of differentially expressed undesirable substances of food safety concern can be established for subsequent targeted analysis.

No regulatory authorities have provided guidance on how to interpret genetic stability data.

Companies may include an evaluation of tumorigenicity evaluation, or justify the lack of tumorigenic potential, for example, due to the absence of viable cells in the final product.

**Industry and expert views on genetic stability and tumorigenicity**

There is not yet consensus on the approach to evaluate cell stability. It is noted that there is natural variation between animals used for conventional meat and seafood, yet testing for genetic stability is not a requirement to demonstrate the safety of meat from conventional animals. Some companies have the view that testing for genetic stability (*i.e.,* with -omics approaches) should not be a requirement. Some companies suggest that the evaluation of phenotypic markers and identification of potential toxic metabolites or allergens that the cells have the capability to produce is adequate to demonstrate the genetic stability and safety of cultivated cells.

The FAO Technical Panel (FAO 2023) concluded that cancer risk from consuming cultivated foods is low due to an inability of cells to survive after harvest and consumption, and there is low potential for viable cells to enter and proliferate in the human body. As stated, "…current scientific knowledge does not support the plausibility of human cancer contagion via introduction of cells". Companies generally agree that tumorigenicity testing is unnecessary due to the low food safety risk in cultivated cells.

Any potential chemical contaminants from the environment (*e.g.,* from equipment, cleaning products) and from the inputs are evaluated for food safety risk.

Finally, the risk assessment includes evaluating any potential allergens produced by the cells during manufacture, introduced from inputs, or resulting from cross-contamination. Labeling is used to communicate the presence of allergens.

**Other information**

Some jurisdictions may use proposed use levels, whereas others may use historical consumption data on similar products (*e.g.,* conventional chicken or ready-to-eat products) to conduct risk assessments.

All jurisdictions require cultivated food companies to have a food safety management system, such as Good Manufacturing Practices (GMP), Good Cell Culture Practices (GCCP), Hazard and Critical Control Point Analysis (HACCP), or Hazard Analysis and Risk-Based Preventive Controls (HARPC, specific to the US). The SFA does not specify requirements for companies to adopt particular types of food safety management systems but requires companies to have a robust food safety management system.

### Documentation recommended by select jurisdictions or experts

There are some potential hazards for which some jurisdictions may have different documentation and testing requirements. Some hazards have been identified by expert groups not currently part of regulatory guidance or review. These include: shelf-life testing, standardized *in vitro* and *in vivo*

toxicity tests, assessment of residual agricultural hormones, quantification of scaffold materials, detection of microbial toxins, and presence of microplastics.

SFA does not require shelf-life testing data to be submitted with a novel food application. However, the data must be available on demand to SFA once the novel food has been reviewed and permitted for sale in Singapore. In the US, UPSIDE Foods provided information regarding its approach to evaluating shelf-life in the public dossier, though UPSIDE did not provide actual shelf-life test data. GOOD Meat provided shelf-life stability data (proximate composition and oxidative state) after freezing and packaging cultivated chicken. FDA did not address shelf-life data in the official Scientific Memos summarizing the FDA evaluation of the GOOD Meat or UPSIDE dossiers. Typically, EFSA does require shelf-life data for novel foods.

Regulatory agencies such as SFA have noted that conventional toxicity testing such as standardized acute, sub-chronic, or chronic feeding studies, carcinogenicity, mutagenicity, reproductive, developmental, and genotoxicity studies may be required if there is, "[…] insufficient data to define with certainty the toxicological profile of the product/ingredient/chemical/molecule under evaluation". No conventional toxicity tests were submitted as part of the FDA UPSIDE or GOOD Meat dossiers. The South Korea MFDS Temporary Standards list the single-dose toxicity testing, the 90-day repeated dose toxicity test, and genotoxicity tests as types of data to be submitted (with reproductive and development toxicity, antigenicity, immunotoxicity, and carcinogenicity tests listed as 'additional information if the safety cannot be confirmed with submitted data.')

**Industry views on conventional toxicity testing**

Standard toxicity tests have historically been conducted for novel food additives. Experts and regulatory agencies have raised concerns that standard testing of whole foods such as cultivated meat or seafood products presents technical challenges and difficulties in relating the toxicity testing results of whole food products to individual substances present in the product (Ong *et al*., 2021, SFA 2023). Companies have expressed the view that toxicology studies are not necessary or appropriate for these products because whole foods do not fit into a paradigm focused on analyzing individual ingredients. Rather, the safety of the finished product may be established through comparative analysis and characterization of the safety of the discrete ingredients used in production, such as media components. Attempting to conduct toxicity tests on the complete chemical composition of a finished meat product is neither practical nor scientifically meaningful. Any attempt would require the consideration of hundreds of analyte tests that have no bearing on the safety of the consumer.

Animal-derived inputs may contain residual levels of synthetic hormones. For example, bovine serum may contain residual concentrations of synthetic hormones administered to the cattle from which the serum was derived. GOOD Meat tested their cultivated chicken product to confirm that any residual synthetic hormones were below approved Maximum Residue Limits (MRLs, or the maximum acceptable levels of veterinary drugs permitted in food and agricultural products in the

US). UPSIDE did not submit these data in the FDA public dossier; SFA does not include this testing in the SFA Requirements document. However, if a company has identified synthetic hormones as a potential food safety issue in their product, then this type of analysis may be considered.

The FAO/WHO Expert Consultation identified additional hazards, including microbial toxins and microplastics. To date, no regulatory agencies have required data collection for these potential hazards.

Microbial toxin data has not been included in regulatory agency guidance documents or in public dossiers. Microbial toxins are toxic compounds naturally produced by some microbes under certain conditions. Microbial toxins may be present due to microbiological contamination during production or can be introduced from host animals used for cell sourcing. For example, some types of fish and shellfish can harbor symbiotic microorganisms capable of producing toxins. Existing requirements for cell sourcing and microbiological contamination testing may eliminate the need for specific microbial toxin testing. If cell sourcing documentation confirms that the source animal was healthy and testing demonstrates a lack of microbiological contamination during manufacturing or in the final product, then the likelihood that microbial toxins are present is low. If a manufacturer uses a species known to harbor symbiotic microorganisms capable of producing toxins as a source animal, companies may need to demonstrate the absence of microbial toxins.

It is noted in the FAO/WHO report that microplastics in food are not unique to cultivated meat and seafood products, are ubiquitous, and are a potential concern for most food products derived from plants, animals, and seafood. Microplastics are not typically evaluated in conventional or other novel foods. Similarly, regulatory agencies have not identified a need to conduct this evaluation in cultivated meat and seafood products.

## SECTION 2. MANUFACTURING PROCESSES AND INPUTS

### Introduction

This section describes the findings of a literature review of papers published between 2020 and 2023 describing the procedures and substances used to manufacture different cultivated meat and seafood products, and information from surveys and interviews with companies. Here we summarize information on procedures reported for different species (*e.g.,* chicken, beef, pork, seafood), cell types (*e.g.,* embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), myoblasts/myosatellite cells, fibroblasts, mesenchymal stem cells (MSCs)), and approaches to optimized cells (*e.g.,* immortalization, genetic modification). This section also includes insights from companies shared during the interviews. Some case studies were developed from the responses collected during company interviews and the literature review.

### 2.1. Cell Sourcing

Cell sourcing is the process of selecting a species and obtaining the desired cell type for cultivated meat production (FAO 2023). Bovine, porcine, poultry, seafood, and fish represent some of the most common cell lines for cultivated meat production (Reiss *et al.,* 2021). Cells that can be used to produce cultivated meat include mature cells like fibroblasts; unipotent (satellite) cells; multipotent (adult) stem cells like mesenchymal stem cells; and pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). In general, cells with lower differentiation capacity (*i.e.,* can give rise to few cell types) possess higher proliferation capacity but are easier to obtain (*e.g.,* can be isolated through muscle biopsy). Meanwhile, cells with higher differentiation capacity are less proliferative but may be more challenging to obtain (*e.g.,* they must be extracted from embryos or bone marrow).

#### *2.1.1. Anatomical sources for different cell types*

Fibroblasts may be obtained from embryos or a skin or skeletal muscle tissue biopsy from a live or recently slaughtered animal (or in the case of fish and seafood, the aquatic animal can be euthanized via ice bath). Similarly to fibroblasts, muscle satellite cells (*i.e.,* myosatellite cells, muscle stem cells) may also be obtained from a muscle tissue biopsy (Reiss *et al.,* 2021). Mesenchymal stem cells are commonly isolated from bone marrow but can also be found in skeletal muscles and adipose tissue (Reiss *et al.,* 2021).

ESCs and iPSCs are isolated from the inner cell mass of developing embryos (blastocysts) and generated by somatic cell reprogramming, respectively. ESCs and IPSCs can differentiate into any somatic cell type (Reiss *et al.,* 2021). They are highly desired for cultivated meat and seafood production due to their ability to replicate indefinitely (Huang *et al.*, 2014; Hochedlinger and Jaenisch, 2015).

Companies may source their cells from cell line providers rather than performing biopsies themselves. Cell lines from some species commonly consumed by humans (*e.g.,* bovine, avian) are

available (ATCC, Cellosaurus). However, commercial cell lines may require further development (*e.g.,* adaptation to serum-free media, adaptation to suspension culture, optimization of sensory characteristics) in order to be suitable for cultivated meat production. Additionally, fewer fish and crustacean cell lines exist than cell lines from species more commonly used for biomedical/pharmaceutical applications (Rubio *et al.,* 2019, ATCC, Cellosaurus). Fish cells have a high propensity for spontaneous immortalization due to their high telomerase activity and regenerative capacity, so in-house development of fish cell lines might give companies more flexibility in cell line development (Futami *et al*., 2021).

UK-based biotech firm PluriCells offers several pluripotent ESC lines from pigs, sheep, and cattle for cultivated meat production. The cell lines are available under research and commercial licenses (Sorrells, 2023). UPSIDE Foods cultivated chicken is isolated from a myoblast cell line derived from adult chicken muscle tissue or a fibroblast-like cell line derived from skin tissue from mid-stage fertilized chicken eggs (UPSIDE 2021). The GOOD Meat master cell bank is derived from the fibroblast chicken cell line UMNSAH/DF11 deposited at the American Type Culture Collection in 1996 (GOOD Meat 2022). Mosa Meat uses muscle satellite cells (Mosa Meat).

### *2.1.2. Advantages/disadvantages of different cell types*

Each cell type (*i.e.,* fibroblasts, satellite cells, mesenchymal stem cells, ESCs, and iPSCs) has specific advantages and limitations for cultivated meat production. Some key advantages of using fibroblasts for cultivated meat production include the availability of well-established cultivation protocols and the availability of numerous fibroblast cell lines derived from agriculturally relevant species (*e.g.,* bovine, porcine, avian) (ATCC, Cellosaurus). Fibroblasts also possess other qualities that may be advantageous for developing cultivated meat. For example, fibroblasts can be transdifferentiated into muscle cells or reprogrammed into induced pluripotent stem cells (iPSC) (Zhao *et al.,* 2015, Lee *et al.*, 2023a, Ito *et al.,* 2017). Fibroblasts also promote the proliferation and differentiation of some cell types when co-cultured and may therefore facilitate the development of a cultivated meat product comprised of multiple cell types (David *et al.,* 2023). Lastly, the ability of fibroblasts to secrete components of the extracellular matrix may provide additional texture, structure, and nutritional value to cultivated products (Guan *et al.,* 2022). The primary drawback of using fibroblasts for cultivated meat is the limited proliferation capacity of primary fibroblast cells, which need to be immortalized to be suitable for cultivated meat production (Pasitka *et al.,* 2022). Furthermore, fibroblasts exhibit limited differentiation capacity unless reprogrammed into induced pluripotent stem cells (Zhao *et al.,* 2015).

Satellite cells are capable of self-renewal and possess greater proliferative capacity than terminally differentiated cells but lower proliferative capacity compared to multipotent (*e.g.,* mesenchymal stem cells) and pluripotent stem cells (Yin *et al.,* 2013). Immortalization of satellite cells may increase their proliferative capacity (Stout *et al.,* 2023). Some companies may produce cultivated meat from muscle satellite cells that have not been immortalized, as immortalized cells may not be able to differentiate efficiently into mature tissues (Mosa Meat 2022).

Mesenchymal stem cells have been isolated from several agriculturally relevant species, including bovine, galline, ovine, piscine, and porcine organisms (Reiss *et al.,* 2021). One of the advantages of using adult stem cells is the ability to differentiate into cell types present in the tissue environment, including skeletal myocytes, adipocytes, chondrocytes, and fibroblasts. However, mesenchymal stem cells have limited proliferative and differentiation capacity compared to ESCs and iPSCs (Reiss *et al.,* 2021). Methods for immortalizing human mesenchymal stem cells may be adapted to animal cells (Merlo *et al.,* 2022, Stricker *et al.,* 2021).

Unlike fibroblasts and adult stem cells, ESCs and iPSCs are pluripotent and possess unlimited proliferation capacity. ESCs have been isolated from bovine, galline, ovine, piscine, and porcine species, while iPSCs have been derived from bovine, galline, ovine, and porcine cells (Reiss *et al.,* 2021). ESCs and iPSCs have a short doubling time compared to adult stem cells (Chatterjee *et al.,* 2015). However, ESCs are often challenging to obtain, as they must be isolated from a specific stage within embryonic development (*i.e.,* the blastocyst stage), and isolation procedures can be laborious, expensive, and time-consuming (Khan *et al.,* 2018). Meanwhile, iPSC reprogramming tends to result in a low yield of iPSCs, and further studies are required to comprehensively characterize reprogrammed cells, especially those derived from livestock species (Reiss *et al.,* 2021). Additionally, protocols for differentiating ESCs and iPSCs into relevant progenitor and mature cell types may need to be developed or adapted from those available for human and mouse cell lines (Reiss *et al.,* 2021).

### 2.2. Cell Isolation

Following biopsy, the desired cell types must be isolated within the sample (Figure 3). Two standard methods for cell isolation are adhesion or enzyme digestion:

1. Adhesion: Target tissue is harvested from the animal, dissected into small sections, and allowed to attach to a tissue culture surface. The cells within the explant proliferate, migrate, and adhere to the substrate.
2. Enzyme digestion: Digestive enzymes are incubated with the dissected explants to degrade the tissue into single cells in suspension. For example, muscle cells are isolated from larger pieces of muscle via enzymatic digestion using trypsin or collagenase to release cells from muscle samples (FAO 2023).

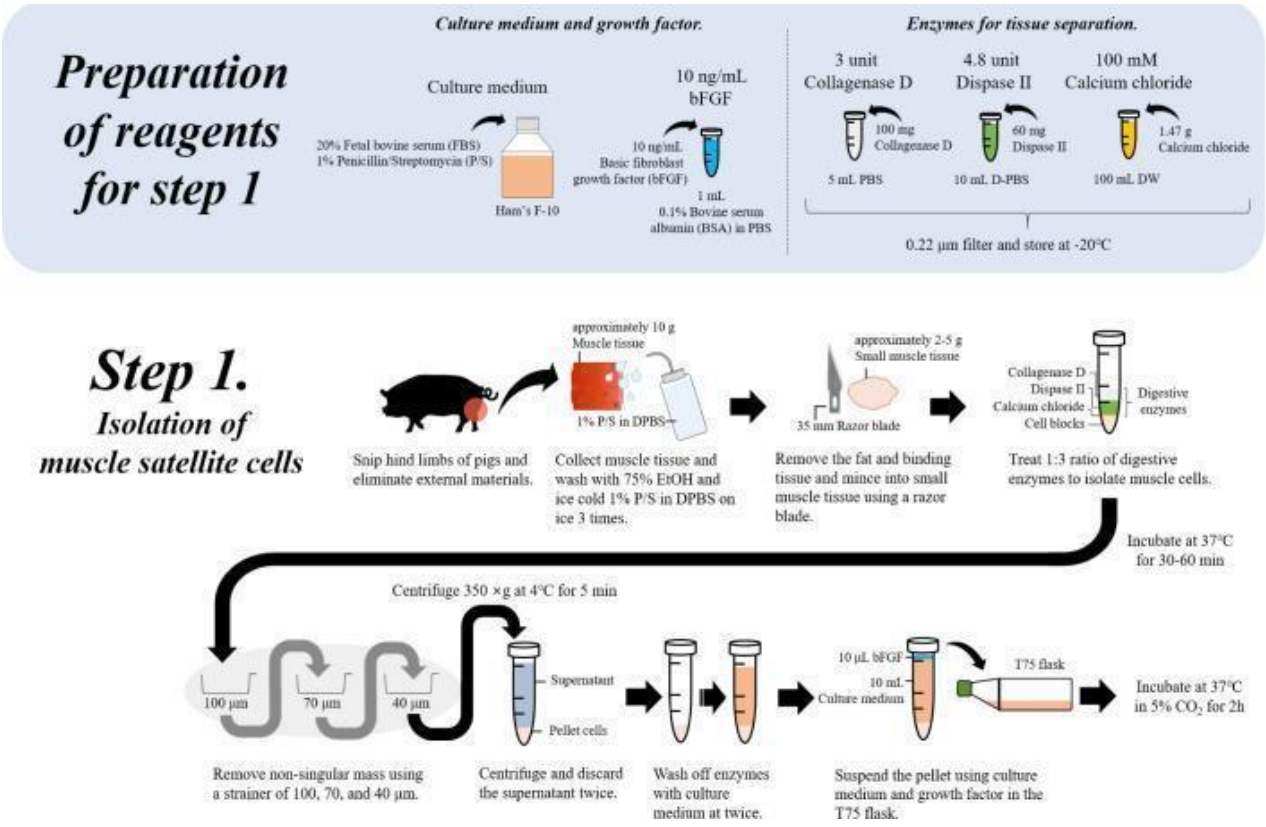



Figure 3. Representative isolation of muscle satellite cells (Lee *et al.,* 2021a).

## 2.3. Establishment of cell lines

Cells may undergo further development following cell sourcing and isolation to generate robust production cell lines. Cell lines capable of prolonged cultivation, suspension growth, and growth in serum-free media are highly desirable for cultivated food production. Approaches to cell line development include genetic engineering and the selection of spontaneous mutants through serial passaging. The time required for cell line development can vary depending on the availability of established protocols for cell cultivation and genetic manipulation. Knowledge of mammalian cell lines is widely available; however, there is a lack of academic literature on continuous fish cell lines, making it more of a challenge for companies to establish cell lines and optimize media (GFI 2023b).

Common methods for cell line immortalization include serial passaging and genetic modification. Serial passaging selects for cells that have acquired spontaneous mutations, increasing proliferative capacity. Cell line immortalization through serial passaging may be accelerated by exposure to chemical or physical mutagens. Immortalization can also be achieved through genetic engineering to introduce viral oncogenes (*e.g.,* SV40 Large T Antigen) or increase the expression of endogenous oncogenes (*e.g.,* telomerase reverse transcriptase) (Guo *et al.,* 2022). Different methods for cell line establishment may also vary in their likelihood of producing off-target or pleiotropic effects. CRISPR/Cas9-mediated mutagenesis, for example, can be used to generate cell lines with more precise genetic modifications than chemically- or physically-induced mutagenesis.

UPSIDE and GOOD Meat describe the use of immortalized cell lines in their dossiers to the FDA for pre-market consultation. Specifically, UPSIDE Foods cultivated chicken can be produced from chicken myoblast, and fibroblast-like cell lines immortalized spontaneously by selection in culture or by constitutive expression of the TERT protein. GOOD Meat cultivated chicken is produced from a spontaneously immortalized fibroblast cell line.

Stem cells are also being explored for cultivated meat production. Stem cells may be isolated as primary cells from the source animal or be generated from other cell types, like fibroblasts, through cell reprogramming (*i.e.,* induced pluripotent stem cells (iPSCs)). Different strategies for stem cell reprogramming differ in their use of genetic modification or potentially hazardous chemicals. Reprogramming factors may be delivered in the form of DNA through viral vector- or transposon-based systems (*e.g.,* through the use of lentivirus) or as a protein, small molecule, or RNA (Bailly *et al.,* 2022). iPSCs have been generated from some mammalian species, but iPSCs from non-mammalian species are challenging and not yet available (Rosselló *et al.,* 2013).

Induced pluripotent stem cells (iPSCs) are produced by reprogramming somatic cells to express the Yamanaka factors, which are a range of transcription factors (OCT4, KLF4, C-MYC, and SOX2). These factors can be expressed using viral vectors, episomes, or mRNA transfection. These reprogramming techniques may result in the unintended expression of other factors and cause genetic modifications (Zehorai *et al.,* 2023).

Embryonic stem cells (ESCs) present specific challenges related to serum replacement in cell media, cell differentiation, and specific nutrient needs across different species. One challenge specific to ESCs is establishing the ideal cell media for maintaining a stable karyotype, pluripotency, and stable transcriptome (Bogliotti *et al.,* 2018). ESCs usually need specialized growth cell media and tighter controls to mature into the desired tissue types, such as muscle and fat. Several environmental factors play a critical role in promoting ESC differentiation. Specific growth factors, scaffolds, oxygen tension, and mechanical stimulation work cooperatively to direct stem cell differentiation into desired cell types (Brown *et al.,* 2013).

Adapting cells to grow in media and in suspension remains a technological challenge for companies. Different cell lines require different media additives for efficient cell adaptation. Media must be tailored to facilitate fast cell adaptation while maintaining cell purity and specific cell signaling pathways. Research suggests that cell lines from marine fish are easier to establish in conventional (*i.e*., mammalian) media than freshwater fish (GFI 2023b). Developing one media standard for adapting cultivated meat cells is not feasible. For more details on culture media inputs, see *Section 2.5.2.*

### 2.4. Cell banking and cell storage

Cell lines are stored by cryopreservation in cell banks. Successful cryopreservation and cell recovery are achieved through slow freezing and quick thawing. Single clone isolates are expanded, re-suspended in cryopreservation media, and frozen as aliquots to generate the Master Cell Bank

(MCB). Individual vials of MCBs can then be used to create the Working Cell Bank (WCB) (Ong *et al.,* 2021).

Banked cells can be stored in liquid-phase nitrogen, vapor-phase nitrogen, or, less commonly, in a specialized electric freezer at ultra-low temperatures. Some cryopreservation media contain animal serum, though animal-free alternatives exist and are widely used, such as Cell Freezing Medium-DMSO Serum free 1x (Sigma-Aldrich).

### 2.5. Mass cultivation: Cell proliferation and differentiation

Scale-up of cultivated meat production is typically carried out through a seed train culture process (Figure 4 and Figure 5). Cells are initially grown at small volumes and are progressively scaled up by successive rounds of passaging into larger cultivation systems to increase cell quantity (Figure 4) (Allan *et al.,* 2019). Based on available industry information, it is possible to cultivate meat on a large scale using bioreactors. For instance, Mosa Meat's facility in Maastricht (Netherlands) uses 1000L bioreactors. There have been announcements to scale up to much larger bioreactors, such as 250,000L, though this is yet to be demonstrated (GOOD Meat 2022a). Production at large-scale may involve using microcarriers or an alternative substrate for anchorage-dependent cells.

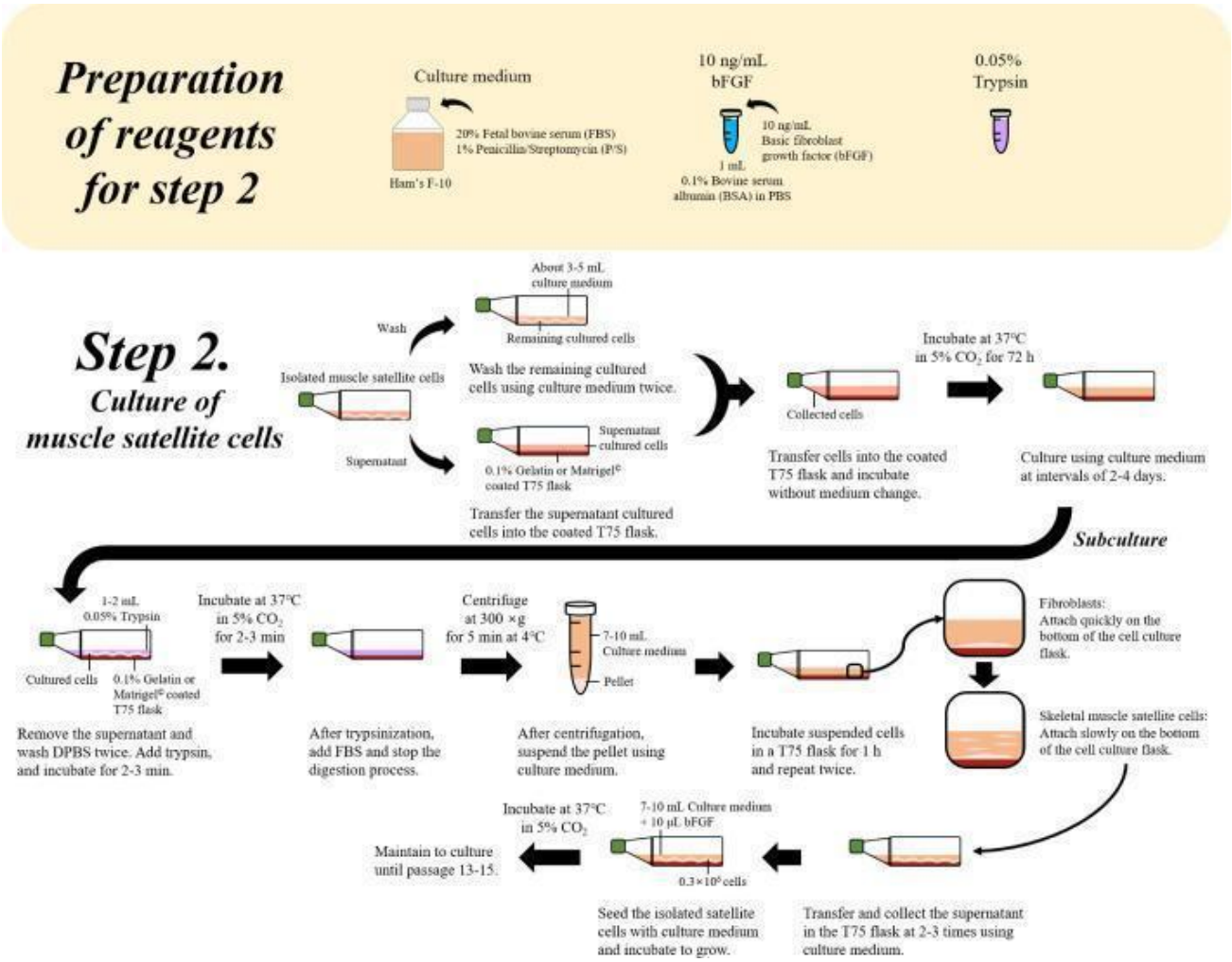



Figure 4. Cultivation of muscle satellite cells at laboratory-scale (Lee *et al.,* 2021a).

Modes of operation for bioreactor cell culture processes include (GFI 2021a, Mayrhofer and Kunert, 2020):

1. Batch culture: A vessel is filled with a fixed volume of media and cells grown to their maximum density and harvested or transferred to a larger vessel in one batch.
2. Fed-batch culture: A vessel is fed fresh media at variable rates to maximize cell growth or cell densities.
3. Continuous culture: A vessel is fed fresh media at an optimized flow rate, and cells may be collected continuously, resulting in a constant cell density.
4. Perfusion culture (a type of continuous culture): Cells are kept in the vessel by a cell retention device, and the medium is harvested, processed (*i.e.,* toxic waste products are removed and nutrients are replenished), and reused. Perfusion bioreactors therefore enable higher viable cell densities for a given bioreactor volume compared to other modes of operation.

### *2.5.1. Bioreactors*

Manufacturing cultivated meat at scale will require the use of large bioreactors. Stirred tank reactors (STRs) are the most widely used bioreactors (GFI 2021a). Cells in STRs are grown in suspension via mechanical stirring, which maintains a high mass transfer of oxygen. Microcarrier-based suspension culture can be used to grow anchorage-dependent cells in STRs. Another commonly used bioreactor is the rocking bioreactor. For example, German Celltainer Biosolutions GmbH is collaborating with Mosa Meat to develop a scalable bioprocess using a rocking bioreactor to produce cultivated meat (Celltainer Biotech 2020).

Other reactors that are being tested are packed bed, airlift/aerated, fluidized bed, and hollow-fiber bioreactors, the latter two being more complex to build (Hubalek *et al.,* 2022, Ellis 2021, Bellani *et al.*, 2020, Mendonca da Silva *et al.,* 2020).

- Packed bed bioreactor – The vessel is packed with fixed microcarriers or porous fibers. Cells are seeded on the packed bed with fresh media continuously circulating to transfer oxygen and nutrients.
- Airlift bioreactor – Oxygen mass transfer and mixing are achieved by bubbling pressurized air through an internal draft tube that generates lift.
- Fluidized bed bioreactor – Culture medium is moved upward through a packed bed of immobilized cells to promote mixing and high heat and mass transfer.
- Hollow-fiber bioreactor – Cylindrical chamber packed with permeable hollow fibers. Cells are inoculated inside or outside fibers. Media is added, and waste is removed continuously while cells are retained.

Israel-based Ever After Foods uses proprietary, packed-bed bioreactors that enable a high solid-to-liquid ratio to achieve higher productivity (Southey 2023). Meanwhile, UK-based Cellular Agriculture Ltd uses a hollow-fiber bioreactor for cultivated meat production (Heles 2019).

IntegriCulture, a Tokyo-based company, has developed an alternative to bioreactors and the use of FBS to produce cultivated meat. Their setup mimics the natural process in the body where organs secrete growth factors that are then circulated to other tissues via the bloodstream. The central tank holds the cells that produce cultivated meat, such as muscle and fat cells. The "feeder" tanks contain other cell types, such as liver or placental cells, that secrete growth factors. These growth factors are then fed into the central tank (AgFunder News, 2023).

Some types of bioreactors being tested or already used by the industry are depicted in Figure 5.

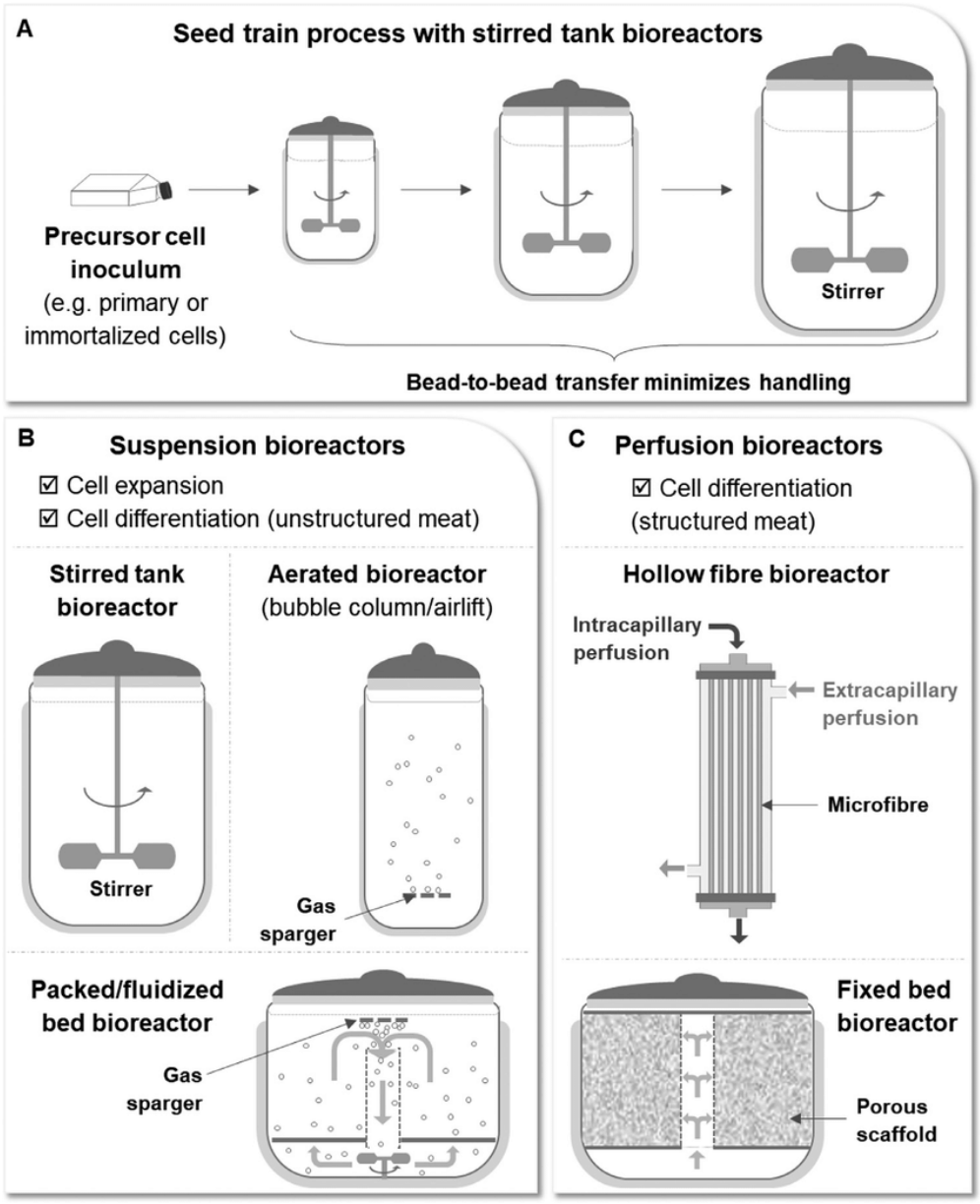



Figure 5. Seed train process and examples of different types of bioreactors (Ng and Kurisawa, 2021).

### *2.5.2. Culture Media*

Cell culture media consists of compounds and nutrients intentionally designed to support cellular growth and proliferation. Cultivated meat standard media comprises basal media supplemented with growth media additives. Basal media provides the basic requirements for the growth and proliferation of cells. Basal media is a buffered solution containing glucose, inorganic salts, vitamins, amino acids, and other nutrients. Three widely used basal media include Eagle's Minimum Essential Medium (MEM), Dulbecco's Modified Eagle Medium (DMEM), and RPMI 1640.

Each type of basal media is different in its concentrations and types of nutrients for each formulation, and these differences are optimized for different types of cell culture.

Basal media may be supplied in liquid or powder form. Powdered culture media is easier to transport and store for large-scale production but must be sterilized before use. Culture media is commonly sterilized by filtration to avoid component degradation (GFI 2021b, Sigma Aldrich). Some cell culture media sterilization methods include high-temperature short time (HTST) pasteurization, filtration, and/or circulating a sterilant (*e.g*., chlorine dioxide gas, fluid) in an enclosed vessel that holds the cell culture media (Leung *et al.*, 2022, UPSIDE 2021). Some companies sterilize their media solution via filtration using 0.1-0.2 µm filters (UPSIDE 2021). Other methods for culture media sterilization include autoclaving and irradiation, which may degrade unstable or heat-labile components (GFI 2021b, Millipore Sigma). Without proper sterilization, introduced pathogens would probably outcompete or affect cell growth, which can easily be detected via real-time monitoring. Media additives tend to vary depending on the cell type and stage in the process. Additives may include animal serum, hydrolysates, lipids, antioxidants, recombinant growth factors, vitamins, amino acids, and trace minerals (FAO 2023). Some media components have no history of safe use as a processing aid or additive in conventional food. However, they may already exist as a natural component of certain foods (*e.g*., growth factors, proteins, serum).

Different ways to induce cell differentiation exist, such as changing the culture media, the environmental conditions, or the scaffold/microcarriers. Changes in the cell media can include the addition or removal of growth factors, vitamins, amino acids, or trace minerals. In some cases, different cell types may be co-cultured, such as muscle and fat cells, to mimic the structure and characteristics of meat. (FAO 2023). Co-culturing may inherently induce differentiation because the different cell types secrete factors to induce the proliferation and differentiation of other cell types, reducing the need for growth factor addition (Balasubramanian *et al.,* 2021; David *et al.,* 2023).

While plasma and animal serum, such as FBS or BSA, are commonly used as a culture media additives, it does not align with companies working towards animal-slaughter-free production. Thus, cell lines may be adapted to grow in serum-free media through serial passaging in media that contains progressively reduced serum concentrations (O'Neill *et al.*, 2021) or genetically engineer the cells to reduce growth factor requirements (Stout *et al*., 2023). Additionally, serum may be replaced with other media additives, such as recombinant growth factors and plant- or yeast-based hydrolysates (Ho *et al*., 2021, Stout *et al*., 2023, O'Neill *et al.*, 2021).

One alternative approach to using animal-based but slaughter-free media has been developed by LA-based startup Omeat. Their product, Plenty, is an alternative to FBS and is created using plasma withdrawn from cows that graze freely on Omeat's farm. The product is available for purchase by cultivated meat companies as a B2B product (Watson 2023).

Some researchers have shown that it is possible to genetically engineer cells to endogenously produce phytonutrients such as the antioxidant carotenoids phytoene, lycopene, and β-carotene (Stout *et al.,* 2020).

Compared to cell media for mammalian cell culture, less is known about the media requirements for cells from fish and other seafood. There is a lack of public information on media formulations for cultivated fish and seafood. A possible media formulation to grow fish cell lines for research and development consists of FBS, fish serum, fish embryo extract, fibroblast growth factor 2 (FGF2) and Leukemia inhibitory factor (LIF) (GFI 2021b), though commercial formulations may be different. A representative comparison of media requirements for different types of cells can be found in Table 3.

Cell culture additives may also include common meat proteins to improve the visual and sensory properties of cultivated meat. Adding heme proteins to culture media may improve cell growth nutrition and color profile of myosatellite cells (Simsa *et al*., 2019). Adding myoglobin to bovine muscle satellite cells (BSCs) significantly increases the proliferation, metabolic activity, and color profile of the cells (Simsa *et al*., 2019).

Table 3. Examples of media components for different types of cells

| Media Categorization | Culture Media Input Category | Purpose | Examples | Cell Type | Cell Species |
|---|---|---|---|---|---|
| Basal Medium | Amino acids | Building blocks of proteins | L-glutamine | All | All |
| | | | Arginine, histidine, threonine | All | Seafood |
| | | | L-Thyroxine | Adipocyte | All |
| | Vitamins | Co-factors for enzymes | Folic acid, B vitamins | All | All |
| | | | Vitamin E | All | Seafood |
| | Inorganic Salts/ Minerals | Provide minerals, retain osmotic balance | Ferric nitrate, sodium pyruvate, putrescine hydrochloride, sodium hydroxide, sodium chloride | All | All |
| | Carbohydrate | Metabolism | Dextrose | All | Mammalian |
| | | | Inositol | All | Seafood |
| Media Additive | Serum | Proliferation, differentiation | FBS, BSA, horse serum | All | Mammalian |
| | | | Fish serum, fish embryo extract, FBS | All | Seafood |
| | Hydrolysate | Serum substitute | Chickpea, yeast, soy | All | All |
| | Lipid | Membrane function | Fatty acids | All | All |
| | | | Omega-3 | All | Seafood |
| | Antioxidant | Prevent cell damage | Lipoic acid | All | All |
| | Proteins (possibly recombinant) | Proliferation, differentiation | Albumin, transferrin, heme | All | Mammalian |
| | | | Insulin, heparin | Adipocyte | Mammalian |

| Media Categorization | Culture Media Input Category | Purpose | Examples | Cell Type | Cell Species |
|---|---|---|---|---|---|
| | Growth Factors | Signaling for proliferation, differentiation | EGF, FGF2, TGF | All | Mammalian |
| | | | Leukemia inhibitory factor, FGF2 | All | Seafood |
| | Processing aids | Support growth in a bioreactor | Defoaming agents, Pluronic F-68 | All | All |
| | Other | Proliferation, differentiation | Thymidine | All | All |
| | | | Glucocorticoids | Adipocyte | Mammalian |

### *2.5.3. Scaffolds and microcarriers*

Large-scale cultivation of adherent cells will require the scaffold/microcarrier to: (i) adhere to cells temporarily and be separated from cells through a removal process, (ii) dissolve once the cells successfully differentiate and proliferate, or (iii) be edible and remain attached to the cells after differentiation and proliferation (Lee *et al.*, 2021b).

Scaffolds or microcarriers may be used to develop more complex structures. Cells can be grown on a scaffold that allows cell attachment, differentiation, and maturation. They can be manufactured from various materials, such as cellulose, alginate, textured vegetable protein, chitosan (derived from crustaceans or fungi), silk, or decellularized animal or plant tissue (GFI 2023a). Microcarriers are small bead-like structures (~100 to 400 µm diameter) modified to mimic the cell's extracellular matrix properties (*e.g.,* stiffness, topography, and porosity). They are used to anchor cells that require attachment to proliferate. The nucleus of microcarriers can be made from materials such as gelatin, dextran, collagen, chitosan, alginate, polystyrene, or glass (Derakhti *et al.,* 2019, Bodiou *et al.,* 2020). The nucleus is covered by materials that stimulate cell adherence, such as collagen, poly-lysine, laminin, fibronectin, vitronectin, thrombospondins, and glycosaminoglycans (Nicolas *et al.*, 2020).

Microcarriers have the potential to enhance differentiation through the encapsulation and release of appropriate growth or differentiation factors into the cultured tissue (Lee *et al.,* 2021b). For example, research has demonstrated that adding curcumin to scaffolds can promote the differentiation of induced human iPSCs into smooth muscle cells (Mokhames *et al.,* 2020). However, the applicability to large-scale cell culture for cultivated meat production is still being determined, and the nutritional benefit would also depend on whether the scaffold is edible and consumed or only used for production and removed during cell harvest (Ye *et al.,* 2022).

If microcarriers are not edible and must be removed, enzymes such as trypsin–EDTA are used to separate the cultured cells from the microcarriers.

The production of thick tissues resembling a beef steak or fish filet remains a challenge. To create dense tissue, cells must receive sufficient nutrients and oxygen. The challenge lies in enabling the

flow of nutrients and oxygen to the cells. Companies have taken two approaches to overcome this hurdle: porous scaffolds infused with cells or layering units of scaffold, or cells constructed into a final shape (GFI 2023d). These approaches are currently practiced at a small R&D scale.

**CASE STUDY: Plant protein scaffolds for cultivated meat production**

Aleph Farms has reported using wheat and textured soy protein (TSP) scaffolds for seeding bovine satellite cells, smooth muscle cells, and endothelial cells (Ben-Arye *et al.,* 2020). TSP, produced via low-moisture extrusion, offers a porous structure that supports cell proliferation and differentiation into muscle cells and myotube formation (Lee *et al.,* 2021b).

Mixtures of pea and soy protein isolate, and one or a few polysaccharide(s) such as alginate, starch, bean, gum, gellan-gum, hyaluronic acid, cellulose, chitin, chitosan, xanthan gum, agar, agarose, pectin, dextran, and carrageenan have also been evaluated as 3D-printed scaffolds for bovine satellite cell cultivation and bioinks for cellular printing. These proteins and polysaccharides would be edible, thereby forming an edible scaffold (Levenberg *et al.,* 2022; Ianovici *et al.,* 2022). Wheat protein could represent another source of plant protein for the production of scaffolds (Wollschlaeger *et al.,* 2022).

The polysaccharides listed above are derived from/produced by plants, algae, or bacteria. The safety of these production organisms would have to be evaluated, *e.g.,* for the potential to cause allergic reactions or to release toxins, particularly if these organisms or their derivatives are consumed as part of the edible scaffold.

Lastly, while synthetic polymers could be an alternative to animal-derived polymers, synthetic polymers are often hydrophobic and lack cell recognition sites, such as the arginylglycylaspartic acid (RGD) peptide motif (Tallawi *et al.,* 2015).

### *2.5.4. Cell culture conditions*

Culturing conditions differ between cell lines. Factors such as temperature, oxygen and other gasses, pressure, pH, and osmolarity influence cell proliferation and differentiation. For instance, fish are adapted to lower-oxygen environments. Generally, the incubation temperature for invertebrates is lower than for vertebrates (Rubio *et al.,* 2021). Mammalian cells typically grow around 37 °C, and fish cells grow between 15 °C and 30 °C. Fish cell lines tend to have longer doubling periods than mammalian cells (GFI 2020).

## 2.6. Cell harvest

Following cell growth and expansion, cells are harvested and may undergo additional processing. Cells can be harvested using various techniques, such as centrifugation, sedimentation, or filtration (FAO 2023). When cells are grown on non-edible, non-biodegradable scaffolds, the cells have to be detached from the 3D structure using enzymatic, chemical, or mechanical methods (Allan *et al*.,

2019, Bodiou *et al.*, 2020, Rodrigues *et al.*, 2019). Cells may be washed after harvested, *e.g.,* with a PBS solution to remove residual culture media components from the tissue (UPSIDE 2021).

### 2.7. Food processing

Harvested cells undergo further processing prior to commercialization as a food product. This may include the addition of preservatives or other food ingredients for flavor, color, or texture. Harvested cells may be supplemented with vitamins, minerals, and other micronutrients to improve the nutritional composition of the final product.

Food processing may also include combining different cell types and altering the biomass to impart shape and structure (*e.g.*, 3D bioprinting, molding, extrusion) or combining the harvested cells with plant-based components (*e.g.,* textured soy, wheat, or pea protein) to produce blended products.

### 2.8. Product packaging and distribution

The final product is packaged and labeled before being sent off to customers. This process is expected to be similar to the current needs for food/meat products in terms of packaging materials, storage, and transport. The final product will be packaged in food contact safe material that maintains food quality and improves shelf life. Depending on the country of marketing and distribution, the label might have to be adjusted to reflect a country's labeling regulations.

### 2.9. Alternative approaches to manufacturing

Some companies use manufacturing processes that use less 'traditional' cultivation steps, such as 3D printing of bioinks made of myocytes and adipocytes (Ferreira 2023) or electro-mechanical stimulation techniques during cell proliferation and differentiation (Lee *et al.*, 2021b).

Hydrogels are another 3D structure that is being explored. These are networks of polymer chains that can absorb water due to their hydrophilic properties. Their high permeability allows for easier flow of oxygen and water-soluble molecules to all cells. Hydrogels can also be dissolved into a mixture of cell media and cells to facilitate cell attachment and spreading. Mosa Meat has used this technique for cell differentiation or structuring (Breemhaar and Post, 2019). However, hydrogels might not work on a large scale in bioreactors; therefore, other avenues are being explored.

Some research teams have also been working on creating scaffold-free cultivated meat using "cell sheet technology," *i.e.,* stacked bovine myoblast cell sheets to fabricate 3D tissue (Tanaka *et al.,* 2022). The benefit of this approach is that no scaffold is needed, which removes the risks associated with scaffold materials.

## Conclusion

Section 2 summarizes commonly used methods and substances to produce cultivated food products, from cell sourcing to final product formulation. In the next section, we identify hazards

associated with the manufacturing methods and materials, as well as safety testing and hazard control measures for each hazard. We also include case studies from the literature or obtained during the interviews.

## SECTION 3. HAZARD IDENTIFICATION

### Introduction

This section describes food safety hazards and associated testing and controls for each manufacturing stage, as identified by experts and companies. Hazards are described according to their stage of manufacture, with an emphasis on hazards specific to cultivated meat and seafood production. Table 4 summarizes the hazards potentially introduced at each manufacturing step, whether the hazard is specific to cultivated meat and seafood production, and potential testing and control measures.

Table 4. Main hazards associated with cultivated meat and seafood and potential testing and control measures

| Manufacturing step | Hazard | Specific to cultivated meat & seafood? | Potential testing and control measures |
|---|---|---|---|
| **Cell sourcing** | Adventitious agents (viruses, bacteria, prions, microbial toxins, yeast, mold) | The same hazard is present in conventional meat & seafood products (FAO 2023). | Evaluation of source animal health; testing for adventitious agents |
| | Allergenicity (from source animal) | The same hazard is present in conventional meat & seafood products (FAO 2023). | Allergen labelling in final product |
| **Cell isolation** | Adventitious agents (viruses, bacteria, mycoplasma) from inputs | The same or similar hazard may be present in products of fermentation or precision fermentation (FAO 2023). | Food safety management systems, testing for adventitious agents in the inputs |
| | Hazardous substances from cell media and reagents | The same or similar hazard may be present in products of fermentation, precision fermentation, fortified foods, novel and conventional proteins, and other processed foodstuffs (FAO 2023). | Analysis of inputs for safe use in food; residue testing of potentially hazardous substances in harvested cells |
| **Establishment of cell lines** | Adventitious agents (viruses, bacteria, mycoplasma) from personnel and environment | The same hazard is present in conventional meat & seafood products and in products of precision fermentation. | Food safety management systems, testing for adventitious agents |
| | Hazardous chemicals/molecules from inputs | The same hazard is also present in the production processes for conventional livestock production and aquaculture (FAO 2023) | Analysis of inputs for safe use in food; residue testing of potentially hazardous substances in harvested cells |
| | Cell line cross-contamination or misidentification | A similar hazard may be present in GM foods and products of precision fermentation. | Cell line identity verification |
| | Genetic instability as a result of genetic engineering | The same or similar hazard may be present in GM foods and products of precision fermentation, and may be similar to genetic | Monitoring of cell growth and morphology; evaluation of stability of integrated or modified genes |

| Manufacturing step | Hazard | Specific to cultivated meat & seafood? | Potential testing and control measures |
|---|---|---|---|
| | | variation in conventional breeding or cloning processes (FAO 2023) | |
| **Cell banking and cell storage** | Adventitious agents (viruses, bacteria, mycoplasma) from contaminated liquid nitrogen | The same or similar hazard may be present in production of conventional foods and cell culture for therapeutics (FAO 2023) | Use vapor-phase nitrogen storage rather than liquid phase; monitoring and adventitious agent testing of cells |
| | Hazardous chemicals (*e.g.,* cryoprotectants) | The same or similar hazard may be present in GM foods and/or fermented foods. | Analysis of inputs for safe use in food; residue testing of potentially hazardous substances in harvested cells |
| | Cell line cross-contamination or misidentification | A similar hazard may be present in GM foods and products of precision fermentation. | Cell line identity verification; separate storage of different cell lines |
| **Mass cultivation: Cell proliferation and differentiation** | Adventitious agents (viruses, bacteria, mycoplasma) from the environment, cell media, and/or scaffolds/microcarriers | The same or similar hazards may be present in products of fermentation, precision fermentation, new food ingredients and additives (FAO 2023). | Food safety management systems; testing for adventitious agents; supplier approval programs; source ingredients from health-screened herds, sterile filter cell media |
| | Chemical hazards from equipment, handling, or inputs | Chemical hazards from equipment may also be present in conventional meat & seafood. Inputs may be qualified similar to qualifying new substances and materials as new food ingredients and additives (FAO 2023). | Food safety management systems; analysis of inputs for safe use in food; residue testing of potentially hazardous substances in harvested cells |
| | Allergenicity from inputs and/or scaffolds/microcarriers | The same hazard is present in conventional meat, seafood, and other foods (FAO 2023). | Testing inputs and final product for allergens; label final product for potential allergens |
| | Genetic instability as a result of prolonged culture | The same or similar hazard may be present in GM foods and products of precision fermentation, and may be similar to genetic | Monitoring of cell growth and morphology; establishment of maximum passage number; testing such |

| Manufacturing step | Hazard | Specific to cultivated meat & seafood? | Potential testing and control measures |
|---|---|---|---|
| | | variation in conventional breeding or cloning processes (FAO 2023) | as karyotyping, genomics, transcriptomics, proteomics, metabolomics |
| **Cell harvest** | Adventitious agents (viruses, bacteria, mycoplasma) introduced during cell harvest | The same hazard is present when harvesting conventional meat & seafood products (FAO 2023). | Food safety management systems; testing harvested cells for adventitious agents |
| **Food processing** | Adventitious agents (viruses, bacteria, mycoplasma) from handling of harvested cells | The same hazards are present in conventional meat & seafood products (FAO 2023). | Food safety management systems; environmental monitoring programs; testing for adventitious agents in final product |
| | Hazardous substances from added ingredients | The same or similar hazard may be present in products of fermentation, precision fermentation, fortified foods, novel and conventional proteins, and other processed foodstuffs (FAO 2023). | Analysis of inputs for safe use in food; residue testing |
| | Allergenicity from preservatives/other added ingredients | The same hazard is present in conventional meat, seafood, and other foods (FAO 2023). | Residue testing; allergen labelling in final product |
| **Product packaging and distribution** | Physical contamination from poor packaging or during storage and distribution | The same hazard is present in most processed food stuffs (FAO 2023). | Food safety management systems; inspection and monitoring of food and packaging |
| | Adventitious agents from improper or damaged packaging | The same hazard is present in conventional meat & seafood products. | Food safety management systems; testing final product for adventitious agents; shelf-life analysis |

## 3.1. Cell sourcing

Cells obtained from animals or cell banks may carry infectious diseases, chemical residues, or allergens from the source animal or be introduced during the sourcing process.

### *3.1.1. Hazards*

Hazards associated with cell sourcing include:

- *Adventitious agents:* Contamination with infectious agents, including viruses, bacteria, and prions may originate from infected source animals, or be introduced during handling (*e.g.,* from personnel or cross-contamination during biopsy). Infectious agents of concern may vary depending on the source animal.
- *Allergenicity:* Cells may be sourced from animals with allergenic potential (*e.g.,* shellfish).

### *3.1.2. Testing and control measures*

#### *3.1.2.1. Adventitious agents*

Control measures include evaluation of source animal health, as obtaining cells from healthy animals minimizes the risk of pathogen transfer, and adherence to standard laboratory practices, including aseptic technique.

Some considerations in identifying relevant adventitious agents include whether the pathogenic agent can be introduced (*i.e.,* the likelihood of its presence in source tissue or introduction during sourcing) and the zoonotic potential (*i.e.*, can it be transmitted to humans). Companies may also test for non-zoonotic adventitious agents as part of quality control measures. Currently, companies are being relatively conservative and testing for a panel of adventitious agents to confirm no carryover of zoonotic and non-zoonotic pathogens during sourcing or at the cell banking stage (refer to Section 3.3.2.2. for more details).

**CASE STUDY: Evaluation of animal health in fish and seafood species**
While it is possible to obtain certification of source animal health for land-based species, it is not possible to have a health certificate for many seafood species, such as a single fish, shrimp, or prawn. The health certificate is usually from the source population, e.g., the fish farm, instead of a specific organism. Certification may state that the source population is in a low-risk country for certain infectious agents.

The concern for prions arises from transmissible spongiform encephalopathies (TSE). This disease affects some animal species (*e.g.,* cattle, sheep, goats). However, the only prion disease known to be zoonotic and transmissible to humans is bovine spongiform encephalopathy (BSE) (EFSA 2011, Houston and Andreoletti, 2018, Kamali-Jamil *et al.,* 2021). BSE is caused by a misfolded form of the otherwise non-infectious prion proteins ($PrP^{C}$) known as $PrP^{Sc}$. Prions have been found in the brain,

spinal cord, lymphoid tissues, tonsils, appendix, enteric nervous system, and the blood of afflicted animals (Gough and Maddison, 2010). Cultivated meat products made with bovine-derived manufacturing inputs, including cells and cell culture reagents, have the potential to harbor $PrP^{Sc}$ and therefore pose a food safety hazard. $PrP^{Sc}$ may be introduced into cultivated meat through the use of infected biopsy tissue or the use of contaminated cell culture reagents.

There is some evidence that prion infection is challenging to propagate *in vitro* (Krance *et al.,* 2020). Additionally, while many studies suggest that the risk of propagation of TSE agents is restricted to neurons or brain-derived cell cultures (Pauwels *et al.,* 2007), there have been suggestions that non-neuronal cells such as epithelial or fibroblast cells can support TSE infection (Vilette *et al.*, 2001, Vorberg *et al.*, 2004). Cell lines expressing normal host prion protein could potentially support the propagation of TSE agents introduced from contaminated culture media components or biopsy samples. Prions are highly resistant to degradation, including heating to high temperatures (Antloga *et al.*, 2000, Giles *et al.*, 2017, Langeveld *et al.*, 2003, Sakudo *et al.*, 2020). Typical cooking processes are likely insufficient for prion inactivation.

The risk of prion transmission may be mitigated by sourcing cows from certified TSE-free herds and isolating cells from non-prion-harboring tissues (EFSA 2006). When cells are sourced from embryos, it may be unnecessary to test for prions since the likelihood of misfolded prion formation is low.

Well-established cell lines may have less available information on source animal health as they have been cultured for extended periods. The cells of some commonly used research cell lines were derived from biopsies decades ago. Therefore, companies may conduct more thorough testing of these cell lines to confirm the absence of source-animal pathogens and evaluate the identity of the cells to confirm the species. In addition, these cells may have undergone significant genetic drift over time. Genetic stability testing may be conducted to evaluate the potential for changes (see Sections 3.3.2.4. and 3.5.4.2. for more details).

#### *3.1.2.2. Allergenicity*

Cells sourced from animals known to be allergenic (*e.g.,* shellfish) may cause an allergic reaction in individuals who are usually susceptible to these foods. The final product will require labeling to inform consumers of potential allergenicity.

## 3.2. Cell isolation

### *3.2.1. Hazards*

Hazards associated with cell isolation include:

- *Adventitious agents:* Contamination with infectious agents, including viruses, bacteria, and mycoplasma may originate from inputs, or be introduced during cell isolation.
- *Hazardous chemicals:* Culture media and reagents used to isolate the cell lines may result in residues in the final product.

### *3.2.2. Testing and control measures*

#### *3.2.2.1. Adventitious agents*

Similar to the other production stages, adventitious agents can be controlled through measures like Good Manufacturing Practices (GMP), Good Cell Culture Practices (GCCP), and Hazard Analysis and Critical Control Point (HACCP) (see Section 3.9. for more details on these measures). Testing for adventitious agents may occur at the cell banking stage (refer to Section 3.4.2. for more information).

#### *3.2.2.2. Hazardous chemicals*

An evaluation is conducted to confirm that all the inputs used in food production are safe for consumption. If there are any potentially hazardous residues, a calculation may be conducted to determine their potential presence in the final product. Alternatively, residue testing may be performed on the harvested cells or at earlier stages. Substances used in these early stages may be diluted or washed out during production and may not be present in the final product.

## 3.3. Establishment of cell lines

During cell line establishment, different strategies for adaptation, cell line immortalization (*e.g.,* spontaneous mutation, oncogene expression), and stem cell reprogramming *(e.g.,* use of integrative or non-integrative methods for introducing reprogramming factors) can introduce adventitious agents, hazardous chemicals/molecules, and affect the genetic stability of the cells.

### *3.3.1. Hazards*

Hazards associated with cell line establishment include:

- *Adventitious agents:* Contamination with infectious agents, including viruses, bacteria, and mycoplasma may originate from inputs or be introduced during handling (*e.g.,* from personnel or cross-contamination during biopsy).
- *Hazardous chemicals/molecules:* Media and other reagents may not be suitable for food production.
- *Cell line misidentification:* Cell line cross-contamination or misidentification can occur in spaces involving work with multiple cell lines and/or species.
- *Genetic stability:* Genetic engineering, including transgene expression and off-target and/or pleiotropic effects, may be evaluated to determine if the modifications lead to intentional or unintentional production of proteins/metabolites/allergens that pose a food safety risk. The use of cell lines harboring recombinant DNA that contains antimicrobial resistance (AMR) genes may be evaluated to determine if they could lead to increased antimicrobial resistance in the unlikely event of gene transfer between the cultivated food product and human gut microorganisms or microorganisms in the environment (Ong *et al.,* 2021).

### *3.3.2. Testing and control measures*

#### *3.3.2.1. Adventitious agents*

The inputs, equipment, or handling may introduce adventitious agents during this stage. Food safety management systems may be established to control and prevent contamination. In addition, the cells are often tested for adventitious agents at the cell banking stage (refer to Section 3.4.2.) or final product stage (refer to Section 3.6.3.1.).

#### *3.3.2.2. Hazardous chemicals/molecules*

An evaluation is conducted to confirm that all the inputs used in food production are safe for consumption. If there are any potentially hazardous residues, a calculation may be conducted to determine their potential presence or concentration in the final product. Alternatively, residue testing can be performed on the harvested cells (see Section 3.6.3.). Substances used in the early stages may be diluted or washed away during production and may not be present in the final product.

Antibiotics may be used during the early stages of cell line development. Also, reagents used to generate cell lines (*e.g*., viral vectors, transgenes including viral genes like SV40T, small molecules, synthetic RNAs, and protein-based reprogramming factors) may be evaluated for food safety hazards.

#### *3.3.2.3. Cell line misidentification*

Control measures include verifying cell line identity, which may be performed using species-specific PCR amplification and DNA sequencing. For example, cytochrome c oxidase subunit 1 (CO1) barcoding may be used to establish the species-level identity of mammalian and insect cell lines (, Ward *et al.,* 2005).

Cell lines may be authenticated by comparing the DNA sequence of a company's cell lines to the sequence of reference DNA. Cell line identity testing provides additional assurance in facilities or laboratories conducting work with multiple cell lines, where there is an increased risk of cross-contamination with allergenic species.

**CASE STUDY: Hazards associated with using SV40T for cell line immortalization**

Strategies used for cell line development influence food safety risks associated with cultivated food products. For example, expressing the Simian Virus 40 Large T antigen is a common method of immortalizing cells. Simian Virus 40 has been associated with the formation of tumors in humans, although it is unclear whether it has a causative effect (Rotondo 2019, Shah 2004, American Cancer Society 2023).

Food safety risks associated with consuming SV40T-immortalized cells may depend on:

1. *Levels of SV40T antigen in the food product*. Levels of SV40T antigen will depend on the copy number of the SV40T expression vector and vector design (*e.g.,* promoter strength, codon usage). Given that continuous expression is required to maintain immortality (May

2004, Ozer 1999) and the use of a multi-copy SV40T expression vector, there is a possibility that cultivated meat produced from of SV40T-immortalized cells will have measurable amounts of SV40T.

2. *Whether the antigen alone (without any other SV40 genes) poses a health risk.* It has been shown that expression of SV40T alone is sufficient to cause a stress response in human cells (Hein 2009, Forero 2014). This suggests that SV40T may have cause adverse health effects even in the absence of other viral genes when expressed in human cells.
3. *Whether the antigen poses a risk if ingested.* There is limited literature on whether the SV40T antigen alone would adversely affect cells in the human body if ingested.

Research is needed to establish whether SV40T poses a food safety risk. A key consideration in assessing the risks of using SV40T for immortalization is that viral vectors conventionally used in SV40T-mediated immortalization do not encode other SV40 genes, including those required for SV40 replication. Uncertainties around SV40T safety due to its viral origin can be avoided by using alternative immortalization methods. For example, immortalization by the over-expression of the endogenous Telomerase Reverse Transcriptase Protein (TERT) avoids concerns associated with expressing genes of viral origin. TERT-mediated immortalization may also avoid concerns associated with transgene expression depending on the method of overexpression and whether exogenous DNA is introduced. Another alternative to SV40T-immortalization is spontaneous immortalization by serial passaging, which does not involve genetic engineering or transgene expression. However, serial passaging may lead to phenotypic and genetic drift associated with changes in food safety (*e.g.,* increased production of hazardous proteins or metabolites) and/or quality (*e.g.,* changes in cellular performance, such as the ability to efficiently differentiate into mature muscle cells). Although genes of viral origin do not necessarily pose a food safety risk, it may be desirable to use immortalization methods that avoid the use of viral genes as their presence in CM products may be viewed negatively by consumers.

*3.3.2.4. Genetic stability*

Similar to the safety assessment of genetically engineered microorganisms or plants, an evaluation of the stability of the integrated or modified genes is conducted to confirm that the intended genes are introduced in the desired location and with the correct copy number in the genome, and to demonstrate the absence of extraneous DNA (*e.g.,* antibiotic resistance genes or other vector backbone components). Characterization of the stability of the modifications may also be confirmed by monitoring consistency in cell growth rate, morphology, and phenotype. A targeted analysis for proteins expressed by the modified cells may be conducted. Further characterization of cells after extensive *in vitro* cultivation through methods such as whole genome sequencing (WGS), single nucleotide variant (SNV) analysis, karyotyping, transcriptomics, proteomics, and metabolomics may also help identify genetic and phenotypic changes of potential concern (see Section 3.6.3.4. for more details).

**CASE STUDY: Approaches to evaluating genetic stability**
UPSIDE conducted genetic characterization to ensure genetic integration and stability after genetic amendments expressing the chicken telomerase reverse transcriptase (TERT) gene. This characterization verified that the genes are introduced in the desired location and with the correct copy number, and that extraneous DNA is absent (*e.g.,* antibiotic resistance genes or other vector backbone components) in the plasmids. Expression of the inserted gene and the expected phenotype are confirmed through passage assay *i.e.,* monitoring growth and viability of immortalized cell lines (UPSIDE 2021). In its response, FDA acknowledged once taken out of the bioreactor the cells are expected to lose their proliferative capacity, and that cooking would further break down cellular structures and digestion would break down any remaining cellular structures. In human cells, dysregulation of TERT (telomerase reverse transcriptase) has been associated with tumorigenesis. However, should any residual TERT protein be present in food, it would be destroyed during cooking by the heat and digestion (FDA 2022).

## 3.4. Cell banking and cell storage

### *3.4.1. Hazards*

Hazards associated with cell banking and cell storage include:

- *Adventitious agents*: Cell banks can also be contaminated if exposed to contaminated liquid nitrogen, which has the potential to transfer mycoplasma and other microorganisms to cells (Ong *et al.,* 2021).
- *Hazardous chemicals:* Cryoprotectants may consist of hazardous chemicals.
- *Cell line contamination/misidentification:* Cell line cross-contamination or misidentification can occur in spaces involving work with multiple cell lines and/or species. Leakage of cryopreservation bags during cell banking can lead to cross-contamination (Ong *et al.,* 2021).

### *3.4.2. Testing and control measures*

#### *3.4.2.1. Adventitious agents*

When cell banks are cryopreserved using liquid nitrogen, potential contamination can come from the liquid nitrogen transferring pathogens to the cells (Soice and Johnston, 2021). Cross-contamination during cryostorage may be mitigated by using vapor-phase nitrogen storage rather than liquid phase (Ong *et al.,* 2021).

#### *3.4.2.2. Hazardous chemicals*

Conduct an evaluation to confirm that all the inputs used in the cell bank are safe for consumption. If there are any potentially hazardous residues, a calculation may be conducted to determine their potential presence and level in the final product. Alternatively, residue testing can be performed on

the harvested cells (see Section 3.6.2.). Substances only used in cell banking may be diluted during production and may not be detectable in the final product.

#### *3.4.2.3. Cell line contamination/misidentification*

Cell bank release testing may include species verification (if there is a risk of cross-contamination) and phenotype analysis (e.g., viability, doubling time, morphology) to ensure cells are healthy and correctly identified. Different cell lines may be stored separately to minimize the risk of cell line cross-contamination.

**CASE STUDY: Cell Bank Release Testing – adventitious agents**

GOOD Meat cell lines and cell banks are tested for sterility, mycoplasma, and human and avian viruses and bacteria, including avian influenza (type A), avian reovirus, avian adenoviruses (Groups I-III), avian encephalomyelitis virus, fowl pox, Newcastle disease virus, paramyxovirus (type 2), mycoplasma, and *Salmonella* spp. GOOD Meat also ensures that bovine serum, which may be used to generate cell banks, is tested for bovine viruses, is not sourced from materials with the potential to transmit bovine spongiform encephalopathy (BSE), and is produced in USDA-approved facilities (GOOD Meat 2022b).

UPSIDE cell lines and cell banks are tested for pathogens of clinical importance in traditional food manufacturing, including *E. coli*, *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes*. Cell banks are additionally screened for aerobic plate counts, Enterobacteriaceae, yeasts and molds, mycoplasma, and avian and zoonotic viruses. Additional testing for species-specific viruses is conducted with animal component exposure, such as when bovine or porcine-derived media components are used to generate banked cell lines (UPSIDE 2021).

## 3.5. Mass cultivation: Cell proliferation and differentiation

### *3.5.1. Hazards*

*3.5.1.1. Environmental contamination*

- *Adventitious agents*: Adventitious agents can be introduced during the handling of the cells, from exposure to air contaminants or via improperly cleaned equipment. Introducing microbial contaminants into the closed system of a bioreactor can lead to the rapid growth of the microorganisms.
- *Chemical hazards*: Hazardous cleaning agents or residues may remain on equipment or tools in contact with cells or inputs.

*3.5.1.2. Culture media*

- *Adventitious agents*: Adventitious agents may be introduced through the media. Using animal-derived components may harbor infectious agents originating from the source animal.
- *Hazardous substances*: Media used for cell proliferation and differentiation may contain hazardous substances or substances at elevated concentrations.
- *Allergenicity*: Inputs used to culture cells may be allergenic.

*3.5.1.3. Scaffold and microcarriers*

- *Adventitious agents*: Scaffolds and microcarriers may introduce contaminants.
- *Hazardous materials*: Scaffolds and microcarriers may be composed of materials not suitable for use in food.
- *Allergenicity*: Materials that make up the scaffolds and microcarriers may be allergenic.

*3.5.1.4. Cell stability*

- *Genetic stability:* Prolonged cultivation naturally results in genetic and epigenetic drift, which may result in altered cell characteristics.

### *3.5.2. Testing and control measures*

*3.5.2.1. Environmental contamination*

*3.5.2.1.1. Adventitious agents and chemical hazards*

Food safety programs are in place to control potential contamination of equipment. Microbial and chemical contamination can be limited through routine environmental monitoring, routine washing and sterilization of equipment with cleaning agents, sterilization or filtration of input materials, appropriate biosafety equipment and personal protective equipment, and routine testing for microbial contamination. Use of cleaning substances that are safe for food is recommended and ensuring that surfaces are rinsed properly with potable water, if necessary.

*3.5.2.2. Culture media*

*3.5.2.2.1. Adventitious agents*

The inputs to a culture media formulation are evaluated for their purity and suitability for use in food. Supplier approval programs ensure that inputs are produced in appropriate facilities and meet food safety standards. Supply chain programs facilitate the assurance that input material meets the requisite specifications. “Pharma-grade” media is currently more available than “food-grade” media; however, the sterility and purity specifications for 'pharma-grade' inputs may or may not be more stringent than required for food manufacturing.

Animal-derived ingredients can potentially harbor bacterial or viral disease agents or prions from the source animal. To control for microorganisms, the ingredients may be sourced from health-screened herds, sterile filtered, and tested to be free of microorganisms. Some companies test for residual synthetic hormones that could be introduced by serum components, as hormonal substances may have been administered to the animal from which the serum was derived. For example, GOOD Meat tested for a range of hormones that may have been administered to cattle before the collection of bovine serum albumin (GOOD Meat 2022b).

*3.5.2.2.2. Hazardous substances*

Currently, the safety assessment of media components occurs on a case-by-case basis. For any components that are not already authorized for use in food, a safety assessment may consist of the following:

1. An *in silico* evaluation of safety based on chemical structure or activity;
2. An analysis of the level in the final cultivated product compared to levels in conventional foods;
3. A ‘worst-case’ theoretical calculation of inputs in the final product; and/or
4. Measurement of the input in the final product (mass balance and analytical testing).

A literature analysis may also be conducted to determine safe levels of residual media components in the final product when used in food.

**CASE STUDY: Categorizing the culture media components based on risk level**

Many companies use a tiered approach to demonstrate the safety of their culture media components.

Category 1 components are substances that already have a safe history of food use and have an approved regulatory status for use in food without a restrictive limit. There may be established food additive identity and purity specifications for these compounds, such as those published by the Food Chemicals Codex (FCC) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). No further safety assessment is required if the substances are used at levels allowed for use in food and meet food specifications. Some companies also consider substances that are naturally occurring and used in normal cell function to be Category 1 substances. These

compounds are metabolized by the cells and result in safe metabolites. Compounds in this category include sugars, pH buffers, water-soluble vitamins, and common antioxidants such as tocopherols.

Category 2 components are substances that are common dietary nutrients permitted by regulation, though they may have limits for use in food or are being used in a novel way. Companies ensure that the levels of these substances are within regulatory limits in the final product. These substances can be measured using common, validated food composition analytical methods, and batch analyses of multiple lots of the finished product are obtained to validate the above assumptions. Similar to Category 1 substances, there may be established food additive identity and purity specifications for these compounds. The use of the substance may be considered safe if intake is lower than or equal to that of Reference Intake Values (the amount of a nutrient that contributes to a healthy, balanced diet), considering intake for all dietary sources. Safety may also be evaluated using a Margin of Exposure (MOE) approach. In this approach, the exposure level of the substance is compared to No Observed Adverse Effect Levels (NOAEL) from peer-reviewed toxicology studies (often 90-day subchronic dietary rat studies or clinical trials). For substances that are not genotoxic nor carcinogenic, a MOE of 100-fold or greater is considered adequate to support safe use in food. If the MOE is < 100-fold, additional risk assessment is conducted, such as comparison to levels in conventional foods, evaluation of absorption, distribution, metabolism, excretion (ADME), and stability assessment in the cultivated product, particularly after processing, cooking, and digestion. Compounds in this category may include most inorganic salts and macronutrients.

Category 3 substances are substances without a history as an additive to food and do not have regulatory status. Companies may either demonstrate the substance is not present in the final product (and therefore not a food safety hazard) or do a full safety evaluation to demonstrate safety. In this case, companies may conduct an *in silico* evaluation of safety based on the chemical structure or activity, compare the final levels in cultivated food to those in conventional foods, conduct a MOE analysis (perhaps with a more stringent ratio due to lack of history), evaluate the ADME profile, and assess the stability of the substance and its metabolites in the final product. Compounds in this category may include bioactive substances such as growth factors.

The safety of recombinant proteins may be conducted using the same approach by which recombinant food additives or processing aids are assessed, where the safety of the protein and the host organism is evaluated (*e.g.,* Codex Guidelines on the Conduct of Food Safety Assessment of Foods Produced Using Recombinant DNA Microorganisms (CAC/GL 46-2003).

The safety assessment of the host organism includes identifying its scientific name, detailing the genetic modification process, providing allergenicity and toxicity information of the host organism, and the presence of the host organism or host organism DNA in the recombinant protein formulation. If host organism is present in the recombinant protein formulation, its presence may be minimal in the final cultivated cells due to dilution throughout the manufacturing process. The

assessment of the genetic modification process includes evaluating whether the inserted DNA codes for known toxins, allergens, or anti-nutrients. Organisms may be assessed for pathogenic, allergenic, or toxigenic potential.

The safety of recombinant proteins may be assessed by comparing sequence similarity to the wild-type protein. Any differences (*i.e.,* sequence modifications, the use of tags, structure, function) can be evaluated for any potential food safety hazards such as allergenicity, toxic potential, stability, *etc.*

Bioactive substances, such as growth factors, are required to culture cells. If any bioactive substances are present in the final product, then further safety assessment may be conducted. This may include comparing levels in cultivated products to levels in conventional meats and seafoods and amounts found in the human body, and/or an evaluation of the stability and activity after food processing, cooking and digestion. Growth factors may be detected using approaches such as immunoassays (*e.g.,* ELISA and Western blots), bioactivity assays, and mass spectrometry methods. If growth factors are present in the final product, the stability or bioactivity of the growth factors may be evaluated using representative cooking scenarios, or with *in vitro* digestion assays (*e.g.,* Minekus *et al.,* 2014).

Complex mixtures, such as lysates derived from plants, algae, or other biological sources, may be challenging to characterize. An assessment of potential antinutrients, allergens, and contaminants (*e.g.,* pesticides) is conducted to evaluate their suitability for use in food.

#### *3.5.3.3. Scaffolds and microcarriers*

##### *3.5.3.3.1. Adventitious agents*

Some scaffolds are manufactured from animal sources. For example, collagen is mostly extracted from bovine and porcine tissue; thus, there is risk of transmission of zoonotic diseases from the source animal (Singh *et al.,* 2023). Some cultivated meat scaffolds may be sterilized prior to cell seeding to minimize the presence of potential microbial contaminants. Sterilization procedures may include gamma and UV irradiation or ethylene oxide; however, these procedures may denature or damage certain biomaterials (Caliari and Burdick, 2016, Dai *et al.,* 2016). Careful sourcing of materials, sterilization, monitoring for contamination, and testing for adventitious agents may be conducted on scaffold and microcarriers.

##### *3.5.3.3.2. Hazardous materials*

The materials used to create scaffolds and microcarriers are assessed for safety and suitability for use in food. Companies may use materials that are already authorized for use in foods. Some materials may not be suitable for consumption (*e.g.*, some synthetic polymers); in this case, testing may be conducted to demonstrate the complete removal of the scaffold. Similarly, substances such as enzymes used for microcarrier removal may be evaluated, and their presence in the final product may be measured.

*3.5.3.3.3. Allergenicity*

Some of the materials used for scaffold, such as soy and wheat, are common food allergens. If potentially allergenic materials remain in the final product, companies may have to provide clear information on the presence of these allergens on the product label. This may be important for cultivated meat because individuals may not anticipate soy or wheat allergens in meat-like products.

*3.5.3.4. Cell stability*

*3.5.3.4.1. Genetic stability*

Changes in cell characteristics throughout product manufacture that indicate instability can be detected by monitoring consistency in cell growth rate, cell growth parameters (*e.g.,* oxygen levels, pH), morphology, and phenotypic characterization. If any cell instability is detected, companies may establish maximum passage specifications to mitigate genetic drift that could directly affect other final product characteristics. Testing of cell stability may occur on the harvested cells (see Section 3.6.3.3. for more details). Table 5 lists the cell growth parameters GOOD Meat monitors during cell culture as hallmarks of a well-controlled and consistent process.

Table 5. GOOD Meat: Parameters monitored during cell culture (from GOOD Meat 2022b)

| In-Process Parameters | Monitored/Controlled | Rationale |
|---|---|---|
| Cell density | Monitored | - Monitor cell growth<br>- Trend process performance |
| Cell viability | Monitored | - Monitor cell health<br>- Trend process performance |
| Glucose | Monitored & controlled | - Monitor carbon source<br>- Controlled addition to medium<br>- Trend process performance |
| Glutamine | Monitored & controlled | - Monitor key nutrient source<br>- Controlled addition to medium<br>- Trend process performance |
| Lactate | Monitored | - Monitor by-product accumulation<br>- Trend process performance |
| Temperature | Monitored & controlled | - Provide optimal temperature for growth |
| Gassing | Monitored & controlled | - Dissolved oxygen control<br>- pH control |
| Agitation | Monitored & controlled | - Homogenous mixing<br>- Gas-liquid mass transfer and dissolved control |
| Dissolved oxygen | Monitored & controlled | - Provide oxygen for growth<br>- Trend process performance |
| pH | Monitored & controlled | - Provide optimal pH for growth<br>- Trend process performance |

## 3.6. Cell harvest

### *3.6.1. Hazards*

The key hazard associated with cell harvest includes:

- *Adventitious agents:* Cell handling during harvest, which may include transfers between equipment (*e.g.,* bioreactor and centrifuge), can lead to adventitious agent contamination. Microbial contamination may also originate from earlier manufacturing stages.

### *3.6.2. Testing and control measures*

#### *3.6.2.1. Adventitious agents*

Microbiological contamination is generally controlled through a food safety program (HACCP, GMP, GCCP) that ensures proper handling of cells, sanitation of equipment, and environmental controls (*e.g.,* positive pressure). Environmental monitoring programs detect potential contamination issues, including air and surface monitoring for indicator organisms. Testing is conducted in the final product (see Section 3.7.2. for more details).

### *3.6.3. Testing conducted on the harvested cells*

#### *3.6.3.1. Microbiological testing*

Companies identify relevant adventitious agents considering their potential to be introduced during manufacturing and the zoonotic potential (*i.e.*, can it be transmitted to humans). Companies may also test for non-zoonotic adventitious agents as part of quality control measures. Currently, some companies are being relatively conservative and testing for a panel of adventitious agents to confirm no carryover of zoonotic and non-zoonotic pathogens during sourcing or at the cell banking and cell storage stage (see Section 3.1.2.1. for testing during cell sourcing and Section 3.4.2. for testing during cell banking).

Companies use standard methods such as the Association of Official Analytical Chemists (AOAC) Official Methods of Analysis, American Public Health Association (APHA) Compendium Methods Microbiological Examination Foods (CMMEF), FDA Bacteriological Analytical Manual (BAM), International Organization of Standardization (ISO), Pharmacopoeia methods (*e.g.,* US [USP], British [BP], European [EP], or methods such as rt-PCR to detect adventitious agents.

**CASE STUDY: Microbiological testing on harvested cultivated chicken cells**

GOOD Meat batch release specifications include aerobic plate count (<10,000 cfu/g), yeast (<100 cfu/g), mold (<100 cfu/g), coliforms (<24 MPN/g), *E. coli* (<3 MPN/g), Enterococcus (<10 cfu/g), and *Salmonella spp.* (negative/25g). All batches of cultivated chicken are tested for microbiological safety and are only released for use as a food ingredient if microbiological specifications are met.

GOOD Meat additionally provided data from multiple batches on *Campylobacter* spp. (negative/25g), a human pathogen common in conventional poultry products, and numerous human and avian viruses in their US premarket notice. Human viruses tested include adeno-associated virus, hepatitis A/B/C, herpes simplex 1 and 2, herpesvirus 6/7/8, HIV-1, HIV-2, HPV-16, HPV-18, human cytomegalovirus, human foamy virus, human T-lymphotropic virus, John Cunningham virus, parvovirus B19, and *Mycoplasma* spp. Avian viruses tested include Avian reticuloendotheliosis virus, avian encephalomyelitis virus, avian leukosis virus A, avian leukosis virus B, avian leukosis virus J, fowl adenovirus 1, fowl adenovirus 3, chicken anemia virus, avian reovirus, *Salmonella pullorum,* and avian *Mycoplasma* spp. (GOOD Meat 2022b).

The UPSIDE Foods batch release specification for microorganisms is aerobic plate count (<100 cfu/g). If the aerobic plate count exceeds this limit, UPSIDE tests for *Enterobacteriaceae* (<10 cfu/g) and *Salmonella* spp. (negative/25g).

UPSIDE additionally provided data from multiple batches on coliforms (<10 cfu/g), *E. coli* (<10 cfu/g), mold (<10 cfu/g), yeast (<10 cfu/g), *Enterobacter cloacae* complex (negative), and Influenza Type A and Type B (negative) in their premarket notice to FDA (UPSIDE 2021).

Cultivated meat and seafood products are manufactured in conditions that reduce the potential for microbial contamination. Therefore, cultivated meat and seafood products have the potential to have lower microbial load compared to conventional products. Currently, the manufacturing processes for cultivated meat and seafood products are sometimes adapted from pharmaceutical manufacturing (*e.g.,* clean rooms), which can be excessively strict and arduous for food manufacturers. Some companies suggest that overly conservative batch release specifications may not be practical for commercial production of cultivated meat and seafood, and that microbial limits should be established to be closer to those of traditional products. Similar to conventional meat and seafood, cooking can eliminate many adventitious agents, while products that are intended to be consumed raw are likely to have more stringent specifications.

*3.6.3.2. Residue testing*

Companies measure the residue levels of potentially hazardous culture media substances after the cells are harvested. These are typically substances that do not have a history of use as a food additive or processing aid in food, such as antimicrobials, metals, anti-foaming agents, pH control agents, and growth factors.

**CASE STUDY: Histamines**

Histamine is important in many physiological processes, such as immune signaling. Still, it can pose a food safety risk in individuals with impaired ability to metabolize histamine or when ingested in high levels (Cleveland Clinic 2023, Comas-Basté *et al.,* 2020, EFSA 2011). Foods processed in unhygienic conditions or conditions inadequate for controlling bacterial growth may contain high levels of histamine due to the conversion of histidine to histamine by bacterial decarboxylases. The mitigation and control of bacteria is crucial for cultivated fish and seafood production, therefore histamines are not it expected to be a food safety concern. Histamine poisoning is most commonly associated with the consumption of spoiled fish, especially scombroid-type fish (*e.g.,* tuna, herring, mackerel, skipjack, bonito), which contain high levels of histidine (Taylor *et al.*, 1989, Comas-Basté *et al.,* 2020).

A Scientific Opinion by EFSA (2011) concludes that histamine levels below detectable limits can be considered safe for individuals with histamine intolerance. No adverse effects have been observed in healthy individuals exposed to 25-50 mg of histamine per meal.

Histamine is heat stable and cannot be removed in the production or cooking process. Cultivated products produced from cells originating from scombroid fish may contain higher histamine levels compared to those derived from other organisms. Cultivated products with high histamine content, as determined by amino acid analysis, may be labeled as an indicator for individuals with histamine intolerance. However, this is typically not required for conventional foods.

#### *3.6.3.3. Allergenicity testing*

Companies identify potential allergens based on cell type, potential cell expression (due to genetic drift or genetic modifications), allergenic inputs, or potential cross-contamination.

Testing for potentially hazardous proteins (*e.g.,* allergens, proteins added to culture media, or expressed in cells as part of cell line development) may be conducted via mass spectroscopy, ELISA analysis, or other immunoassays.

Safety documentation of raw materials combined with a well-established Supplier Approval Program also provide companies with information on allergenic agents and trace elements that can be contained in raw materials and compounds. The final product packaging is required to have proper labeling of allergens.

#### *3.6.3.4. Cell stability*

Prolonged cultivation naturally results in genetic and epigenetic drift (similar to that in animal breeding), which may result in altered cell characteristics. This could theoretically result in altered expression of allergenic proteins, toxins, or hazardous metabolites, as well as changes in the nutritional profile of the final product.

(Epi)genetic changes may cause different cell types to produce novel metabolites, some of which could be toxic or allergenic. Most species used for cultivated food production do not produce toxins. Thus, (epi)genetic changes are not likely to induce toxin production.

Changes in cell characteristics throughout product manufacture that indicate instability can be detected by monitoring consistency in cell growth rate, nutrient usage, morphology, and composition characterization. Some companies measure specific gene markers over multiple generations to ensure that cellular processes are constant. Genetic and epigenetic drift may be minimized through the use of quality-controlled cell banks and setting passage limits.

There has yet to be a consensus on how to evaluate cell stability and potential to produce hazardous substances. The SFA and other experts have suggested a combination of the following strategies:

1. Conduct a systematic scientific literature review to identify all known undesirable substances of food safety concern associated with the animal species of the cell culture and establish a list of such substances for subsequent targeted analysis.
2. Perform an *in silico* genome screen against relevant databases to establish a list of potential toxins/allergens for subsequent targeted analysis.
3. Carry out a quantitative comparison of the end-product cells against the starter cells through methodologies such as transcriptomics, proteomics, or metabolomics so that a list of differentially expressed undesirable substances of food safety concern can be established.

If any undesirable substances are identified in these analyses, or if there are any known toxins or allergens endogenously coded by a cell line, the final product may be tested for these substances. For example, a gene expression analysis with RNA-seq could be conducted and compared against expression in conventional animals. If the expression is within the range of natural variation, then it may be concluded there is no food safety concern. If the expression is higher than the normal range, or if the expression is different, then quantification of the proteins, hormones, or small molecules may be conducted (*e.g.*, with ELISA, LC-MS, GC-MS, HPLC, *etc*.).

*3.6.3.5. Tumorigenicity*

Companies do not use cancerous animal cells as their source cells. The food safety risk associated with the consumption of tumor cells in CM is anticipated to be low, as cells would need to survive food processing (*e.g*., cooking) and be capable of survival and growth in a different species. Additionally, consumers may already consume microtumours or precancerous lesions present in conventional meat; there is no evidence linking this exposure to cancer formation in humans (FAO 2023).

Cells may potentially accumulate genetic mutations, gene amplifications, and karyotypic abnormalities or rearrangement during cultivation that lead to tumorigenic transformation (Sato *et al.*, 2019). However, in the unlikely scenario of tumorigenic transformation, the food safety risk of

the cultivated meat product remains low, as cells must remain viable to result in teratoma formation in humans.

**Expert and regulatory views: Low risk of tumorigenicity**

Experts on the FAO/WHO Panel do not consider tumorigenic potential to be a significant food safety risk. According to the FAO/WHO report, the likelihood of tumor formation in cultivated foods is low because once the cells are harvested *i.e*., taken out of the bioreactor, they do not have a steady supply of nutrients, oxygen, and a fixed temperature to keep them alive. The cells would also need to survive food processing post-harvest, survive the gastrointestinal tract, enter into the bloodstream, and evade the body's immune systems to proliferate in the body and form a tumor. The likelihood of cells to survive any or all of these steps is low (FAO 2023).

The FDA's Scientific Memo on UPSIDE Foods' safety dossier noted, "The information reported was consistent with chicken-derived cells that displayed enhanced replicative capacity under *in vitro* conditions. However, once removed from the protected and controlled environment of the bioreactor the cells quickly die, removing any replicative capacity. Subsequent food processing (such as cooking) would further break down cellular structures and contents. Digestion after consuming food made from this cell material would also break down any residual cellular structure. No information presented by the firm or otherwise available to us indicated any mechanism by which this cellular material, once rendered non-living, heated, consumed, and digested, would retain any replicative capacity or the ability to induce replicative capacity in living cells exposed to this material." Therefore, even in the unlikely case of chromosomal restructuring, potential mutations, or cancer cell formation, the cells are not live when consumed, and even if they were, the stomach would further denature those cells and render them unviable (FDA 2022).

Indicators of possible tumorigenic transformation include increased chromosomal aberrations, altered cell morphology, increased genetic instability, and changes in cell growth. To assess the possibility of tumorigenic transformation, cultivated food manufacturers conduct routine monitoring of cell growth and morphology. They may also conduct karyotype analysis to detect the formation of chromosomal aberrations and further characterize production cell lines through whole genome sequencing, transcriptomics, proteomics, and metabolomics, which may reveal changes in cell characteristics (*e.g.*, gene expression, genome sequence) associated with tumorigenic transformation.

Potential mutational hotspots identified in human cancer cell lines may provide information for potential target sequences in animal cell lines to detect oncogenic mutations. Techniques such as total RNA sequencing, flow cytometry, genome-wide sequencing, and quantitative PCR can be employed to detect changes and activation of tumorigenic profiles in cells.

Cell therapy products (non-food) may be evaluated for the risk of tumorigenicity using *in vitro* and *in vivo* assays. For example, the *in vitro* soft agar colony formation assay measures the ability of

cells to grow in an anchorage-independent manner, a hallmark of cancerous cells. Manufacturers may also evaluate tumorigenicity *in vivo* by assessing the ability of cells to form tumors when injected into laboratory animals. This method, however, is conventionally used to assess the tumorigenicity of cell therapy products and may not accurately reflect the risk of tumorigenicity in products intended for use as food (Sato *et al*., 2019, GOOD Meat 2022).

Some cell lines deposited in cell banks are intended for biomedical work, for which standard tumorigenicity tests are more common. For example, GOOD Meat sourced their cells from a cell line deposited at the American Type Culture Collection that had been tested for tumorigenic potential, including the soft agarose colony formation assay and an *in vivo* tumorigenicity test (injection into adult chickens) (GOOD Meat 2022).

*3.6.3.6. Composition and nutrition*

Cultivated meat companies typically conduct composition and nutritional testing of their final harvested product. The testing compares the compositional and nutritional characteristics of a cultivated product to conventional products. Compositional testing includes proximate analysis for properties such as protein, fat, carbohydrate, moisture, ash, and caloric content. Cultivated meat companies may also analyze amino acids, fatty acids (*e.g.,* saturated, mono-unsaturated, poly-unsaturated, and trans), vitamins, and minerals. Heavy metal residue testing may also be part of a safety assessment to ensure no toxic heavy metals are present in the product above set regulatory thresholds. Cultivated meat companies can also use the nutritional and compositional testing results as a manufacturing monitoring tool to demonstrate uniformity in the production process and identify differences in composition, which are then further assessed for potential food safety hazards and/or used to set recommended dietary intake/recommended dietary allowances (RDI/RDA) levels and upper limit (UL) levels of the cultivated food. Composition databases for common foods such as meat and seafood already exist, such as the Japan Ministry of Education, Culture, Sports, Science, and Technology (MEXT) Standards Food Composition, USDA Food Data Central Database; Singapore Health Promotion Board (HPB) Energy and Nutrient Composition of Food database, and the Food Standards Australia New Zealand (FSANZ) Australian Food Composition Database.

Drawing from historical approaches to identify comparative parameters may support safety and nutritional assessment of cultivated meat and seafoods. These parameters may be derived from evaluating traditional food, food additives, genetically modified plants and animals, and drugs. The Codex Alimentarius Commission provides guidelines for the conduct of food safety assessment of foods derived from recombinant-DNA plants and recombinant-DNA animals (Codex Alimentarius Commission 2003a, 2003b). The assessments include a compositional analysis of key components. In evaluating genetically engineered animals like the AquAdvantage salmon and GalSafe pig intended for food, the proximate, vitamin, mineral, amino acid, and fatty acid parameters of the edible tissue were considered as part of the FDA safety assessment (FDA 2015, 2017). The assessment also involved examining the growth hormones present in tissue.

Different labeling may be necessary for specific species. If common food allergens are used in the inputs and present in the final product, these will have to be on the final product label.

It may or may not be appropriate to compare the nutritional value of cultivated meat and seafood to conventional animal products. What is important is to have a broader understanding of the nutritional composition of the cultivated product from a total diet perspective, but comparing it to existing meat/animal products may not be adequate because cultivated meat and seafood is usually only composed of a single cell culture, this cell culture is not representative of, *e.g.,* a whole cut of meat which is made up of various cell types. When the cultivated meat product is not intended to be an exact substitute for conventional meat, a comparison of the nutritional value of cultivated meat vs. conventional meat should not be part of the safety evaluation. Additionally, if the aim is to create meat products that are different or grown from an exotic animal, there might not be a lot of data available to conduct a nutritional comparison. It is a challenge to obtain that data ethically.

**CASE STUDY: Nutrient content in cultivated quail**

Australian cultivated food company Vow submitted an application to FSANZ to obtain permission to commercialize its cultivated quail as a novel food. In its "Hazard and risk assessment" of Vow cultivated quail, FSANZ did not identify any nutritional issues for the majority of nutrients assessed in the application. Still, FSANZ undertook a more detailed evaluation for a few specific nutrients that were higher in the cultivated quail cells when compared to their conventional counterpart, namely cobalamin, biotin, folate, iron, and sodium.

Vow proposes/assumes the serving size will be 150 g to 300 g. Per 300 g serving of cultivated quail, the consumed amount of cobalamin and biotin would be up to 929 times the estimated average requirement (EAR) and nine times the adequate intake (AI) respectively per serving. However, no upper limits (UL) exist for these vitamins, and no adverse effects have been reported when they are consumed in high quantities.

The folic acid content per 300 g serving size may exceed the UL in individuals aged 14 to 18. However, FSANZ does not see this as a health concern because 300 g serving size per day is likely an overestimation, and the product would be consumed infrequently.

Iron and sodium in the harvested quail cells were higher than in chicken breast, but the total iron intake would not exceed the UL for all the Australia and New Zealand population subgroups assessed, even if consumers eat 300 g of the harvested cells daily in addition to other conventional meats. Sodium consumption would be 8% to 19% higher for the Australian population aged 2 to 3 years; however, as with iron, a 300 g serving size is likely to be an overestimation for this age group.

FSANZ concluded that, "there were no nutritional risks identified from the consumption of the harvested [cultivated quail] cells containing the levels of nutrients provided in the application, particularly given the likely infrequent consumption of the harvested cells" (FSANZ 2023).

**Industry View: Benefits of cultivated meat**

There are potential health benefits of cultivated foods when compared to conventional products because cultivated meat and seafood are less likely to contain (or contain lower levels of) microbes (such as *Listeria*, yeast, coliforms, and mold), heavy metals (such as arsenic, lead, and mercury), and in the case of fish/seafood also microplastics and other items occasionally ingested by fish/seafood.

## 3.7. Food processing

After the cells are harvested, the cells may undergo more processing, and/or additional ingredients are added to create a hybrid product to give the cells more taste, texture, and nutritional qualities.

### *3.7.1. Hazards*

- *Adventitious agents:* Unhygienic handling of harvested cells can lead to microbial contamination.
- *Hazardous substances*: Ingredients added to the harvested cells may not be suitable for use in food.
- *Allergenicity*: Preservatives or other food ingredients used in food processing may be allergenic.

### *3.7.2. Testing and control measures*

*3.7.2.1. Adventitious agents*

Microbiological contamination is controlled through a food safety program (HACCP, GMP, GCCP) that ensures proper handling of cells, sanitation of equipment, and environmental controls (*e.g.,* positive pressure). Environmental monitoring programs detect potential contamination issues, including air and surface monitoring for indicator organisms. Testing may be conducted in the final product.

**CASE STUDY: Microbiological contamination in cultivated quail**

In December 2023, FSANZ published its "Hazard and risk assessment" of Vow cultivated quail in advance of the public consultation on Vow cultivated quail derived from embryonic fibroblast cells originating from *Coturnix japonica* (Japanese quail).

FSANZ identified that the main microbiological risk stems from the post-harvest process, where the harvested cells are exposed to the food production environment and foodborne pathogens. FSANZ identified *Listeria monocytogenes* as a concern during harvesting and final food, as this pathogen can grow at refrigeration temperatures. Other foodborne pathogens FSANZ highlights include *Salmonella* and *E. coli,* which could be introduced by personnel or other ingredients added during food processing.

FSANZ requires that the harvested cells undergo a microbiological control step, *e.g.,* cooking before consumption. FSANZ concluded that there is less microbial risk when the cells are isolated from the Japanese quail eggs as embryonic cells rather than from adult birds, *e.g.,* via a biopsy, as only pathogens in the reproductive system of the adult bird can be transmitted via vertical transmission to the embryonic cells.

For more details on microbial hazards per production step and possible risk mitigation strategies, see FSANZ's 'Hazard and risk assessment' of Vow cultivated quail - Appendix-IV: Microbiological Hazard Identification (FSANZ 2023).

*3.7.2.2. Hazardous substances*

The ingredients added to the final products are assessed for safety and suitability for use in food. Safety documentation of raw materials and a well-established Supplier Approval Program can provide companies with information on potential impurities.

*3.7.2.3. Allergenicity*

The ingredients added to the final products can be assessed for potential allergenicity. The final product packaging is required to have proper labeling of allergens. Safety documentation of raw materials and a well-established Supplier Approval Program can provide companies with information on allergenic agents.

## 3.8. Product packaging and distribution

The final product has to be packaged, stored, and distributed to reach the final consumers.

### *3.8.1. Hazards*

- *Physical contamination:* Foreign materials may be introduced due to poor packaging or during storage and distribution.
- *Adventitious agents:* Improper or damaged packaging can introduce environmental pathogens, especially if storage areas are unclean.

### *3.8.2. Testing and control methods*

*3.8.2.1. Physical contamination*

A standard food safety program includes approaches to control physical contamination during packaging and distribution.

*3.8.2.2. Adventitious agents*

Final product handling, packaging, and storage may introduce microbial contaminants. A food safety program includes approaches to control contamination during packaging and distribution, including the use of appropriate packaging materials for storing meat and seafood, storage of food

in conditions to prevent contamination and growth of microbes, cleaning in storage areas, inspection of storage areas/warehouses, and shipping inspection to verify the finished product is properly packaged and not damaged.

Currently, not all jurisdictions require shelf-life testing of the cultivated food product as part of the dossier, but some jurisdictions may require that it be available on demand (*e.g.,* SFA, US FDA). However, other jurisdictions (*e.g.,* EFSA) do require that the stability of the novel food be evaluated to identify the physicochemical, biochemical, and microbiological stability of the novel food under normal conditions of storage.

A shelf-life analysis may be conducted after representative storage conditions. A study may include testing of microbial quality, product organoleptic (sensory) properties, and physicochemical properties.

### 3.9. Food safety programs

The principles of Food Safety Programs such as GMP, HACCP, and GCCP generally apply to cultivated meat and seafood. Food Safety Programs help prevent, mitigate, and control microbiological, chemical, and physical hazards by establishing preventative measures for food safety within the manufacturing process, from the raw material procurement stage to product distribution. Similar to other foods, companies may have HACCP (or HACCP-like) plans analyzing hazards and controls, and environmental monitoring, employee safety training, supply chain, sanitation, product release, and traceability programs. Ovissipour *et al.* (2023) have published a sample food safety plan for a cultivated fish product. The food safety plan is applicable to all cultivated products.

**CASE STUDY: Supply Chain Program – incoming ingredients, materials, and non-food chemicals**

A supply chain program ensures that incoming ingredients, materials, and non-food chemicals are appropriate and safe for manufacturing food. The program includes checking expiration dates, reviewing allergen statements, and physically inspecting materials. Cultivated meat and seafood companies vet suppliers, confirming that suppliers hold appropriate facility certifications, and have food safety management systems. Materials must be manufactured, transported, and stored to prevent microbiological, chemical, and physical damage. Any incoming materials must meet identity, quality, and purity specifications, and suppliers may be asked to supply Certificates of Analysis (CoA).

Many companies do not consider the “pharma-grade” standard necessary or appropriate for ingredients used in food production. The specifications are unnecessarily stringent for food development. Cultivated meat and seafood companies may use existing specifications (*e.g*., FCC, JECFA) or develop their own specifications. Some companies note that wider specifications may be applied to culture media ingredients compared to pharma-grade, as some batch-to-batch variability in food is acceptable if the variability does not introduce food hazards and is within the producers’ specifications.

Packaging materials and labels of all incoming raw materials should be inspected for signs of damage, spoilage, or contamination. To avoid cross-contamination, ingredients with allergenic components should be stored together, separated from the other ingredients, and labeled properly. Pest control inspections should be regularly conducted in the ingredient storage areas (Ovissipour *et al.*, 2023).

Good Manufacturing Practices prevent the introduction of foreign object contamination (*e.g.,* plastic, metal, hair, jewelry, glass*, etc*.). An X-ray inspection system is sometimes implemented to detect foreign contaminants such as metal or glass fragments.

Water should be suitable for use in food production. Some companies filter or treat water with UV before it enters the facility, and water samples can be regularly tested for adventitious agents, such as total coliforms and *E. coli* (Ovissipour *et al.*, 2023, FDA 2023a).

**CASE STUDY: Environmental monitoring**

GOOD Meat environmental monitoring includes viable air and surface monitoring in processing areas and during operation in biosafety cabinets; aerobic plate count and *Enterobacteriaceae* swab tests on bioreactor outlet tubings, and *Listeria* and *Salmonella* swab tests on sink drains (GOOD Meat 2022). Similarly, UPSIDE describes indicator and pathogen organism monitoring, including swab tests, as part of their environmental monitoring plan (UPSIDE 2021).

In its scientific memos, FDA summarized the management strategies used by Upside and GOOD Meat to address potential food safety issues during manufacturing. A robust food safety program was implemented by the companies that included appropriate testing and monitoring, aseptic procedures, sterilization, supplier management, and controlled manufacturing processes. These were summarized by the FDA (Table 6 and 7).

Table 6. FDA summary of Upside's strategies to manage potential food safety issues (from FDA 2022)

| Process Step | Potential Issues | Management Strategies |
|---|---|---|
| Cell Isolation | Cell identity; contaminants from source, reagents, or environment | Antibiotics, aseptic procedures, documentation, sterilization, supplier management, testing program |
| Establishment of Cell Lines | Cell identity; contaminants from materials or environment; appropriate adaptation to culture; introduced genetic material and expression product | Aseptic procedures, documentation, genetic sequencing, food safety assessment[4], process and environmental monitoring, sterilization, supplier management, testing program |
| Establishment of Master Cell Banks (MCB) | Cell identity; contaminants from materials or environment; appropriate adaptation to culture | Aseptic procedures, documentation process and environmental monitoring, sterilization, supplier management, testing program |
| Proliferation Phase | Contaminants from materials, equipment, or environment; media components | Aseptic procedures, documentation, food safety assessment, process and environmental monitoring, sterilization, supplier management, testing program |
| Differentiation Phase | Contaminants from materials, equipment, or environment; media components | Aseptic procedures, documentation, food safety assessment, process and environmental monitoring, sterilization, supplier management testing program |
| Harvest of Cell Material | Contaminants from materials, equipment, or environment; media components | Compositional analysis, controlled temperature conditions, food safety assessment, specifications, sterilization, supplier management, testing program, washing |

Table 7. FDA summary of GOOD Meat's strategies to manage potential food safety issues (from FDA 2023b)

| Process Step | Potential Issue | Management Strategy |
|---|---|---|
| Cell Procurement | Cell identity; contamination from source, reagents, or environment | Aseptic procedures, documentation, genetic testing, supplier management, testing program, filtration of media, controlled temperature conditions |
| Establishment of Cell Lines | Cell identity; contaminants from materials or environment; appropriate adaptation to culture | Aseptic procedures, documentation, genetic sequencing, process and environmental monitoring, supplier management, testing program |
| Establishment of Master Cell Banks (MCB) and Master Working Cell Banks (MWCB) | Cell identity; contaminants from materials or environment; media components; appropriate adaptation to culture | Aseptic procedures, documentation, genetic sequencing, controlled temperature conditions, process and environmental monitoring, testing program |
| Cell Proliferation using Suspension Culture | Cell identity; contaminants from materials, equipment, or environment; contamination during transportation, media components | Aseptic procedures, controlled temperature conditions, documentation, genetic testing, process and environmental monitoring, supplier control, testing program |
| Harvest of Cell Material | Contaminants from materials or environment; media components | Aseptic procedures, compositional analysis, controlled temperature conditions, documentation, testing program, washing |

Novel manufacturing methods

Novel manufacturing methods need to be assessed for their potential to introduce microbial, chemical, or physical hazards or produce novel hazardous substances. As with all manufacturing approaches, the development of HACCP plans can identify potential hazards and risk mitigation and control strategies.

When additional equipment is introduced to produce cultivated foods, this can present another opportunity to expose the foods to contaminants. For example, during 3D printing of cultivated meat, the bioink comes into contact with several parts of the printer, such as the extruder, nozzle, and piston. These parts are located inside the printer, making them hard to clean. This may promote the growth of microorganisms inside the printer that could be passed onto the ink, such as *Staphylococcus aureus* and *E. coli* (Dong *et al.,* 2023). If possible, nozzles should still be changed

between each production batch and washed and sanitized at the end of the shift. The bioink holder and bioink preparation vessels should also be washed and sanitized at the end of each production cycle (Ovissipour *et al.*, 2023).

Media recycling is currently not common practice, but it could be implemented to reduce resource use and production costs. Control measures, including UV treatment, filtration, and testing, may be implemented to reduce contamination.

## SECTION 4. RECOMMENDATIONS

This section provides a summary of recommendations for information requirements to be included in a regulatory submission for approval of cultivated meat and seafood products (Table 8). This information was developed by analyzing current requirements from regulatory agencies, expert recommendations, literature, and industry interviews.

Companies encourage regulatory agencies to provide official guidance on information requirements as it provides clarity and supports more efficient regulatory approval processes. Companies also encourage the establishment of clear guidance on submission and review processes, if the need of submission and review is justified by the local authority, including instructions on the submission process, *i.e.,* how to submit the dossier, where to submit the dossier, who will review the dossier, etc. and developing statutory timeframes for the dossier review, *i.e.,* processing periods, length of dossier review, *etc.* If jurisdictions need more resources and expertise for the dossier reviews, a recommendation is to establish expert groups to review technical questions.

Collaboration and harmonization across regulatory agencies are encouraged, so companies only have to develop one dossier appropriate for multiple jurisdictions. It was suggested that relevant ministries could consider regulatory acceptance from other jurisdictions as part of the review process, for example, fast-tracking reviews that have already been authorized in other countries.

Companies are strongly opposed to *in vivo* testing and believe that safety can be demonstrated without animal testing.

Companies expressed opinions on the information that should be made available to the public as part of the dossier review process. All companies agree that intellectual property (IP) and commercially sensitive information should be maintained as confidential business information. Safety information that is not IP or commercially sensitive could be made public, as it can help instill public confidence in cultivated meat and seafood. However, it was recognized that some information is not easily digestible or understood by the public, *e.g.,* genetic drift or immortalization. If complex scientific information is shared publicly, it could be accompanied by descriptions that are more easily understood by the general public.

Generally, it would be beneficial for companies if regulatory bodies provide guidelines on submitting and approving dossiers. Companies could submit more complete dossiers, easing the review process for regulatory agencies.

Table 8 represents a sample documentation checklist to be included in the dossier. It may serve as a template checklist for companies to follow to prepare for effectively and efficiently communicating with the resource-limited government. The template can even be used by the authority as guidance for a company that intends to apply for authorization to commercialize its cultivated meat and seafood products in Japan, if any approval system is officially established.

The specific documentation each company will be required/recommended to provide may vary depending on the cell type and animal species used. Companies noted that general information requirements would be similar across cell lines and production processes, though some details would differ. For example, the adventitious agents tested may vary depending on the origin of the cells being used.

Table 8. Recommended safety information requirements for a dossier

| Manufacturing step | Documentation information | Description |
|---|---|---|
| **Cell sourcing** | Cell origin | Description of cell origin (species, biopsy, slaughtered animal, cell line provider, *etc*.) |
| | Type of cell | Description of type of cell (GMO, immortalized, stem cell, tissue, *etc.*) |
| | Species identity | Verification of species identity |
| | Source animal health | Demonstration that biopsies/cell sourcing comply with animal health and food safety requirements. Health of the sample animal (if possible) |
| | Prions | Description of prevention/mitigation steps to avoid prion contamination (if applicable – bovine sources) |
| | Analysis of inputs | Listing of substances used (antibiotics, substances for sterilization, *etc*.) and safety assessment |
| **Establishment of cell lines** | Cell characteristics | Documentation of cell characteristics, *e.g.,* morphology, cell viability, doubling time, cell stability, cell density, protein yield |
| | Genetic modification | If genetically modified, description of genetic modification process and safety evaluation |
| | Analysis of inputs | Listing of substances used and safety assessment |
| | Adventitious agents | Microbiological safety assessment - testing for viruses, bacteria, and mycoplasma |
| **Cell storage** | Analysis of inputs | Safety assessment of substances (cryoprotectant, antibiotics, substances for sterilization, *etc*.) |
| | Adventitious agents | Microbiological safety assessment of the cells - testing for viruses, bacteria, and mycoplasma |
| **Mass cultivation: Cell proliferation** | Analysis of inputs | Safety assessment of media components, scaffold, and other added substances demonstrating that the substance is food-safe |

| Manufacturing step | Documentation information | Description |
|---|---|---|
| **and differentiation**<br><br>**Mass cultivation: Cell proliferation and differentiation** | | Animal derived components: Documentation demonstrating that animal-derived substances do not contain disease-agents or other hazardous substances<br><br>Biological agents: Documentation demonstrating safe use<br><br>Components derived from genetically modified organisms: Documentation demonstrating safe use |
| | Cell contamination | Monitor for microbiological or chemical contamination |
| | Chemical contaminants | Mitigation or measurement of chemical contaminants from equipment, cleaning products, ingredients, *etc.* |
| | Genetic stability* | Monitor genetic stability |
| **Cell harvest** | Composition | Analysis of nutritional composition (proximate, amino acid, vitamins, minerals, fatty acids) |
| | Residue analysis | Measurement of potentially hazardous residues and safety assessment |
| | Adventitious agents | Measurement of viruses, bacteria, yeast, mold |
| | Genetic stability* | Assessment of genetic stability and evaluate the potential for production of unintended toxins or allergens. |
| | Chemical contaminants | Measurement of chemical contaminants (*e.g.*, heavy metals, cleaning substances, *etc.*) |
| | Food allergens | Assessment for food allergens |
| **Other information** | Manufacturing process | Detailed description of the manufacturing process |
| | Estimated dietary intake and Intended use | Proposed maximum use level/serving size portion, or calculation of potential exposure |
| | Food Safety programs | Description of food safety programs, including Good Manufacturing Practices (GMP), Hazard Analysis Critical Control Points (HACCP), Hazard Analysis and Risk-Based Preventive |

| Manufacturing step | Documentation information | Description |
|---|---|---|
| | | Controls (HARPC), Quality control measures, Good Cell Culture Practices (GCCP) |

*Note that there is not consensus on this requirement. Some experts do not consider genetic instability to be a feasible food risk.

The flowchart in Figure 6 provides an overview of the safety documentation checklist and the potential workflow from development and implementation of a food safety program, to testing and gathering information, to stages of review and revisions of the dossier, through to the final submission of the dossier.


Figure 6. Overview of the recommended safety information requirements and process flow for submission of a dossier.

## REFERENCES


Aleph Farms. (n.d.). Our Technology. https://aleph-farms.com/our-recipe/

Allan, S. J., De Bank, P. A., & Ellis, M. J. (2019). Bioprocess design considerations for cultured meat production with a focus on the expansion bioreactor. *Frontiers in Sustainable Food Systems*, 3, 44. https://doi.org/10.3389/fsufs.2019.00044

American Cancer Society. (2023). Viruses that Can Lead to Cancer. *American Cancer Society.* https://www.cancer.org/cancer/risk-prevention/infections/infections-that-can-lead-to-cancer/viruses.html

Antloga, K., Meszaros, J., Malchesky, P. S., & McDonnell, G. E. (2000). Prion disease and medical devices. *ASAIO journal*, 46(6), S69-S72. http://doi.org/10.1097/00002480-200011000-00040

ATCC. (n.d.). Animal Cells – ATCC animal cells provide taxonomic equivalents resembling human physiology. *ATCC*. https://www.atcc.org/cell-products/animal-cells#t=productTab&numberOfResults=24

Bailly, A., Milhavet, O., & Lemaitre, J. M. (2022). RNA-based strategies for cell reprogramming toward pluripotency. *Pharmaceutics*, 14(2), 317. https://doi.org/10.3390/pharmaceutics14020317

Balasubramanian, B., Liu, W., Pushparaj, K., & Park, S. (2021). The epic of in vitro meat production—a fiction into reality. *Foods*, 10(6), 1395. https://doi.org/10.3390/foods10061395

Bellani, C. F., Ajeian, J., Duffy, L., Miotto, M., Groenewegen, L., & Connon, C. J. (2020). Scale-up technologies for the manufacture of adherent cells. *Frontiers in nutrition*, 7, 575146. https://doi.org/10.3389/fnut.2020.575146

Ben-Arye, T., Shandalov, Y., Ben-Shaul, S., Landau, S., Zagury, Y., Ianovici, I., ... & Levenberg, S. (2020). Textured soy protein scaffolds enable the generation of three-dimensional bovine skeletal muscle tissue for cell-based meat. *Nature Food*, 1(4), 210-220. https://doi.org/10.1038/s43016-020-0046-5

Bodiou, V., Moutsatsou, P., & Post, M. J. (2020). Microcarriers for upscaling cultured meat production. *Frontiers in nutrition*, 7, 10. https://doi.org/10.3389/fnut.2020.00010

Bogliotti, Y. S., Wu, J., Vilarino, M., Okamura, D., Soto, D. A., Zhong, C., ... & Ross, P. J. (2018). Efficient derivation of stable primed pluripotent embryonic stem cells from bovine blastocysts. *Proceedings of the National Academy of Sciences*, 115(9), 2090-2095. https://doi.org/10.1073/pnas.1716161115

Bomkamp, C., Carter, M., Cohen, M., Gertner, D., Ignaszewski, E., …& Voss, S. (2022). Cultivated meat and seafood: 2022 State of the Industry. *The Good Food Institute*. https://gfi.org/wp-content/uploads/2023/01/2022-Cultivated-Meat-State-of-the-Industry-Report.pdf

Breemhaar, J. J., & Post, M. (2022). U.S. Patent No. 11,512,273 - Apparatus and process for production of tissue from cells. Washington, DC: *U.S. Patent and Trademark Office.* https://patents.google.com/patent/US11512273B2/en

Brown, P. T., Handorf, A. M., Bae Jeon, W., & Li, W. J. (2013). Stem cell-based tissue engineering approaches for musculoskeletal regeneration. *Current pharmaceutical design*, 19(19), 3429-3445. https://doi.org/10.2174/13816128113199990350

Caliari, S. R., & Burdick, J. A. (2016). A practical guide to hydrogels for cell culture. *Nature methods*, 13(5), 405-414. https://doi.org/10.1038/nmeth.3839

Cellosaurus. (n.d.). Cellosaurus: Browse by cell line group. *Cellosaurus.* https://www.cellosaurus.org/browse_by_group

Celltainer Biotech. (2020). CELL-tainer single-use rocking bioreactor applied for cultured meat. *Celltainer Biotech.* https://celltainer.com/cell-tainer-single-use-rocking-bioreactor-applied-for-cultured-meat/

Chatterjee, I., Li, F., Kohler, E. E., Rehman, J., Malik, A. B., & Wary, K. K. (2016). Induced pluripotent stem (iPS) cell culture methods and induction of differentiation into endothelial cells. *Induced Pluripotent Stem (iPS) Cells: Methods and Protocols,* 311-327. https://doi.org/10.1007/7651_2015_203

Cleveland Clinic. (2023). Histamine. *Cleveland Clinic.* https://my.clevelandclinic.org/health/articles/24854-histamine

Comas-Basté, O., Sánchez-Pérez, S., Veciana-Nogués, M. T., Latorre-Moratalla, M., & Vidal-Carou, M. D. C. (2020). Histamine intolerance: The current state of the art. *Biomolecules*, *10*(8), 1181. https://doi.org/10.3390/biom10081181

Dai, Z., Ronholm, J., Tian, Y., Sethi, B., & Cao, X. (2016). Sterilization techniques for biodegradable scaffolds in tissue engineering applications. *Journal of tissue engineering*, 7, 2041731416648810. https://doi.org/10.1177/2041731416648881

David, S., Tsukerman, A., Safina, D., Maor-Shoshani, A., Lavon, N., & Levenberg, S. (2023). Co-culture approaches for cultivated meat production. *Nature Reviews Bioengineering*, 1-15. https://doi.org/10.1038/s44222-023-00077-x

Derakhti, S., Safiabadi-Tali, S. H., Amoabediny, G., & Sheikhpour, M. (2019). Attachment and detachment strategies in microcarrier-based cell culture technology: A comprehensive review. *Materials Science and Engineering*: *C*, 103, 109782. https://doi.org/10.1016/j.msec.2019.109782

Dong, N., Xue, C., Yang, Y., Chang, Y., Wang, Y., Guo, H., ... & Wang, Y. (2023). Auxenochlorella pyrenoidosa extract supplementation replacing fetal bovine serum for Carassius auratus muscle cell culture under low-serum conditions. *Food Research International*, 164, 112438. https://doi.org/10.1016/j.foodres.2022.112438

EFSA. (2011). Scientific Opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Journal*. https://doi.org/10.2903/j.efsa.2011.2393

EFSA. (2016). Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. *EFSA Journal.* https://doi.org/10.2903/j.efsa.2016.4594

EFSA. (2006). Opinion of the Scientific Panel on biological hazards (BIOHAZ) on the BSE risk from cohort animals: bovine hides and skins for technical purposes. *EFSA Journal*, 4(6), 367. https://doi.org/10.2903/j.efsa.2006.367

EFSA. (2011). Joint Scientific Opinion on any possible epidemiological or molecular association between TSEs in animals and humans. *EFSA Journal*, 9(1), 1945. http://doi.org/10.2903/j.efsa.2011.1945

EFSA. (2016). Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. *EFSA Journal*, 14(11), e04594. https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2016.4594

EFSA. (2021). Novel foods: alternative proteins and their sources. *EFSAchannel*. https://www.youtube.com/watch?v=FQO0gi9aM6s&t=1209s

Ellis, M. (2021). Designing large-scale bioreactors and bioprocesses for cultivated meat. *The Good Food Institute*. https://www.youtube.com/watch?v=_9HwoIpNuCM

EMA. (2011). Reflection paper on stem cell-based medicinal products. *European Medicines Agency/Committee for Advanced Therapies*, 571134. https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-stem-cell-based-medicinal-products_en.pdf

FAO. (2023). Food safety aspects of cell-based food. *World Health Organization.* https://doi.org/10.4060/cc4855en

FDA. (2015). AquAdvantage Salmon Approval Letter and Appendix; FDA: College Park, MD, USA.

FDA. (2017). Guidance for Industry: Regulation of Intentionally Altered Genomic DNA in Animals; U.S. Department of Health and Human Services: Rockville, MD, USA; pp. 1–30.

FDA. (2022). Memorandum - Cell Culture Consultation (CCC) 000002, Cultured *Gallus gallus* cell material. *U.S. Food and Drug Administration.* https://www.fda.gov/media/163261/download

FDA. (2023a). Import Alert 16-125. *U.S. Food and Drug Administration.* https://www.accessdata.fda.gov/cms_ia/importalert_28.html

FDA. (2023b). Memorandum - Cell Culture Consultation (CCC) 000001, Cultured *Gallus gallus* cell material. *U.S. Food and Drug Administration.* https://www.fda.gov/media/166348/download

Ferreira, F., (2023). Manufacturing cultured fish fillet. *The Good Food Institute.* https://www.youtube.com/watch?v=GrsJ1YPMxH4

Food Safety Magazine. (2023). UK FSA Seeking Food Industry Respondents for Survey on Cell-Based Product Authorization. *Food Safety.* https://www.food-safety.com/articles/9179-uk-fsa-seeking-food-industry-respondents-for-survey-on-cell-based-product-authorization

Forero, A., Giacobbi, N. S., McCormick, K. D., Gjoerup, O. V., Bakkenist, C. J., Pipas, J. M., & Sarkar, S. N. (2014). Simian virus 40 large T antigen induces IFN-stimulated genes through ATR kinase. *The Journal of Immunology*, 192(12), 5933-5942. https://doi.org/10.4049/jimmunol.1303470

FSA. (2023). Cell-cultivated products. *U.K. Food Standards Agency*. https://www.food.gov.uk/business-guidance/cell-cultivated-products

FSANZ. (2019). Application Handbook. *Food Standards Australia New Zealand*. https://www.foodstandards.gov.au/code/changes/Documents/Application%20Handbook%20at%201%20July%202019.pdf.

FSANZ. (2023). Supporting document 1: Hazard and risk assessment – Application A1269 Cultured quail as a novel food. *Food Standards Australia New Zealand*. https://www.foodstandards.gov.au/sites/default/files/2023-12/SD1%20-%20Hazard%20and%20risk%20assessment_0.pdf

FSIS. (2023a). FSIS Directive 7800.1: Responsibilities in Establishments Producing Cell-Cultured Meat and Poultry Food Products. *U.S.Department of Agriculture.* https://www.fsis.usda.gov/sites/default/files/media_file/documents/7800.1.pdf

FSIS. (2023b). FSIS Notice 31-23: Updated-Cell-Cultured Meat and Poultry Food Products Sampling Program. *U.S.Department of Agriculture.*. https://www.fsis.usda.gov/sites/default/files/media_file/documents/31-23.pdf

GFI. (2020). Promoting stemness and proliferation in fish cell cultures. *The Good Food Institute.* https://gfi.org/solutions/fish-cell-proliferation/

GFI. (2021a). Deep dive: Cultivated meat bioprocess design. *The Good Food Institute.* https://gfi.org/science/the-science-of-cultivated-meat/deep-dive-cultivated-meat-bioprocess-design/

GFI. (2021b). Deep dive: Cultivated meat cell culture media. *The Good Food Institute.* https://gfi.org/science/the-science-of-cultivated-meat/deep-dive-cultivated-meat-cell-culture-media/

GFI. (2021c). Incorporating omega-3s into cultivated seafood. *The Good Food Institute.* https://gfi.org/solutions/incorporating-omega-3s-into-cultivated-seafood/

GFI. (2022a). Cultivated meat and seafood: 2022 State of the Industry. *The Good Food Institute.* https://www.youtube.com/watch?v=V5U2lCRxS3M

GFI. (2022b). Ensuring appropriate food safety controls for cultivated meat. *The Good Food Institute*. https://gfi.org/solutions/cultivated-meat-food-safety-controls/

GFI. (2023a). Deep dive: Cultivated meat scaffolding. *The Good Food Institute.* https://gfi.org/science/the-science-of-cultivated-meat/deep-dive-cultivated-meat-scaffolding/

GFI. (2023b). Promoting stemness and proliferation in fish cell cultures. *The Good Food Institute.* https://gfi.org/wp-content/uploads/2023/01/External-Promoting-stemness-and-proliferation-in-fish-cell-cultures_-Workshop-summary.pdf

GFI. (2023c). Reimagining Meat: Pathways for Cell Biologists in the Cultivated Meat Field. *The Good Food Institute*. https://www.youtube.com/watch?v=-UTOHYyOFQk

GFI. (2023d). The science of cultivated meat. *The Good Food Institute.* https://gfi.org/science/the-science-of-cultivated-meat/

GFI. (2024). The Ministry of Food and Drug Safety of Republic of Korea is now accepting applications for regulatory approval of cultivated meat! *The Good Food Institute.* https://www.linkedin.com/posts/gfi-apac_%EC%8B%9D%EC%95%BD%EC%B2%98-%EC%84%B8%ED%8F%AC%EB%B0%B0%EC%96%91-%EC%8B%9D%ED%92%88-%EC%9B%90%EB%A3%8C-%EC%8B%9D%ED%92%88-%EC%9D%B8%EC%A0%95-%EC%A0%88%EC%B0%A8-%EB%A7%88%EB%A0%A8-activity-7166309659761463299-TK7E/?u

Giles, K., Woerman, A. L., Berry, D. B., & Prusiner, S. B. (2017). Bioassays and inactivation of prions. *Cold Spring Harbor perspectives in biology*, 9(8), a023499. http://doi.org/10.1101/cshperspect.a023499

GMAC (Genetic Modification Advisory Committee of Singapore (1999). Singapore Guidelines on the Release of Agriculture-Related Genetically Modified Organisms. https://www.gmac.sg/wp-content/uploads/2022/03/Agriculture_Guidelines.pdf

GOOD Meat. (2022a). GOOD Meat Partners with ABEC Plans to Build the World's First Large-Scale Cultivated Meat Facility. https://www.goodmeat.co/all-news/good-meat-partners-with-industry-leader-to-build-first-large-scale-cultivated-meat-facility

GOOD Meat. (2022b). Dossier in Support of the Safety of GOOD Meat Cultured Chicken as a Human Food Ingredient. *U.S. Food and Drug Administration.* https://www.fda.gov/media/166346/download?mod=article_inline

Gough, K. C., & Maddison, B. C. (2010). Prion transmission: prion excretion and occurrence in the environment. *Prion*, 4(4), 275-282. https://doi.org/10.4161/pri.4.4.13678

Guan, X., Zhou, J., Du, G., & Chen, J. (2022). Bioprocessing technology of muscle stem cells: Implications for cultured meat. *Trends in Biotechnology*, 40(6), 721-734. https://www.cell.com/trends/biotechnology/abstract/S0167-7799(21)00265-1

Guo, D., Zhang, L., Wang, X., Zheng, J., & Lin, S. (2022). Establishment methods and research progress of livestock and poultry immortalized cell lines: A review. *Frontiers in Veterinary Science*, 9, 956357. http://doi.org/10.3389/fvets.2022.956357

Hein, J., Boichuk, S., Wu, J., Cheng, Y., Freire, R., Jat, P. S., ... & Gjoerup, O. V. (2009). Simian virus 40 large T antigen disrupts genome integrity and activates a DNA damage response via Bub1 binding. *Journal of virology*, 83(1), 117-127. https://doi.org/10.1128/jvi.01515-08

Heles, T. (2019). Feeding the world: the story of Cellular Agriculture. *Global University Venturing*. https://globalventuring.com/university/feeding-the-world-the-story-of-cellular-agriculture/

Ho, Y. Y., Lu, H. K., Lim, Z. F. S., Lim, H. W., Ho, Y. S., & Ng, S. K. (2021). Applications and analysis of hydrolysates in animal cell culture. *Bioresources and Bioprocessing*, 8, 1-15. https://doi.org/10.1186/s40643-021-00443-w

Hochedlinger, K., & Jaenisch, R. (2015). Induced pluripotency and epigenetic reprogramming. *Cold Spring Harbor perspectives in biology*, 7(12), a019448. https://doi.org/10.1101/cshperspect.a019448

Houston, F., & Andréoletti, O. (2018). The zoonotic potential of animal prion diseases. In Handbook of clinical neurology (Vol. 153, pp. 447-462). *Elsevier.* https://doi.org/10.1016/B978-0-444-63945-5.00025-8

Huang, Y., Liang, P., Liu, D., Huang, J., & Songyang, Z. (2014). Telomere regulation in pluripotent stem cells. *Protein & Cell*, 5(3), 194-202. https://doi.org/10.1007/s13238-014-0028-1

Hubalek, S., Post, M. J., & Moutsatsou, P. (2022). Towards resource-efficient and cost-efficient cultured meat. *Current Opinion in Food Science,* 47, 100885. https://doi.org/10.1016/j.cofs.2022.100885

Ianovici, I., Zagury, Y., Redenski, I., Lavon, N., & Levenberg, S. (2022). 3D-printable plant protein-enriched scaffolds for cultivated meat development. *Biomaterials,* 284, 121487. https://doi.org/10.1016/j.biomaterials.2022.121487

Ito, N., Kii, I., Shimizu, N., Tanaka, H., & Takeda, S. I. (2017). Direct reprogramming of fibroblasts into skeletal muscle progenitor cells by transcription factors enriched in undifferentiated subpopulation of satellite cells. *Scientific Reports*, 7(1), 8097. https://www.nature.com/articles/s41598-017-08232-2

Jaime-Rodríguez, M., Cadena-Hernández, A. L., Rosales-Valencia, L. D., Padilla-Sánchez, J. M., & Chavez-Santoscoy, R. A. (2023). Are genetic drift and stem cell adherence in laboratory culture issues for cultivated meat production? *Frontiers in Nutrition*, 10. http://doi.org/10.3389/fnut.2023.1189664

Kamali-Jamil, R., Vázquez-Fernández, E., Tancowny, B., Rathod, V., Amidian, S., Wang, X., ... & Wille, H. (2021). The ultrastructure of infectious L-type bovine spongiform encephalopathy prions

constrains molecular models. *PLoS Pathogens*, 17(6), e1009628. https://doi.org/10.1371/journal.ppat.1009628

Khan, F. A., Almohazey, D., Alomari, M., & Almofty, S. A. (2018). Isolation, culture, and functional characterization of human embryonic stem cells: current trends and challenges. *Stem cells international*, 2018. https://doi.org/10.1155/2018/1429351

Krance, S. H., Luke, R., Shenouda, M., Israwi, A. R., Colpitts, S. J., Darwish, L., ... & Watts, J. C. (2020). Cellular models for discovering prion disease therapeutics: Progress and challenges. *Journal of Neurochemistry*, 153(2), 150-172. https://doi.org/10.1111/jnc.14956

Krieger, J. (2022). Cultivated meat science and product development. The Good Food Institute. https://www.youtube.com/watch?v=dxwk3EAWZ3I

Langeveld, J. P., Wang, J. J., Van de Wiel, D. F., Shih, G. C., Garssen, G. J., Bossers, A., & Shih, J. C. (2003). Enzymatic degradation of prion protein in brain stem from infected cattle and sheep. *The Journal of infectious diseases*, 188(11), 1782-1789. https://doi.org/10.1086/379664

Lee, A. S., Tang, C., Rao, M. S., Weissman, I. L., & Wu, J. C. (2013). Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. *Nature medicine*, 19(8), 998-1004. https://www.nature.com/articles/nm.3267

Lee, D. K., Kim, M., Jeong, J., Lee, Y. S., Yoon, J. W., An, M. J., ... & Lee, C. K. (2023a). Unlocking the potential of stem cells: Their crucial role in the production of cultivated meat. *Current Research in Food Science*, 100551. https://doi.org/10.1016/j.crfs.2023.100551

Lee, S. Y., Kang, H. J., Kang, J. H., Ramani, S., Park, S., & Hur, S. J. (2021a). Principal protocols for the processing of cultured meat. *Journal of animal science and technology*, 63(4), 673. https://doi.org/10.5187/jast.2021.e40

Lee, K. Y., Loh, H. X., & Wan, A. C. (2021b). Systems for muscle cell differentiation: from bioengineering to future food. *Micromachines*, 13(1), 71. https://doi.org/10.3390/mi13010071

Lee, D. K., Kim, M., Jeong, J., Lee, Y. S., Yoon, J. W., An, M. J., ... & Lee, C. K. (2023a). Unlocking the potential of stem cells: Their crucial role in the production of cultivated meat. *Current Research in Food Science*, 100551. https://www.sciencedirect.com/science/article/pii/S2665927123001193

Lee, S. Y., Jeong, J. W., Kim, J. H., Yun, S. H., Mariano Jr, E., Lee, J., ... & Hur, S. J. (2023b). Current technologies, regulation, and future perspective of animal product analogs—A review. *Animal Bioscience*, 36(10), 1465. https://doi.org/10.5713/ab.23.0029

Leung, M., Benton, P. R., Copeland, C. G., & JOHNSON, K. L. (2022). U.S. Patent Application No. 16/996,567 - *Systems, Devices, and Methods for Sterilizing Bioreactors and Culture Media.* https://patents.google.com/patent/WO2022039998A1/en

Levenberg, S., Ianovici, I., Zagury, Y., Lavon N., & David S. (2022). U.S. Patent Application No. 63/142.511- 3d-printable protein-enriched scaffolds. https://patents.google.com/patent/WO2022162662A1/en?oq=WO2022162662A1

May, T., Hauser, H., & Wirth, D. (2004). Transcriptional control of SV40 T-antigen expression allows a complete reversion of immortalization. *Nucleic acids research*, 32(18), 5529-5538. https://doi.org/10.1093/nar/gkh887

Mayrhofer, P. & Kunert, R. (2020). Perfusion Medium Development for Continuous Bioprocessing of Animal Cell Cultures. *American Pharmaceutical Review.* https://www.americanpharmaceuticalreview.com/Featured-Articles/562913-Perfusion-Medium-Development-for-Continuous-Bioprocessing-of-Animal-Cell-Cultures/

Mendonça da Silva, J., Erro, E., Awan, M., Chalmers, S. A., Fuller, B., & Selden, C. (2020). Small-scale fluidized bed bioreactor for long-term dynamic culture of 3D cell constructs and in vitro testing. *Frontiers in Bioengineering and Biotechnology*, *8*, 895. https://www.frontiersin.org/articles/10.3389/fbioe.2020.00895/full

Merlo, B., Gugole, P. M., & Iacono, E. (2022). An update on applications of cattle mesenchymal stromal cells. *Animals*, 12(15), 1956. https://doi.org/10.3390/ani12151956

MFDS. (2024). Notification of partial revision of temporary standards and standards for food, etc. (No. 2024-13, February 21, 2024). *Ministry of Food and Drug Safety.* https://www.mfds.go.kr/brd/m_207/view.do?seq=14965&srchFr=&srchTo=&srchWord=&srchTp=&itm_seq_1=0&itm_seq_2=0&multi_itm_seq=0&company_cd=&company_nm=&page=1

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, Bourlieu, C., ... & Brodkorb, A. (2014). A standardised static *in vitro* digestion method suitable for food–an international consensus. *Food & function*, *5*(6), 1113-1124.

Mokhames, Z., Rezaie, Z., Ardeshirylajimi, A., Basiri, A., Taheri, M., & Omrani, M. D. (2020). Efficient smooth muscle cell differentiation of iPS cells on curcumin-incorporated chitosan/collagen/polyvinyl-alcohol nanofibers. *In Vitro Cellular & Developmental Biology-Animal*, 56, 313-321.

Mosa Meat. (n.d.). Growing Beef. *Mosa Meat*. https://mosameat.com/growing-beef

Mosa Meat. (2022). Cultivating Meat: The Mosa Approach. *Mosa Meat*. https://mosameat.com/blog/cultivating-meat-the-mosa-approach

Ng, S., & Kurisawa, M. (2021). Integrating biomaterials and food biopolymers for cultured meat production. *Acta Biomaterialia*, 124, 108-129. https://doi.org/10.1016/j.actbio.2021.01.017

Nicolas, J., Magli, S., Rabbachin, L., Sampaolesi, S., Nicotra, F., & Russo, L. (2020). 3D extracellular matrix mimics: fundamental concepts and role of materials chemistry to influence stem cell fate. *Biomacromolecules*, 21(6), 1968-1994. https://doi.org/10.1021/acs.biomac.0c00045

Nims, R. (2015). Cell Line Identification – The Applicability of Cytochrome C Oxidase 1 Barcoding in Cell Bank Authentication. *American Pharmaceutical Review.* https://www.americanpharmaceuticalreview.com/Featured-Articles/179229-Cell-Line-Identification-The-Applicability-of-Cytochrome-C-Oxidase-1-Barcoding-in-Cell-Bank-Authentication/

O'Neill, E. N., Cosenza, Z. A., Baar, K., & Block, D. E. (2021). Considerations for the development of cost-effective cell culture media for cultivated meat production. *Comprehensive Reviews in Food Science and Food Safety,* 20(1), 686-709. https://doi.org/10.1111/1541-4337.12678

Ong, K. J., Johnston, J., Datar, I., Sewalt, V., Holmes, D., & Shatkin, J. A. (2021). Food safety considerations and research priorities for the cultured meat and seafood industry. *Comprehensive reviews in food science and food safety*, 20(6), 5421-5448. https://doi.org/10.1071/RD23169

Ong, K. J., Tejeda-Saldana, Y., Duffy, B., Holmes, D., Kukk, K., & Shatkin, J. A. (2023). Cultured Meat Safety Research Priorities: Regulatory and Governmental Perspectives. https://www.mdpi.com/2304-8158/12/14/2645

Ovissipour, R., Yang, X., Saldana, Y. T., Kaplan, D. L., Nitin, N., Shirazi, A., ... & Rasco, B. A. (2023). Cell-Based Fish Production Case Study for Developing a Food Safety Plan. *SSRN*. https://dx.doi.org/10.2139/ssrn.4596174

Ozer, H. L. (2000). SV40-mediated immortalization. *Cell Immortalization*, 121-153. https://doi.org/10.1007/978-3-662-06227-2_6

Pasitka, L., Cohen, M., Ehrlich, A., Gildor, B., Reuveni, E., Ayyash, M., ... & Nahmias, Y. (2023). Spontaneous immortalization of chicken fibroblasts generates stable, high-yield cell lines for serum-free production of cultured meat. *Nature Food*, 4(1), 35-50. https://www.nature.com/articles/s43016-022-00658-w

Pauwels, K., Herman, P., Van sVaerenbergh, B., Dai Do Thi, C., Berghmans, L., Waeterloos, G., ... & Sneyers, M. (2007). Animal cell cultures: risk assessment and biosafety recommendations. *Applied Biosafety*, 12(1), 26-38. http://doi.org/10.1177/153567600701200105

Ravikumar, M., & Powell, D. (2023). Cell line development and utilisation trends in the cultivated meat industry. *The Good Food Institute*. https://gfi-apac.org/cell-line-development-and-utilisation-trends-in-the-cultivated-meat-industry/

Reiss, J., Robertson, S., & Suzuki, M. (2021). Cell sources for cultivated meat: applications and considerations throughout the production workflow. *International Journal of Molecular Sciences*, 22(14), 7513. https://doi.org/ 10.3390/ijms22147513

Rodrigues, A. L., Rodrigues, C. A., Gomes, A. R., Vieira, S. F., Badenes, S. M., Diogo, M. M., & Cabral, J. M. (2019). Dissolvable microcarriers allow scalable expansion and harvesting of human induced pluripotent stem cells under xeno-free conditions. *Biotechnology journal*, 14(4), 1800461. https://doi.org/10.1002/biot.201800461

Rosselló, R. A., Chen, C. C., Dai, R., Howard, J. T., Hochgeschwender, U., & Jarvis, E. D. (2013). Mammalian genes induce partially reprogrammed pluripotent stem cells in non-mammalian vertebrate and invertebrate species. *elife*, 2, e00036. https://doi.org/10.7554/eLife.00036

Rotondo, J. C., Mazzoni, E., Bononi, I., Tognon, M., & Martini, F. (2019). Association between simian virus 40 and human tumors. *Frontiers in oncology*, 9, 670. https://doi.org/10.3389/fonc.2019.00670

Rubio, N., Datar, I., Stachura, D., Kaplan, D., & Krueger, K. (2019). Cell-Based Fish: A Novel Approach to Seafood Production and an Opportunity for Cellular Agriculture. *Frontiers in Sustainable Food Systems*, 3, 43. https://doi.org/10.3389/fsufs.2019.00043

Sakudo, A. (2020). Inactivation methods for prions. Current Issues in Molecular Biology, 36(1), 23-32. https://doi.org/10.21775/cimb.036.023

Sant'Ana, A.S., Leitolis, A., Ambiel, C., Habowski, K., Bruna da Silva, A., Franzen Matte, B., … & Swiech Antonietto, K. (2023). Assuring the Safety of Cultivated Meat: HACCP plan development and application to a cultivated meat target-product. *The Good Food Institute Brazil*. E-Book: PDF, 72 p.; IL. https://doi.org/10.22491/application_cultivated_meat

Sato, Y., Bando, H., Di Piazza, M., Gowing, G., Herberts, C., Jackman, S., ... & Van der Laan, J. W. (2019). Tumorigenicity assessment of cell therapy products: The need for global consensus and points to consider. *Cytotherapy*, 21(11), 1095-1111. https://doi.org/10.1016/j.jcyt.2019.10.001

SFA. (2023). Requirements for the Safety Assessment of Novel Foods and Novel Food Ingredients. *Singapore Food Agency*. https://www.sfa.gov.sg/docs/default-source/food-information/requirements-for-the-safety-assessment-of-novel-foods-and-novel-food-ingredients.pdf

SFA. (2024). Microbiological standards for ready-to-eat food. *Singapore Food Agency*. https://www.sfa.gov.sg/docs/default-source/default-document-library/microbiological-standards-for-ready-to-eat-food.pdf

Shah, K. V. (2004). Simian virus 40 and human disease. *The Journal of infectious diseases*, 190(12), 2061-2064. https://doi.org/10.1086/425999

Sigma-Aldrich. (n.d.). Cell Freezing Medium-DMSO Serum free 1x. *Sigma-Aldrich*. https://www.sigmaaldrich.com/FR/fr/product/sigma/c6295

Sigma-Aldrich. (n.d.). Media Preparation. *Sigma Aldrich.* https://www.sigmaaldrich.com/FR/en/applications/cell-culture-and-cell-culture-analysis/cell-culture-by-technique/cell-culture-media-preparation

Simsa, R., Yuen, J., Stout, A., Rubio, N., Fogelstrand, P., & Kaplan, D. L. (2019). Extracellular heme proteins influence bovine myosatellite cell proliferation and the color of cell-based meat. *Foods*, 8(10), 521. https://doi.org/10.3390/foods8100521

Singh, A., Kumar, V., Singh, S. K., Gupta, J., Kumar, M., Sarma, D. K., & Verma, V. (2023). Recent advances in bioengineered scaffold for in vitro meat production. *Cell and Tissue Research*, 391(2), 235-247. https://doi.org/10.1007/s00441-022-03718-6

Smith-Uchotski, R., Wanjiru, P., Willis, R., & Adkin, A. (2022). Hazard identification: Identification of hazards in cultured animal cells. *Food Standards Agency.* https://www.food.gov.uk/sites/default/files/media/document/Cultured%20meat%20hazard%20identification%20final_0.pdf

Soice, E., & Johnston, J. (2021). Immortalizing cells for human consumption. *International Journal of Molecular Sciences*, 22(21), 11660. http://doi.org/10.3390/ijms222111660

Sorrells, M. S. (2023). PluriCells launches cell lines. Alt Meat. https://www.alt-meat.net/pluricells-launches-cell-lines?oly_enc_id=0795J6306167I4E

Southey, F. (2023). Bioreactors for meat: Ever After Foods 'overcomes scaling challenge' for cultivated meat players. *Food Navigator.* https://www.foodnavigator.com/Article/2023/03/01/Bioreactors-for-meat-Ever-After-Foods-overcomes-scaling-challenge-for-cultivated-meat-players#:~:text=More%20than%20700%25%20boost%20in%20productivity%E2%80%8B&text=Indeed%2C%20Ever%20After%20Foods%20says,%

Stout, A. J., Mirliani, A. B., Soule-Albridge, E. L., Cohen, J. M., & Kaplan, D. L. (2020). Engineering carotenoid production in mammalian cells for nutritionally enhanced cell-cultured foods. *Metabolic engineering*, 62, 126-137. https://doi.org/10.1016/j.ymben.2020.07.011

Stout, A. J., Rittenberg, M. L., Shub, M., Saad, M. K., Mirliani, A. B., Dolgin, J., & Kaplan, D. L. (2023). A Beefy-R culture medium: replacing albumin with rapeseed protein isolates. *Biomaterials*, 296, 122092. https://doi.org/10.1101/2022.09.02.506409

Stricker, P. E. F., Irioda, A. C., Mogharbel, B. F., Abdelwaid, E., Cavalli, L. R., Zotarelli-Filho, I. J., & Carvalho, K. A. T. (2021). Mesenchymal stem cells immortalization's and state-of-art: a systematic review and meta-analysis. *European Journal of Public Health*, 31(Supplement_2), ckab120-058. https://doi.org/10.1093/eurpub/ckab120.058

Tallawi, M., Rosellini, E., Barbani, N., Cascone, M. G., Rai, R., Saint-Pierre, G., & Boccaccini, A. R. (2015). Strategies for the chemical and biological functionalization of scaffolds for cardiac tissue engineering: a review. *Journal of the Royal Society Interface*, 12(108), 20150254. https://doi.org/10.1098/rsif.2015.0254

Tanaka, R. I., Sakaguchi, K., Yoshida, A., Takahashi, H., Haraguchi, Y., & Shimizu, T. (2022). Production of scaffold-free cell-based meat using cell sheet technology. *npj Science of Food*, 6(1), 41. https://doi.org/10.1038/s41538-022-00155-1

Taylor, S. L., Stratton, J. E., & Nordlee, J. A. (1989). Histamine poisoning (scombroid fish poisoning): an allergy-like intoxication. *Journal of Toxicology: Clinical Toxicology*, 27(4-5), 225-240. https://doi.org/10.3109/15563658908994420

ThermoFisher. (n.d.) Dulbecco's Modified Eagle Medium (DMEM). *ThermoFisher.* https://www.thermofisher.com/fr/fr/home/life-science/cell-culture/mammalian-cell-culture/cell-culture-media/dmem.html

ThermoFisher. (n.d.). Milieu RPMI 1640. *ThermoFisher.* https://www.thermofisher.com/order/catalog/product/11875093

UPSIDE Foods. (2021). Premarket Notice For Integral Tissue Cultured Poultry Meat. *U.S. Food and Drug Administration*. https://www.fda.gov/media/163262/download

Vilette, D., Andréoletti, O., Archer, F., Madelaine, M. F., Vilotte, J. L., Lehmann, S., & Laude, H. (2001). Ex vivo propagation of infectious sheep scrapie agent in heterologous epithelial cells expressing ovine prion protein. *Proceedings of the National Academy of Sciences*, 98(7), 4055-4059. https://doi.org/10.1073/pnas.06133799

Vorberg, I., Raines, A., Story, B., & Priola, S. A. (2004). Susceptibility of common fibroblast cell lines to transmissible spongiform encephalopathy agents. *The Journal of infectious diseases*, 189(3), 431-439. https://doi.org/10.1086/381166

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1847-1857. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1609232/

Watson, E. (2023). Meet the founder – IntegriCulture's Yuki Hanyu: 'We're a cellular ag infrastructure company, not just a cultivated meat company'. *AgFunder News.* https://agfundernews.com/meet-the-founder-integricultures-yuki-hanyu-were-a-cellular-ag-infrastructure-company-not-just-a-cultivated-meat-company

Welch, D., & Swartz, E. (2020). Food safety considerations for cultivated meat. *The Good Food Institute*. https://gfi.org/images/uploads/2020/06/2020.06.10-Food-Safety-Considerations-in-Cultivated-Meat-.pdf

Wollschlaeger, J. O., Maatz, R., Albrecht, F. B., Klatt, A., Heine, S., Blaeser, A., & Kluger, P. J. (2022). Scaffolds for cultured meat on the basis of polysaccharide hydrogels enriched with plant-based proteins. *Gels*, 8(2), 94. https://doi.org/10.3390/gels8020094

Ye, Y., Zhou, J., Guan, X., & Sun, X. (2022). Commercialization of cultured meat products: Current status, challenges, and strategic prospects. *Future Foods*, 6, 100-177. https://doi.org/10.1016/j.fufo.2022.100177

Yin, H., Price, F., & Rudnicki, M. A. (2013). Satellite cells and the muscle stem cell niche. Physiological reviews, 93(1), 23-67. https://doi.org/10.1152/physrev.00043.2011

Zhao, Z., Xu, M., Wu, M., Tian, X., Zhang, C., & Fu, X. (2015). Transdifferentiation of fibroblasts by defined factors. *Cellular Reprogramming*, 17(3), 151-159. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4487681/

Zehorai, E., Maor-Shoshani, A., Molotski, N., Dorojkin, A., Marelly, N., Dvash, T., & Lavon, N. (2023). From fertilised oocyte to cultivated meat–harnessing bovine embryonic stem cells in the cultivated meat industry. Reproduction, *Fertility and Development.* https://doi.org/10.1071/RD23169