Characterization Standards for Probiotics

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Guidance for Industry

Early Clinical Trials with Live Biotherapeutic Products: Chemistry, Manufacturing, and Control Information

DRAFT GUIDANCE
This guidance document is for comment purposes only.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
September 2010
Definitions

**Drug**: includes, but is not limited to, articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals.

**Biological product**: means a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, protein applicable to the prevention, treatment, or cure of a disease or condition of human beings.

“Virus”: is understood to be a product containing the minute living cause of an infectious disease and includes but is not limited to filterable viruses, bacteria, rickettsia, fungi, and protozoa

**Drug substance**: is the unformulated active substance that may be subsequently formulated with other excipients to produce the drug product. The microorganisms contained in an LBP are typically cellular microbes such as bacteria and yeast. Thus, the drug substance for an LBP is typically the unformulated live cells.

**Drug product**: is the finished dosage form of the product.
Live Biotherapeutic Products (LBP)

- The FDA does not classify probiotics as a separate class of products.
- Under current FDA guidelines, probiotics fit within the live biotherapeutic products (LBP) category.
- An LBP is a biological product (non-recombinant) that:
  - contains live microorganisms, such as bacteria or yeast.
  - is applicable to the prevention, treatment, or cure of a disease or condition of human beings.
  - is not a vaccine.
Mechanisms of Actions/Applications

- Dosage forms and routes of administration can vary from ready mixed oral preparations with the appearance of a traditional food or drink to pre-filled vaginal applicators. The proposed mechanisms of action are generally to interfere with the growth of a pathogenic or potentially pathogenic microorganism in the body or to stimulate other potentially beneficial cellular processes as a result of transient persistence and/or long-term colonization with the microorganisms contained in the LBP.

- Investigational objectives vary with respect to prevention versus treatment, and stand-alone therapy versus adjunct therapy to antimicrobial, or other, therapy. For example, potential indications include treatment of bacterial vaginosis in combination with antibiotics, prevention of necrotizing enterocolitis, prevention of allergic rhinitis, and maintenance of remission of acute pouchitis. Intended study populations vary from premature neonates to older adults, and from healthy individuals who may be at risk for specific diseases to individuals severely afflicted with particular diseases or conditions.
LPB Drug Substance - Characterization

A description of the LBP’s drug substance, including its physical, chemical, or biological characteristics, must be included in the IND and should include:

- Biological name and strain designations
- Original source of cells from which the drug substance was derived
- Culture/passage history of the strain(s)
- If cells were obtained from a clinical specimen, a description of the clinical health of the donor(s), if known (merely noting procurement from a commercial provider is not adequate)
- Summary of the phenotype and genotype of the product strains, with special attention to biological activity or genetic loci that may indicate activity or potency
- Documentation and summary of modifications, if any, to the LBP, e.g., intentional introduction of foreign genes or mutations, along with details of the genetic construction
Characterization of an LBP must include a description of the acceptable limits and analytical methods used to assure the identity, strength, quality, and purity of the drug substance. Test results should contain actual laboratory data in tabulated form rather than summaries. Results for quantitative assays should be presented as actual data and not simply as “Pass,” “Satisfactory,” or “Within Specification.”

Production of investigational drug and biological products are subject to section 501(a)(2)(B) of the PHS Act (21 U.S.C. 351(a)(2)(B))
LPB Characterization

- Identification of the cells used to establish the Master Cell Bank. This information should be at the species and strain level with at least two complementary methods of identification, e.g., biochemical identification and genetic identification.

- Minimum inhibitory or minimum bactericidal concentrations for a panel of antibiotics (establish sensitivity or resistance). Assay the extent of antibiotic resistance transferability from a product strain to relevant microbial flora. If antibiotic resistance intentionally introduced - justification for need (potential alternative).

- Provide the methods used to attenuate an otherwise virulent strain, as well as document the stability of such attenuation.

- If the ability of the product strain(s) to cross a mucosal barrier is a critical safety concern. Use reproducible assay for translocation, preferably in an appropriate animal model, such as germ-free mice.

- Product’s mechanism(s) of action(s) (if known): provide data to support the mechanism(s) of action(s). Investigating the mechanism(s) of action(s) can be valuable for developing quality assurance criteria. (potency traceability)
LPB Drug Substance - Characterization

CHEMISTRY, MANUFACTURING, AND CONTROL (CMC) INFORMATION

Manufacturing of the LBP Drug Substance

- **Manufacturing information**: manufacturer, co-productions, processes and methods.

- **Cell Bank System**: Master Cell Bank (MCB) and Working Cell Bank (WCB). Description of the cell banking procedures used, including: the banking system; the size of the cell banks; the methods, reagents, and media used for preparation of the cell banks; the conditions employed for cryopreservation and storage; in-process controls; and storage conditions.

- For both MCB and WCB: The history and characterization should include: the original source of cells used in the establishment of the cell banks and the culture/passage history of the cells; the method used to derive the cell bank; phenotypic and genotypic characterization as a means for identification; biochemical and/or genetic markers that may be potency-indicating; purity of culture (screening procedures for adventitious agents); and a description.

- **Cell growth and Harvesting**: steps for propagation, from retrieval of the cell bank to culture harvest (stages of growth) - media used at each step (including water quality), with details of their preparation and sterilization - inoculation and growth of initial and sub-cultures, including volumes, time and temperature of incubation(s) - methods and criteria used for harvesting and determining yields, and criteria for pooling more than one harvest.
LPB Drug Substance - Characterization

CHEMISTRY, MANUFACTURING, AND CONTROL (CMC) INFORMATION

Manufacturing of the LBP Drug Substance

Purification and Downstream Processing. Description of the methods and materials by which separation and concentration of intermediate forms and final bulk of whole cells from media, solvents, or solutions used in the production process. Description of each step in downstream processing including accompanying analytical tests developed or adopted by the manufacturer to show identity, purity, and concentration, and the levels of product related and non-product related impurities.
LPB Drug Substance - Characterization

CHEMISTRY, MANUFACTURING, AND CONTROL (CMC) INFORMATION

LBP Drug Substance Specifications

Provide preliminary specifications and tests, including, but not limited to, assays for: identity, purity, microbial bioburden/contamination, potency, and/or biochemical or physicochemical measurements thought to predict potency, and where applicable, measures of stability.

- Identity of each microbial strain present in the drug substance determined using a specific and reproducible assay. Testing may be based upon biochemical methods such as fermentation profile or genotypic methods, including such as ribotyping, restriction fragment length polymorphism (RFLP), or both. If one or more genetic loci, either naturally occurring or engineered, are critical for biological activity, develop a specific identity assay.

- Potency of live microbial products is generally a measure of viable cells per unit or dose, i.e., colony-forming units (CFUs). Develop a test for the ratio of live to dead organisms in the drug substance to determine whether dead organisms have any effect on efficacy and safety. Additional measures of product potency may be applicable, depending on the specific product strain(s) and knowledge of the mechanism(s) of action.
LPB Drug Substance - Characterization

CHEMISTRY, MANUFACTURING, AND CONTROL (CMC) INFORMATION

- LBP Drug Substance Specifications

  - **Purity tests of a LBP**: Assessment of endotoxin content, residual antibiotics, and/or the quantification of residual toxic components or contaminants introduced during manufacturing.

  - **Tests for microbial bioburden** should be in accordance with the US Pharmacopeia (31 USP <61>) Microbial Limits Tests.

For example: The specification for non-product bioburden in an oral product should be less than or equal to 200 non-product bacteria and less than or equal to 20 non-product fungi per gram of material or per dose (whichever is less) with evidence for the absence of pathogens. Depending on the nature of other products campaigned at the manufacturing facility, the inclusion of tests specific for other organisms may be necessary.
LPB Drug Product - Characterization
CHEMISTRY, MANUFACTURING, AND CONTROL (CMC) INFORMATION

- Drug Product (containing LBP)
  - Composition
  - Manufacturing
  - Drug Product Specification
    - Identity
    - Purity
    - Microbial bioburden/contamination
    - Appearance/visual inspection
    - Where applicable, additional tests: percent viable cells, determination of particulate matter, pH testing, residual moisture...
  - Stability
  - Placebo
Other sources for Guidance on Characterization

Guidance for Industry: Enzyme Preparations: Recommendations for Submission of Chemical and Technological Data for Food Additive Petitions and GRAS Notices

- Identity
- Manufacturing Process
- Specifications for Identity and Purity
- Intended Technical Effects and Use
- Intake Estimate
- Enzyme Preparations Containing Allergenic Ingredients
Other sources for Guidance on Characterization

- FDA Guidance for Industry: Recommendations for Submission of Chemical and Technological Data for Direct Food Additive Petitions

  - Identity
  - Manufacturing Process
  - Specifications for Identity and Purity
  - Stability of the Food Additive
  - Intended Technical Effects and Use
  - Methodology for Analysis of the Additive in Food
  - Intake Estimate
Special case

- Fecal transplants - microbiota transplants

The guidelines are somewhat inappropriate for this types of LBP

- Identity - Too complex, no methods would be exhaustive and could not guarantee the Microbial Limit Test
- Chemical/microbiological composition changes from “batch to batch”
- Manufacturing process

Where should this type of products be regulated?