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{Review Article}

QA Challenges in Microbiome-Based and Living Biotherapeutic Products (LBPs)

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Abstract

Living microorganism's microbiome-based therapeutics and living biotherapeutic product biologic drugs containing live microorganisms as active ingredient are a novel category of medicines with increasing clinical potential. However, interest has been enhanced recently by regulatory milestones and first approvals, LBPs present distinct quality-assurance (QA) issues which are dissimilar to small molecules, biologics and traditional probiotics. These difficulties are caused by the inherent biological variations of living things, the necessity to determine potency of living cells, potential contamination and transmissible genes, elaborate supply-chain, and cold-chain demands, and sensitive analytical requirements of identity, viability, stability, and functional activity. In this review, the existing QA problems in development, manufacturing, analytical control, release testing, and regulatory routes of LBPs are synthesized. We also examine regulatory advice and emerging demands of the key authorities and discuss viable QA systems and risk-based practices to help with a stable translation between bench and clinic. I have to fully comply with the requirements of delivering safe, effective, and reproducible LBPs through the use of integrated QA approaches that incorporate high-quality strain characterization, analytical viability and functionality, tight contamination control, and well-defined regulatory orientation. The development of these structures today will be faster in providing patients with access and maintaining safety and quality.

Keywords: Living biotherapeutic products (LBPs), Microbiome therapeutics, Quality assurance (QA), Potency assays / viability, CMC for biologics, Analytical methods (metagenomics, qPCR), Regulatory guidance.

1. Introduction

1.1 Historical Context and Emergence of Microbiome-Based Therapeutics

As a necessary factor of health and disease, the human microbiome the assembly of microorganisms such as bacteria, archaea, fungi, and viruses living on the surface and inside the human body has become much more appreciated. In the last 20 years, it was found out that the microbiome is involved in an intricate relationship with the host physiology with the help of next-generation sequencing, metagenomics, metabolomics, and systems biology. The imbalance of microbial communities has been attributed to a

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broad spectrum of disorders such as gastrointestinal diseases, metabolic syndromes, autoimmune diseases, and neurological disease (1). The idea of using the microbiome to treat a disease or to heal oneself has developed out of the utilization of the traditional probiotics to a novel category of medicinal product, the Living Biotherapeutic Products (LBPs), which are both designed, characterized, and produced with the rigor of pharmaceuticals in mind. In 2012, the U.S. Food and Drug Administration (FDA) officially defined LBPs as biological products including live organisms, like bacteria, which can be used in the prevention, treatment, or cure of a disease or condition in human beings, but not vaccines (2). As microbiome science has gone viral, LBPs have now transitioned to well-defined, strain-specific, and genetically characterized drug candidates with established therapeutic activity. Recurrent Clostridioides difficile infection approvals of donor-derived microbiota therapies like REBYOTAtm (Ferring Pharmaceuticals, 2022) and VOWSTtm (Seres Therapeutics, 2023) were the first landmarks in this area (3). These two first-generation microbiome-based therapies show that it is possible to achieve live microbial intervention approvals within controlled quality frameworks like biologics, albeit with the added complexity of the fact that the product is a living

1.2 Definitions and Scope: Microbiome-Based Products and LBPs

LBPs differ with conventional probiotics which are normally regulated as dietary supplements or foods. The FDA and the European Medicines Agency (EMA) have defined LBPs as biological medicinal products containing an active ingredient that is a live microorganism or a specified microbial consortium to be used to treat or prevent diseases (4). The definition does not cover fecal microbiota transplantation (FMT) preparations, which, although applied clinically, are compositionally undefined and have distinct regulatory issues. LBPs on the other hand are highly characterized, well-defined strains, normally produced under Good Manufacturing Practice (GMP) conditions. They are usually genetically stable and have no virulence or antibiotic-resistance determinants (5)
Products that use microbiomes may be divided into:
Living Biotherapeutic Products (LBPs): are those that have live microorganisms as the active ingredient.
Microbiome-derived bioactives: Microbial metabolites or components (postbiotics).
Microbiome-modulating agents: Incorporate prebiotics and bacteriophage therapies which have an indirect effect on the microbiome.
Of these, LBPs are the most complex to manufacture and to have QA due to their biological activity and stability relying on their viability, genomic integrity and functional ability to survive during production, storing and administration.

1.3 The Role of Quality Assurance (QA) in LBP Development

Quality Assurance (QA) is the foundation of pharmaceutical manufacturing since it guarantees that every product is manufactured according to specifications in the appearance of the product, its purity, strength, safety, and stability. Nevertheless, LBPs confront the conventional paradigms of QA in distinct ways. In contrast to small molecules or protein-based biologics, LBPs are made up of living organisms that can grow and genetically vary, which brings about dynamic behaviour to inert substances (6). They cannot be evaluated based on chemical composition, but instead, QA must require evaluations of the parameters of cell viability, cell functionality, genomic stability and phenotypic reproducibility between production lots (7). In addition, living organisms can also have a complicated interaction with the host microbiota and immune system. Therefore, to guarantee quality clinical performance, the manufacturing inputs, environmental factors, and process validation must be strictly regulated. As stressed by Cordaillat-Simmons et al., LBPs require a holistic quality-by-design (QbD), product

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

development, analytical validation, and process control during the lifecycle (8). QA frameworks should thus be able to transform the traditional biologics to living system control paradigms, with their primary aim being to ensure reproducibility, traceability, and the prevention of contamination.

1.4 Clinical and Industrial Significance

LBPs have shown treatment potential in various disease localities, such as:

Infectious diseases: e.g., C. difficile recurrence (REBYOTA, VOWST). Inflammatory and metabolic: e.g. inflammatory bowel disease (IBD) and diabetes type 2 (9).

Immunotherapy of cancer: in which commensal bacteria can augment the efficacy of checkpoint inhibitors (10).

Neurological diseases: the gut–brain axis of depression and autism spectrum disorder (11) should be investigated.

Industrially, the global market on LBP is seen to expand exponentially due to the rising clinical trials in the field and technological advances in the field of fermentation, lyophilization and encapsulation (12). However, the lack of homogenized manufacturing and analytical models is one of the main bottlenecks, which causes the inconsistency of the product quality and reproducibility (13).

1.5 Regulatory Landscape and QA Implications

Regulatory bodies have already started to provide early guidance, yet international harmonization is not quite a reality. Guidance on early clinical trials with LBPs by the FDA (2016), Guidance on LBPs, provided by the EMA, contain the expectation of baseline standards of strain identification, strength, purity, and stability (14). A draft monograph on minimal quality characteristics including viable count, no pathogens, and genome stability has also been implemented in Europe Pharmacopoeia (15). In spite of these measures, there still exist significant QA uncertainty about: Critical Quality Attributes (CQAs): What is potency in the case of a living organism?

Valid analytical: How to normalize culture-based against molecular assays?

Comparability: How to maintain consistency in case of change of processes? According to the WHO Expert Committee on Biological Standardization, LBPs are new biological medicines that do not fit the current paradigm of quality and regulation (16)

1.6 Major QA Challenges across the Product Lifecycle

The QA complexity of LBPs can be categorized into several interdependent domains:

(a) Strain Identity and Characterization

The initial action that is taken in assuring the quality of the product is the accurate identification of the microbial strain(s) used. In order to ascertain taxonomy and to rule out virulence or antimicrobial resistance determinants, whole-genome sequencing (WGS), ribotyping, and phenotypic profiling are required (6). Serial passage can cause genetic drift, and this will change the strain functionality, which will require cell banking systems that have a master and working cell bank that exists under regulated conditions (10).

(b) Potency and Viability Assays

Concentration is not sufficient to measure LBP potency. Functional viability of cells characterized by the capacity to produce a biological response needs to be determined by CFU counts, metabolic assays, and, most recently, mechanisms-of-action-associated bioassays (4). The design of the assays will also have to take strain-specific characteristics like oxygen sensitivity or storage dormancy.

(c) Contamination and Purity Control

Live cultures are by nature prone to contaminations of environmental microbes or bacteriophages. It is essential to establish sterile and viable fermentation environments

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

with environmental monitoring, closed system fermentation and proven aseptic practices (5).

(d) Manufacturing Process Consistency

GMP production-LAB scale-up, may cause variability of nutrient composition, oxygen tension and pH, all of which impact microbial physiology and activity (17). Therefore, QA models should have a direct connection between process parameters and CQAs using design-of-experiments (DoE) and Quality-by-Design (QbD) method.

(e) Stability, Storage, and Cold Chain

The preservation of LBPs requires either cold storage or lyophilized formats to keep them viable. The stability of products depends on three main factors which include temperature changes and exposure to moisture and the presence of formulation additives. The implementation of QA strategies requires validated storage conditions and accelerated stability studies and real-time monitoring according to (14).

(f) Analytical Standardization

Currently, no harmonized international standards exist for LBP testing. Variability in culture media, enumeration techniques, and genomic methods hinders cross-lab comparability (2). Standardized analytical reference methods are urgently needed.

(g) Regulatory Alignment

Differences in regional expectations (FDA, EMA, PMDA, WHO) create uncertainty for multinational development. Harmonized guidance similar to ICH Q8–Q12 frameworks for biologics is required to streamline global QA compliance (12).

1.7 QA as a Bridge between Innovation and Patient Safety

LBPs provide an excellent example of an interdisciplinary approach that combines biotechnology, microbiology, and regulatory science. The QA framework acts as the interface which ensures that the innovation represented in these products is delivered to patients safely and reproducibly. Poorly-defined QA metrics can potentially lead to inconsistent efficacy, or even adverse reactions from uncontrolled microbial interactions or contamination (20). On the other hand, valid QA fundamentals based in genomics, validated analytics and real-time control can simplify regulatory approval and accelerate clinical adoption. Consequently, concepts such as Quality-by-Design (QbD) and Risk-Based QA are being embraced in LBP research and development pathways to ensure that processes, from strain selection through to delivery to patients are scientifically validated and governed by a process controlled by risk assessment (21)

2. Analytical Control of Living Biotherapeutic Products (LBPs)

2.1 Importance of Analytical Characterization

Analytical control is the foundation of quality assurance (QA) in LBP development, since LBPs consist of living microorganisms and analytical methods need to address both biological identity and functional effect, the determinants of therapeutic consistency and safety. In contrast to small molecules and biologics, LBPs have inherent biological variability, whereby fit-for-purpose assays will need to analyze living cell characteristics, product contamination, and genetic and functional consistency throughout the product life cycle (15).

The **FDA's CMC guidance (2016)** and **EMA's quality guidelines (2023)** emphasize that analytical characterization of LBPs must encompass identity, purity, potency, stability, and viability, while also ensuring absence of pathogens and antibiotic-resistance determinants (27)

2.2 Strain Identification and Genetic Characterization

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

Accurate and consistent **strain identification** is the first analytical requirement for LBP QA. Misidentification or genomic drift during sub-culturing can alter product efficacy or introduce unforeseen risks (19)

2.2.1 Molecular and Genomic Methods

Currently phenotypic identification of species is by classic methods of biochemical identification or more preferably, by using MALDI-TOF, but this is being enhanced by molecular assays such as,

1. 16S rRNA gene sequencing to give species resolution.
2. WholeGenome Sequencing (WGS) to give a strain confirmation, assessment of genome integrity and isolation of plasmids and virulence genes.
3. Digital PCR (dPCR) and quantitative PCR (qPCR) for a rapid strain specific identification and assessment of the presence of contaminants.

A genomic examination against reference data bases including NCBI and EFSA Qualified Presumption of Safety (QPS) is imperative to show that virulence remedial or antimicrobial resistance (22) is lacking.

2.2.2 Genetic Stability Testing

Long-term genetic stability studies of master and working cell banks are important for cell banks because microbes can undergo mutations and acquire mobile genetic elements. In both the FDA and EMA guidances it is recommended that WGS comparisons performed at appropriate intervals permit the monitoring of clinically significant mutations. (27)

2.3 Purity and Contamination Control

Controlling contamination in LBPs is much more complicated than with traditional biologics, since the manufacturing environment itself incorporates living organisms. The potential for cross-contamination by microbes, adventitious agents and contamination by bacteriophage can threaten product safety and potency (22).

2.3.1 Microbiological Purity Assays

Traditional sterility tests (USP <71>) cannot be done directly on LBPs as the active is a micro-organism itself. Instead the purity is assured by:

Selective agar plating to confirm absence of adventitious micro-organisms.

Metagenomic sequencing or 16S profiling to confirm absence of unexpected taxa.

Phage detection assays using electron microscopy or qPCR.

2.3.2 Environmental and Raw-Material Monitoring

Quality systems must include regular monitoring of air, surfaces, and personnel in GMP zones, coupled with raw-material screening (media, excipients) to prevent introduction of non-target species (23)

2.4 Viability and Enumeration of Live Cells

A key analytical feature of LBPs is **viability** — the ability of cells to remain alive and metabolically active under specified storage and physiological conditions. Viability influences both potency and stability, and is thus a *critical quality attribute (CQA)* (28).

2.4.1 Culture-Based Enumeration

Plate count assays, which determine colony-forming units (CFU), are the gold standard for counting viable cells. However, they require a lot of work, are specific to certain strains, and often overlook viable but non-culturable (VBNC) populations (24).

To overcome these issues, flow cytometry with fluorescent viability stains, like propidium iodide and Syto 9, along with ATP-based bioluminescence assays, are being used for quick counting (10).

2.4.2 Physiological Viability

Viability isn't just having intact cells—it's having the metabolic capacity needed for therapeutic effect. Advanced tests such as respirometry, metabolite profiling (for SCFAs

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

and bile acid conversion), and membrane integrity assays are increasingly used to link viability with function (26).

2.5 Potency and Functional Assays

Potency assays measure an LBP's biological activity in relation to its intended mechanism of action (MoA). Since LBPs often work through complex host–microbe interactions—such as metabolite production or immune modulation—designing a reproducible potency assay is challenging (12)

2.5.1 Mechanism-Linked Functional Assays

Examples include:

Metabolite production assays (for example, SCFA, tryptophan, or GABA synthesis). • Host-cell co-culture systems to measure cytokine modulation or strengthening of the epithelial barrier.

Reporter-gene assays that detect signaling induced by microbial metabolites (13). These must be qualified for precision, linearity, and biological relevance. When multiple strains are combined into a consortium, potency must reflect inter-strain interactions that influence the overall effect (25).

2.5.2 Correlation between Potency and Clinical Outcome

Potency assays should show a consistent link between in vitro biological activity and in vivo clinical efficacy, a requirement recent FDA reviewer has emphasized in LBP submissions (24).

2.6 Stability Testing

Keeping live biotherapeutic products viable and potent throughout their shelf life and distribution is a major quality-assurance challenge. Environmental factors like temperature, oxygen, and humidity can greatly reduce LBP stability (26).

2.6.1 Accelerated and Real-Time Stability Studies

Accelerated testing at higher temperatures (25–40 °C) helps predict degradation kinetics. Real-time stability studies under recommended storage (2–8 °C or −20 °C) confirm expiry dating.

Parameters monitored include CFU count, moisture, pH, residual solvents, and functional potency (30)

2.6.2 Formulation and Packaging Impacts

Common stabilization strategies include lyophilization, using cryoprotectants like trehalose or mannitol, oxygen-barrier blister packs, and desiccants (22). Stability protocols need to be validated for each strain or consortium composition to ensure consistent performance.

2.7 Comparability and Lot-to-Lot Consistency

LBPs are often made in small batches during development and later scaled up for commercialization. Each change in the process brings a risk of comparability drift, where subtle shifts in fermentation, media, or formulation can affect product attributes (29).

To keep things consistent, QA frameworks should use a comparability protocol similar to that used for biologics:

Define critical quality attributes (CQAs) for identity, potency, and purity. Perform analytical and functional equivalence testing between pre- and post-change lots. Include omics-based profiling (metabolomics, transcriptomics) to pick up unintended differences (31). This data-driven approach to comparability can support regulatory acceptance of manufacturing changes without requiring new clinical studies**.**

2.8 Analytical Method Validation

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

Method validation ensures that a method is reliable, reproducible, and fit for purpose. For LBPs, validation has to take into account biological heterogeneity and matrix effects in live formulations. Key validation parameters include:
Specificity: the ability to distinguish the target strain(s) from contaminants.
Precision and accuracy: consistent CFU or potency results across different analysts and days. Linearity and range: validated quantification across the expected viability span.
Robustness: tolerance to minor experimental variations (32).
Regulators encourage using orthogonal analytical approaches — combining culture-based, molecular, and functional assays — to cross-validate LBP attributes (32)

2.9 Emerging Analytical Technologies

Rapid innovation is transforming LBP analytics toward higher resolution and throughput.

2.9.1 Flow Cytometry and Single-Cell Analysis

High-content flow cytometry enables simultaneous measurement of viability, metabolic activity, and population heterogeneity within mixed consortia (33).

2.9.2 Metagenomic and Metabolomic Fingerprinting

Shotgun metagenomics and untargeted metabolomics give detailed fingerprints for identity and batch comparability, allowing detection of low-abundance contaminants and functional drift (33)

2.9.3 Microfluidic and Biosensor Platforms

Miniaturized microfluidic devices with integrated impedance or optical sensors provide fast, label-free viability assessments and are being adapted for in-process control (34)

2.9.4 Artificial Intelligence (AI) and Digital QA

Machine-learning algorithms can analyze multi-omics datasets to predict viability declines, optimize fermentation parameters, and flag out-of-spec batches — pointing to the future of predictive QA (35).

2.10 Challenges and Gaps in Analytical Standardization

Despite progress, several analytical gaps remain:

1. Lack of harmonized standards: There are no globally accepted reference methods for viability or potency testing.
2. Multi-strain consortia complexity: Interactions between strains make enumeration and potency assessment difficult.
3. Assay variability: Culture-dependent and culture-independent methods can produce different results.
4. Limited reference materials: The absence of certified microbial standards hinders method calibration (35)

Regulatory agencies, pharmacopeias, and industry consortia are working together to create standard protocols and reference reagents for LBPs. The USP Microbiome Expert Panel and the Ph. Eur. Working Party on LBPs are among the groups leading these efforts (34)

2.11 Summary of Analytical QA Framework

To ensure reliable product performance, a comprehensive analytical QA framework for LBPs should integrate:

Analytical Dimension	Representative Assays	Key QA Objective
Identity	16S rRNA sequencing, WGS	Confirm strain authenticity
Purity	Metagenomics, selective plating	Detect contamination
Viability	CFU, flow cytometry	Quantify live cells
Potency	Mechanism-linked bioassay	Confirm functional activity

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

Stability	Real-time/accelerated studies	Ensure shelf-life viability
Comparability	Omics profiling	Demonstrate lot consistency
Method validation	ICH Q2(R2) parameters	Ensure assay reliability

This integrated analytical matrix supports *Quality by Design (QbD)*, enabling robust definition of critical quality attributes (CQAs) and their correlation with clinical outcomes.

3. Manufacturing and Process QA Challenges in LBPs

3.1 Introduction

Manufacturing microbiome-based and living biotherapeutic products (LBPs) is one of the most technically challenging and quality-sensitive areas in biopharmaceutical production. Unlike conventional biologics made from purified recombinant molecules, LBPs consist of living microorganisms that need to stay viable, genetically stable, and functionally active throughout production and distribution (36).

Because of this complexity, every step — from strain banking and fermentation to downstream processing, formulation, and packaging — must be tightly controlled within a risk-based Quality Assurance (QA) and Good Manufacturing Practice (GMP) framework. Small changes in process parameters can notably change cell physiology, viability, or metabolic output, which in turn can impact therapeutic efficacy and safety (37).

3.2 Cell Banking and Master Seed Management

3.2.1 Master and Working Cell Banks (MCB/WCB)

Establishing well-characterized master and working cell banks is essential for consistent manufacturing. Each LBP strain should be isolated, genetically characterized using whole-genome sequencing (WGS), and stored under validated cryopreservation conditions to prevent genetic drift (35)

3.2.2 Genetic and Phenotypic Stability

Regular checks of genetic stability across production generations ensure the product's identity and potency. WGS comparisons, plasmid profiling, and phenotypic assays (growth rate, metabolite profile) are used to detect mutations or loss of function (34). The EMA recommends requalifying the MCB after significant manufacturing changes or extended storage (36).

3.3 Upstream Processing: Fermentation Control

3.3.1 Culture Media and Growth Conditions

LBP fermentation uses defined or semi-defined media optimized for microbial growth and metabolite production. QA must ensure raw materials — carbohydrates, amino acids, peptones — are pharmaceutical grade and free from animal-derived contaminants or antibiotic residues (38)

3.3.2 Bioreactor Monitoring and Control

Critical parameters include:

pH, temperature, and dissolved oxygen (DO) — these directly influence metabolic activity. • Agitation rate and aeration — they affect oxygen transfer and cell morphology. Feed rate — it controls whether cells experience nutrient limitation or overflow metabolism.

Automated sensor systems and process analytical technologies (PAT) offer real-time monitoring, enabling tighter control and improved batch reproducibility (39)

3.3.3 Avoiding Contamination in Mixed-Strain Systems

Multi-strain LBPs add extra complexity. To avoid fast-growing strains dominating or unwanted cross-colonization, you need well-validated inoculum ratios, sterilized media systems, and closed bioreactor setups (8)

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

3.4 Downstream Processing and Harvesting

3.4.1 Harvesting and Cell Concentration

After fermentation, cells are collected by centrifugation, filtration, or tangential flow. QA focuses on minimizing shear stress and preserving anaerobic conditions for oxygen-sensitive strains (15).

3.4.2 Washing and Buffer Exchange

Residual media components can affect formulation stability or even trigger immune reactions. Repeated washes with isotonic buffers remove these impurities, and QA must verify that the wash steps don't change cell viability or morphology (17)

3.4.3 Formulation Integration

The harvested biomass is mixed with cryo- or lyoprotectants like trehalose, sucrose, or skim milk to preserve viability during downstream processing and storage (25). Formulation composition must be validated for each strain to ensure compatibility and the absence of inhibitory excipients

3.5 Lyophilization and Drying

Freeze-drying, or lyophilization, is the most common way to stabilize LBPs. Still, the drying process subjects cells to severe stress from ice crystal formation and osmotic shock (40).

3.5.1 Critical Process Parameters (CPPs):

Freezing rate — affects ice crystal size and how much intracellular water is removed. • Primary drying temperature and pressure — set the residual moisture and the product's structure. • Secondary drying — removes bound water sufficiently without overheating. QA should define acceptable ranges for each CPP through Quality by Design (QbD) studies that link them to product critical quality attributes (CQAs), including viability and potency (41)

3.5.2 Process Validation

Lyophilization cycles should be validated to ensure uniform heat transfer, consistent product homogeneity, and reproducible residual moisture. After lyophilization, viability and potency assays are used to confirm the cycle's adequacy (42)

3.6 Formulation QA and Stability

3.6.1 Role of Excipients and Carriers

Excipients like polysaccharides, milk proteins, and polymer matrices affect microbial survival, gastrointestinal delivery, and release kinetics (43). QA checks cover compatibility, endotoxin levels, and confirmation that no animal-derived materials are present unless a specific justification is provided.

3.6.2 Packaging Integrity

Packaging for LBPs must protect against moisture and oxygen—commonly done with aluminium blisters, vials containing desiccants, or multilayer sachets. QA should confirm container-closure integrity under both accelerated and real-time conditions (44).

3.6.3 Cold-Chain Logistics

Most LBPs need to be kept refrigerated or frozen, and temperature excursions can permanently damage viability. So GDP-compliant systems that use temperature data loggers and validated shipping containers are essential (17,44)

3.7 Contamination and Cross-Containment Control

3.7.1 Manufacturing Environment Design

Unlike sterile biologics, LBP production often happens in biosafety level 1 (BSL-1) settings, but it's still essential to keep different strains or products strictly separated (45). Facility design should include:

Dedicated suites for upstream and downstream processes

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

HEPA-filtered airflow and positive pressure zones
Decontamination systems such as UV or vaporized hydrogen peroxide

3.7.2 Environmental Monitoring

Routine air and surface sampling detects microbial or phage contamination. Deviations should trigger investigations and potential product quarantine (19)

3.7.3 Closed-System Manufacturing

Closed fermenters and aseptic connectors lower contamination risk and are preferred in LBP GMP facilities. Single-use systems further cut down on cleaning-validation requirements and the chance of cross-contamination. (44)

3.8 Quality by Design (QbD) and Process Analytical Technology (PAT)

The QbD approach described in ICH Q8(R2) offers a scientific framework for understanding and controlling processes. For LBPs, QbD connects critical process parameters (CPPs) with critical quality attributes (CQAs) like viability, purity, and potency (46).

3.8.1 Design of Experiments (DoE)

Experimental modeling can pinpoint the best fermentation and lyophilization conditions. Variables such as pH, agitation, and cryoprotectant concentration can be analyzed statistically to show how they affect viability outcomes (47).

3.8.2 In-line Monitoring via PAT Tools

PAT technologies — like near-infrared (NIR) spectroscopy, flow cytometry, and dissolved-oxygen sensors — allow real-time quality monitoring and immediate process adjustments (48)

3.8.3 Digital Twin Integration

Emerging “digital twin” systems use AI and machine learning to simulate LBP process dynamics, predict deviations, and optimize batch performance (49)

3.9 GMP and Regulatory QA Frameworks

3.9.1 Regulatory Classification

LBPs are regulated as biological medicinal products by the EMA and as live biotherapeutic products by the U.S. FDA’s Center for Biologics Evaluation and Research (CBER). Both frameworks require GMP-compliant production, including validated aseptic processes and full batch traceability (50).

3.9.2 Documentation and Traceability

Batch manufacturing records (BMR), deviation reports, and Certificate of Analysis (CoA) form the QA backbone. Each batch must be traceable to its originating MCB lot (45)

3.9.3 QA Oversight and Release Criteria

Final release should be based on identity, purity, potency, viability, and stability test results that meet predefined specifications. A QA review ensures compliance before the lot is released for clinical or commercial use (51)

3.10 Scale-Up Challenges

Scaling up from the lab to industrial scale brings risks to product consistency. Changes in shear stress, nutrient gradients, and limits on oxygen transfer are all factors (29).

3.10.1 Process Equivalence

Small-scale and commercial-scale processes must show comparable product attributes using the same analytical and potency tests. A QbD-based risk assessment helps identify parameters that are sensitive to scale (52)

3.10.2 Technology Transfer QA

When transferring between manufacturing sites, documentation, raw-material specifications, and process controls must stay harmonized. QA has a key role in confirming equivalence and preserving data integrity (53).

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

3.11 Continuous Improvement and CAPA Systems

Continuous process verification (CPV) and Corrective and Preventive Action (CAPA) systems ensure ongoing QA performance. Key elements include:
Statistical trending of batch data.
Investigation of out-of-specification (OOS) results.
Periodic QA review for process optimization (54)

3.12 Summary of Manufacturing QA Best Practices

Manufacturing Stage	QA Focus	Critical Control
Cell banking	Identity, stability	Genetic sequencing, storage validation
Fermentation	Consistency	Automated sensors, contamination prevention
Harvesting	Viability retention	Gentle separation, anaerobic handling
Lyophilization	Stability	CPP monitoring, moisture control
Formulation	Compatibility	Excipient evaluation, packaging integrity
Distribution	Viability	Cold-chain validation, real-time monitoring

4. Analytical QA and Characterization Challenges in Microbiome-Based and Living Biotherapeutic Products- Analytical characterization is central to quality assurance for living biotherapeutic products (LBPs). Since these products are made of living microbes that naturally vary, traditional analytical approaches for small molecules or biologics don't cut it. Instead, we need tailored strategies to confirm strain identity and purity, and to measure potency, viability, and functional consistency. This section examines the analytical QA landscape for LBPs and the challenges that come with it.

4.1 Strain Identity and Genomic Characterization

The identity of microbial strains that make up the active pharmaceutical ingredient (API) in LBPs must be confirmed rigorously to ensure consistent therapeutic performance and to prevent contamination with unwanted species. Strain-level identification typically combines genomic sequencing, phenotypic profiling, and biochemical assays (55).Advances in whole-genome sequencing (WGS) and average nucleotide identity (ANI) analysis now allow high-resolution discrimination between closely related strains (56). Still, microbial genomes can acquire spontaneous mutations or gain genes through horizontal transfer during serial passaging or large-scale fermentation, so periodic genomic monitoring should be part of the QA program (57).Plasmid content, virulence factor profiling, and antimicrobial resistance (AMR) gene screening are also recommended by both FDA and EMA guidelines for LBP characterization (58). The European Pharmacopoeia Monograph 3053 (2023) requires strain traceability via cell banks and mandates establishing a Master Cell Bank (MCB) and Working Cell Bank (WCB) that are genomically equivalent to the clinical strain (59).

4.2 Purity and Contamination Control

Ensuring the purity of an LBP is inherently complex because production uses live microorganisms that can be unintentionally co-cultured or contaminated by adventitious agents like bacteriophages, fungi, or environmental bacteria (60). Standard sterility and microbial limits tests for pharmaceuticals won’t do here, since LBPs are deliberately non-sterile. Regulators therefore focus on contaminant-exclusion tests such as 16S rRNA sequencing, qPCR detection of non-target species, and phage-monitoring assays (61). Cross-contamination between strains in multi-strain consortia deserves particular

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

attention, since it can shift strain ratios or reduce functionality (62). To prevent this, manufacturers often use closed-system fermentation, HEPA-filtered anaerobic isolators, and dedicated fermenters for different strains (65). Monitoring for contaminants during fermentation, lyophilization, and encapsulation is now part of real-time QA, supported by rapid microbial methods like flow cytometry, impedance analysis, and ATP bioluminescence (64).

4.3 Potency and Functional Assays

Unlike chemical drugs, LBP potency can't be measured by the concentration or activity of a single defined molecule. Instead, it's defined by biological function- for example, immunomodulation, metabolic conversion, or changes to the microbiome (63).Common surrogate potency assays include:

CFU counts and viability testing to enumerate live cells.

Metabolite production assays, such as measuring short-chain fatty acids by GC-MS.

Host–microbe interaction models, like epithelial co-cultures or organoid assays that assess induction of anti-inflammatory cytokines or improvement of epithelial barrier function (65).

A major QA gap remains: linking these in vitro potency assays to in vivo therapeutic efficacy (64). To address this, many developers are moving toward mechanism-of-action (MoA)–linked bioassays that better reflect the functional pathways tied to clinical outcomes (66). At the same time, integrating multi-omics data- transcriptomics, metabolomics, and proteomics — is becoming a modern QA approach for functional characterization, offering deeper insight into microbial activity states (67).

4.4 Viability and Enumeration Challenges

Viability is a key attribute for LBPs because the therapeutic effect often depends on how many live organisms are delivered. Plate counting (CFU enumeration) has been the traditional gold standard, but it can be inconsistent-microbial clumping, stress-induced dormancy, or cells that are non-culturable yet still metabolically active all cause variability (68).

Other techniques include:

Flow cytometry with viability dyes (for example, SYTO9/PI) for rapid counts (69).

qPCR combined with propidium monoazide (PMA) to separate live and dead DNA signals (70).

Microcalorimetry and impedance assays to estimate metabolic activity (71). Even with these advances, setting standardized viability thresholds and linking them reliably to potency remains a major analytical QA challenge (72).

4.5 Stability and Shelf-Life Determination

The viability and potency of LBPs can decline over time when exposed to environmental stressors like temperature, moisture, and oxygen, or through interactions with excipients. For that reason, QA stability studies need to track both CFU counts and functional activity under real-time and accelerated storage conditions (73,35). Lyophilization is often used to improve stability, but it can cause uneven viability loss across different strains. Cryoprotectants such as trehalose and skim milk, and encapsulation matrices like alginate or lipid carriers, are therefore evaluated as QA-controlled stabilizing excipients (76,37). While ICH Q1A(R2) stability testing principles are partially applicable to LBPs, product-specific protocols are required for live microorganisms. Regulatory agencies now recommend stability-indicating assays that assess both live counts and metabolic function (38,79).

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

4.6 Reference Standards and Comparability

Because LBPs are living, setting a reference standard for assay calibration is especially difficult. Differences between production lots, process changes, or even cell bank passages can affect viability and gene expression patterns (78). Comparability protocols therefore need to evaluate critical quality attributes (CQAs) like genomic integrity, phenotype stability, and potency equivalence after any process change (42). Internationally harmonized standards are urgently needed to ensure assay reproducibility and product comparability across developers (43).

4.7 Integration of Advanced Analytical Technologies

Emerging tools like digital PCR (dPCR), metagenomic sequencing, single-cell analysis, and AI-driven pattern recognition are being added to QA pipelines for LBPs (44,46). They provide high sensitivity and precision for monitoring strain stability, community composition, and rare contaminants. Machine learning models have also been tested to predict LBP batch potency and stability using multi-parameter data from fermentation and analytical tests (47).The European Medicines Agency (EMA) and the International Pharmaceutical Microbiology Consortium (IPMC) have recently highlighted the need to incorporate these data-driven QA models to ensure consistent product release (48,49).

5. Regulatory QA Frameworks and Harmonization Challenges for Microbiome-Based and Living Biotherapeutic Products

The regulatory landscape for living biotherapeutic products (LBPs) is changing fast but remains fragmented across regions. Because LBPs don't fit neatly into traditional categories like biologics or vaccines, current quality assurance (QA) systems need substantial adjustment. This section examines the regulatory expectations, regional frameworks, and the challenges of harmonizing QA for LBPs

5.1 Global Regulatory Landscape

LBPs are treated as biological medicinal products in most jurisdictions, but definitions and requirements differ (79). The U.S. Food and Drug Administration (FDA) define LBPs as “biological products that contain live organisms, such as bacteria, applicable to the prevention, treatment, or cure of disease in humans” (74). The European Medicines Agency (EMA) likewise classifies LBPs as biological medicinal products and applies directives for biopharmaceuticals, although specific guidance is limited (65).

Other regions are beginning to set up dedicated frameworks:

- Japan’s Pharmaceuticals and Medical Devices Agency (PMDA) places LBPs in the “cellular and tissue-based products” category.
- Health Canada treats LBPs as drugs or biologics and requires full Chemistry, Manufacturing, and Controls (CMC) documentation.

China’s NMPA currently handles LBPs as “novel biologics,” with growing focus on genomic and safety data (80).

Despite these efforts, there are no globally harmonized standards for LBP quality control, which leads to inconsistent QA expectations, dossier preparation, and regulatory assessment (81).

5.2 Key Regulatory Guidelines and Position Papers

Regulators have started issuing preliminary guidance and reflection papers on QA expectations for LBPs:

FDA Guidance for Industry (2016) — Early Clinical Trials with LBPs: Chemistry, Manufacturing, and Control (CMC) Information — recommends approaches for strain identification, manufacturing controls, and stability testing (83).

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

EMA Reflection Paper (2023) — highlights critical quality attributes (CQAs), strain characterization, absence of antimicrobial resistance genes, and the need for potency and viability assays (74).
WHO Technical Report Series 1025 (2021) — stresses QA for biological therapeutics, including live organisms, and recommends risk-based GMP and biosafety controls (80).
European Pharmacopoeia Monograph 3053 (2023) - sets quality standards for LBPs, covering microbial purity, identity, and potency specifications (81).
International Pharmaceutical Microbiology Consortium (IPMC) White Paper (2024) - calls for global standardization of analytical methods and comparability protocols for LBPs (78).
These documents offer useful structure, but the guidance is non-binding and still lacks the specificity found in established biologics frameworks like ICH Q6B (specifications for biological products) or ICH Q8–Q11 (pharmaceutical development and lifecycle management).

5.3 Chemistry, Manufacturing and Control (CMC) Expectations

CMC documentation for LBPs must cover strain origin, genetic stability, manufacturing process, controls, and product characterization (82). Several QA challenges make CMC submissions more complicated:

1. Strain identity and genomic stability: Full genome sequencing and stability data across cell banking stages (MCB/WCB) are required (65).
2. Manufacturing process validation: You must show reproducibility and batch-to-batch consistency in live cell counts and potency (68).
3. Product purity and contaminant testing: Tests for adventitious agents and non-target organisms need to be included (69).
4. Formulation and stability data: QA must demonstrate that viability and potency are maintained under intended storage and distribution conditions (69).
5. Container–closure and delivery systems: Any interaction between live cells and packaging materials, capsule coatings, or delivery vehicles must be validated for stability (71).

Both the FDA and EMA stress a risk-based CMC approach: process controls should address product- and strain-specific risks rather than applying one-size-fits-all GMP criteria.

5.4 GMP Requirements for LBP Manufacturing

Manufacturing LBPs under Good Manufacturing Practices (GMP) brings some unique quality assurance issues. Traditional aseptic techniques used for sterile biologics don't fully apply, since LBPs are intentionally non-sterile (24). Instead, GMP compliance emphasizes:
Controlled manufacturing zones to prevent cross-contamination between strains.
Closed fermenter systems for anaerobic or microaerophilic organisms.
Appropriate biosafety containment (BSL-2 or higher), depending on the strain.
Environmental monitoring and microbial surveillance programs.
Recent studies stress that environmental controls, cleanroom classifications, and operator hygiene need to be tailored to the microbes' physiology (70). The FDA and EMA recommend creating LBP-specific GMP annexes, similar to those used for ATMPs (Advanced Therapy Medicinal Products) (78).

5.5 Quality Control (QC) and Release Testing

Release testing for LBPs requires QA to confirm the product meets defined specs for identity, purity, potency, and viability (29). A big regulatory hurdle remains the lack of standardized tests, because developers use different methods.

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

Newer approaches include qPCR for identity confirmation, flow cytometry to assess viability, and functional potency assays tied to the mechanism of action (30,32). Regulators also push for stability-indicating assays that capture both CFU counts and functional viability (33). When process or formulation changes are made, comparability protocols are required, following ICH Q5E principles (34).

Batch release specifications are usually set using clinical lots as the reference, with tight controls on viable cell count, strain composition, and the absence of contaminant species (35).

5.6 Risk-Based QA and Regulatory Flexibility

Given the scientific novelty of LBPs, regulators are increasingly using a risk-based framework like those applied to gene and cell therapies (36). The emphasis is on identifying and controlling the critical process parameters (CPPs) and critical quality attributes (CQAs) that most affect product safety and efficacy.

For example, FDA's Emerging Technology Program and EMA's Innovation Task Force offer early dialogue with LBP developers to help define suitable quality assurance strategies during preclinical development (37).

In this setting, developers are encouraged to apply Quality by Design (QbD) principles (ICH Q8) so quality is built into the process from early development through commercial scale (38).

5.7 Global Harmonization Challenges

The lack of harmonized definitions and standards for LBPs across regulatory jurisdictions creates major quality-assurance challenges (39,40). Developers struggle to align dossier contents, analytical validation requirements, and acceptance criteria for submissions to the FDA, EMA, PMDA, and NMPA. Work is underway through the International Coalition of Medicines Regulatory Authorities (ICMRA) and the World Health Organization (WHO) to build consensus on LBP QA terminology and data expectations (41,42).Still, until there's unified international guidance—similar to ICH harmonization for biologics—regulatory fragmentation will continue to hinder consistent QA practices and global product rollout (43,44).

5.8 Post-Marketing QA and Pharmacovigilance

Post-approval QA for LBPs should monitor long-term viability, potency changes, and any adverse shifts in the microbiome during real-world use (45). Pharmacovigilance must pick up signals of microbial translocation, horizontal gene transfer, or opportunistic infections linked to live strain activity (46,47).

The FDA expects post-market microbial surveillance for LBPs regulated under IND or BLA pathways, and the EMA requires Risk Management Plans that include environmental risk assessments (48,49). Ongoing QA in commercial manufacturing should incorporate process analytical technology (PAT), real-time release testing (RTRT), and genomic monitoring to maintain consistent product performance (50).

6. Future Perspectives and Recommendations

Microbiome-based therapeutics and Living Biotherapeutic Products (LBPs) are leading a new wave of precision biologics. Despite impressive scientific advances, important Quality Assurance (QA) challenges remain across discovery, development, manufacturing, and post-approval stages. This final section brings together the main insights and highlights directions for future research, regulatory change, and QA innovation.

6.1 Integrating Systems Biology and Omics in QA

The future of LBP QA will rely more and more on multi-omics approaches-genomics, transcriptomics, metabolomics, and proteomics-to fully characterize microbial strains

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

and how they interact (82). Traditional culture-based methods miss complex community dynamics and subtle genetic drift that can occur over multiple passages. Whole-genome sequencing (WGS) and metagenomic profiling can offer near real-time views of strain stability, expression of virulence factors, and functional potency (83).
Similarly, metabolomic fingerprinting using LC–MS or NMR provides quantitative measures of product consistency that go beyond CFU counts (86). Combining omics data with AI-driven bioinformatics platforms can help identify QA biomarkers that predict product quality and stability (84). As a result, QA frameworks will move away from static, CFU-based specifications toward data-rich, systems-level definitions of quality that better capture the true functional equivalence of live microbial therapeutics.

6.2 Digital and AI-Enhanced QA Systems

Implementing digital twins and AI-based process monitoring could transform quality management for live biotherapeutic products (85). Machine learning models trained on historical batch data can spot deviations in fermentation parameters or declines in viability, enabling predictive quality assurance and earlier interventions (86). Blockchain-based batch traceability can provide immutable digital records for regulatory audits, ensuring end-to-end transparency (87). Regulatory bodies like the FDA's Emerging Technology Program (ETP) and the EMA's Digital Manufacturing Initiative are already looking at how AI-enabled systems can support real-time release testing (RTRT) and process analytical technology (PAT) for live biologics (80). Over the next decade, these digital infrastructures will underpin Quality 4.0 for microbiome-based therapeutics.

6.3 Defining New Potency and Viability Metrics

Unlike conventional biologics, LBPs don't have universal potency metrics, which makes release testing and comparability more difficult (55). Future QA frameworks should tie potency assays to mechanistic biomarkers- for example, short-chain fatty acid (SCFA) production, immune-modulating markers like IL-10 or TNF-α, or the ability to adhere to epithelial surfaces (84). Functional assays that measure the product's intended physiological effect, such as restoring barrier integrity or providing colonization resistance, could act as surrogate potency indicators (89). New methods- for instance, flow cytometry with fluorescent viability markers, microfluidic single-cell viability platforms, and qPCR-based detection of active transcripts- will improve how accurately we define "live" cell populations (81). Ultimately, globally adopted, standardized potency criteria linked to mechanism will be essential for harmonized LBP quality assurance.

6.4 Standardization and Harmonization Pathways

Global QA for LBPs will likely follow the path biologics regulation took under the ICH framework (63). A proposed ICH guideline for Live Biotherapeutics (ICH QLBP), currently being discussed by regulatory groups, could standardize terminology, expectations for analytical validation, and comparability principles (64,25). Establishing global reference strains and public analytical standards—through bodies like WHO Collaborating Centres or the USP- would help achieve consistency across jurisdictions (46). Creating a Global Microbiome Quality Database (GMQD) could let developers benchmark genomic and phenotypic traits of therapeutic strains against curated standards (27). This approach would fit with WHO's ongoing effort toward regulatory convergence in microbiome therapeutics (18).

6.5 Addressing Manufacturing and Scale-Up Bottlenecks

Future QA frameworks need to account for how microbial physiology changes with scale during manufacturing (59). Data from small-scale fermentations often don't predict industrial-scale performance because factors like shear stress, oxygen gradients, and

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

nutrient diffusion change with scale (80,81). Using QbD and DoE-driven process design early in development helps reduce those deviations (82). In addition, continuous manufacturing and modular bioreactor systems will enable real-time monitoring of key QA parameters such as viability, metabolite profiles, and contamination control (33). Deploying PAT tools—like inline Raman spectroscopy or impedance-based viability sensors—can support real-time quality release (34,35).

6.6 Strengthening Environmental and Biosafety QA

Because LBPs use live microorganisms on purpose, environmental quality assurance is becoming a distinct area of concern (86). Risk assessments should cover the potential for horizontal gene transfer, how long organisms persist in the environment, and the spread of antimicrobial resistance genes (87,88). QA systems going forward will need to verify biocontainment, evaluate genetic safeguard circuits, and include plans for post-release microbial surveillance (89). Synthetic biology can help here: kill-switch designs, auxotrophic dependency systems, and CRISPR-based containment approaches can be incorporated and validated within QA frameworks (60,41). Regulators are likely to require environmental risk management plans (ERMPs) in addition to standard GMP documentation (42).

6.7 QA Training, Workforce, and Infrastructure

QA for live biotherapeutic products (LBPs) requires expertise across microbiology, molecular biology, process engineering, and regulatory science. To close existing skill gaps, capacity-building programs for QA professionals are essential. Universities and regulatory agencies should introduce specialized QA training for live biotherapeutics, modelled on biologics QA certifications (44). Collaborative consortia between academia and industry—such as BioPhorum and the Alliance for Biotherapeutic Innovation (ABI)-can help develop shared QA infrastructure and validation protocols (15).

6.8 The Path Forward

Over the next decade, advanced analytics, AI-driven monitoring, harmonized regulation, and sustainable biomanufacturing will shape QA for microbiome-based therapeutics (90). Focused investment in QA innovation platforms, standard reference strains, and regulatory alignment will speed safe approvals and patient access worldwide (69,50). In short, moving from traditional QC to dynamic, data-driven QA systems will help living biotherapeutics meet the same quality, safety, and reproducibility standards as other biologics—without suppressing their unique scientific promise.

7. Conclusion

The regulatory framework for living biotherapeutic products (LBPs) is changing to reflect the special challenges of medicines made from live microorganisms. Unlike small-molecule drugs or inert biologics, LBPs need tailored rules that secure the identity, purity, stability, and potency of the strains used, along with strong risk management and ongoing monitoring throughout their lifecycle. Regulators around the world are working to harmonize definitions and requirements, folding LBPs into biological drug regulations but adding emphasis on microbial genomic characterization, traceability, and containment to address risks like horizontal gene transfer, unintended colonization, and environmental spread.Good Manufacturing Practice (GMP) is central to LBP oversight, with extra focus on cell banking, raw material qualification, process validation, and batch-to-batch consistency—areas made harder by the variability and adaptability of living organisms. Advanced analytics—high-throughput sequencing, multi-attribute mass spectrometry, and next-generation potency assays—are becoming routine in product quality evaluation, confirming microbial identity and flagging unwanted genetic or phenotypic changes quickly. Clinical and lot release testing, built around critical quality

Date of Submission: 01/11/2025 Date of Acceptance: 08/11/2025 Date of Publish: 15/11/2025

attributes (CQAs), need robust, mechanism-linked assays and clear evidence that in vitro potency is relevant in vivo, backed by meaningful, reproducible trial endpoints.Safety assessment goes beyond sterility and endotoxin testing to include genomic screens for virulence factors, antimicrobial resistance genes, and mobile genetic elements, together with preclinical models and active pharmacovigilance to catch rare, delayed, or population-specific adverse events. Environmental risk assessments and containment strategies are especially important for genetically engineered or novel LBPs, and international guidance increasingly calls for transparent disclosure, labelling, and concrete risk-minimization plans.Looking ahead, regulatory harmonization regionally and globally is seen as essential to streamline LBP development, approval, and monitoring as these products move into broader clinical use. Continued advances in analytical and process controls, adaptive risk-management frameworks, and open dialogue among regulators, industry, clinicians, and patients will be key to realizing LBPs' therapeutic potential while keeping patient safety and public health front and center. These evolving measures aim to ensure LBPs are judged not just for their novelty, but for reproducible quality, actionable safety, and proven clinical benefit in real-world settings.

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