

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

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## Guidance for Industry

### **This guidance is for immediate implementation**

FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(3) without seeking additional comments after determining that prior public participation is not reasonable or appropriate (see 21 CFR 10.115(g)(2)). FDA notes that we already sought comments on the issues addressed by the revisions in this guidance in the *Federal Register* notice of March 31, 2015 (80 FR 17050) entitled “Early Clinical Trials with Live Biotherapeutic Products: Chemistry, Manufacturing, and Control Information; Guidance for Industry: Request for Comments,” under Docket No. FDA-2010-D-0500. Further delay in implementing these revisions could impede the progress of certain investigations that are of low risk and may be of benefit to the public health.

FDA invites comments on this guidance. Submit one set of either electronic or written comments on this guidance at anytime. Submit electronic comments to <http://www.regulations.gov>. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with docket number FDA-2010-D-0500.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email [ocod@fda.hhs.gov](mailto:ocod@fda.hhs.gov), or from the Internet at <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Biologics Evaluation and Research  
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## Contains Nonbinding Recommendations

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### Guidance for Industry

*This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.*

#### I. INTRODUCTION

##### A. Purpose and Scope

We, FDA (or Agency), are providing you, Investigational New Drug Application (IND) sponsors, with recommendations regarding IND submissions for early clinical trials with live biotherapeutic products (LBPs) in the United States (U.S.), including LBPs lawfully marketed as foods (such as conventional foods and dietary supplements) in the U.S. and proposed for clinical uses regulated under section 351 of the Public Health Service (PHS) Act (42 U.S.C. 262).<sup>1</sup>

This guidance focuses on the chemistry, manufacturing, and control (CMC) information that you should submit in an IND for an LBP. This guidance is applicable to all INDs of LBPs, whether clinical trials are conducted commercially, in an academic setting, or otherwise under Title 21 of the Code of Federal Regulations Part 312 (21 CFR Part 312).<sup>2</sup>

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<sup>1</sup>The term “live biotherapeutic product” is defined in the “definitions” section (section I.B.) of this document.

<sup>2</sup>This guidance is not intended to address when an IND is required prior to conducting clinical trials. For further discussion of that subject see “Guidance for Clinical Investigators, Sponsors, and IRBs: Investigational New Drug Applications (INDs) – Determining Whether Human Research Studies Can Be Conducted Without an IND” dated September 2013, available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM229175.pdf>. A stay is in effect for parts of this guidance document. See “Investigational New Drug Applications – Determining Whether Human Research Studies Can Be Conducted Without an Investigational New Drug; Guidance for Clinical Investigators, Sponsors, and IRBs; Partial Stay and Republication of Guidance” (80 FR 66907, October 30, 2015).

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This guidance is not applicable to LBPs intended as gene therapy vectors, to oncolytic bacteria, or to oncolytic viruses.<sup>3</sup>

This guidance updates the guidance of the same title dated February 2012 (February 2012 guidance) by including the new section IV.D to address when the label on commercially available product(s) would be considered adequate to satisfy the purpose of the CMC information requirements under 21 CFR 312.23(a)(7). In addition, this guidance updates the February 2012 guidance by removing the General Safety Test (21 CFR 610.11) as an example of a test method in section IV.C and by updating certain footnotes, references, and addresses.<sup>4</sup>

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

### B. Definitions

For purposes of this guidance:

A **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. (see section 201(g)(1) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 321(g)(1)) for the full definition).

A **biological product** means a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, protein (except any chemically synthesized polypeptide), or analogous product, or arsphenamine or derivative of arsphenamine (or any other trivalent organic arsenic compound) applicable to the prevention, treatment, or cure of a disease or condition of human beings (see section 351(i) of the Public Health Service (PHS) Act (42 U.S.C. 262(i)). The FD&C Act applies to a biological product subject to regulation under 42 U.S.C. 262, except that, under 42 U.S.C. 262(j), a product for which a license has been approved under subsection (a) of 42 U.S.C. 262 shall not be required to have an approved application under section 505 of the FD&C Act (21 U.S.C. 355).

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<sup>3</sup>As a general matter, the Office of Vaccines Research and Review in the Center for Biologics Evaluation and Research (CBER) reviews the clinical studies for the LBPs described in this guidance. CBER's Office of Cellular, Tissue and Gene Therapies (OCTGT) reviews the clinical studies for certain microbial products, such as microbial vectors for gene therapy (MVGT), oncolytic bacteria and oncolytic viruses. OCTGT encourages potential sponsors of INDs for such products to contact its office for additional guidance prior to submission of their IND.

<sup>4</sup>We are removing reference to the General Safety Test because the underlying regulations were revoked in 2015. See "Revocation of General Safety Test Regulations That Are Duplicative of Requirements in Biologics License Applications" (80 FR 37971, July 2, 2015).

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A **virus** as used in the PHS Act, 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 (21 CFR 600.3(h)(1)).

The **sponsor** of an IND is a person who takes responsibility for and initiates a clinical investigation. The sponsor may be an individual or pharmaceutical company, governmental agency, academic institution, private organization, or other organization (21 CFR 312.3(b)).

An **LBP**, for the purposes of this guidance document, is a biological product that: 1) contains live organisms, such as bacteria; 2) is applicable to the prevention, treatment, or cure of a disease or condition of human beings; and 3) is not a vaccine. For the purposes of this document, LBPs are not filterable viruses, oncolytic bacteria, or products intended as gene therapy agents and, as a general matter, are not administered by injection. An example of an LBP, for the purposes of this document, would be one or more strains of lactobacilli administered orally to treat patients with ulcerative colitis, or administered vaginally to prevent bacterial vaginosis.

A **recombinant LBP** is a live biotherapeutic product composed of microorganisms that have been genetically modified through the purposeful addition, deletion, or modification of genetic material. A recombinant LBP which is subject to this guidance is likely to raise additional considerations and thus would require additional information to be submitted in an IND. Potential sponsors of an IND for a recombinant LBP are encouraged to contact FDA to obtain additional guidance prior to submission of their IND.

The **drug substance** is the unformulated active substance that may be subsequently formulated with excipients to produce the drug product (Refs. 1 and 5). 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.

The **drug product** is the finished dosage form of the product (Refs. 1 and 5).

A **clinical investigation** means any experiment in which a drug is administered or dispensed to, or used involving, one or more human subjects (21 CFR 312.3(b)).

An **experiment** is any use of a drug except for the use of a marketed drug in the course of medical practice (21 CFR 312.3(b)).

## II. BACKGROUND

Proposed clinical trials to evaluate LBPs vary in design, depending on the product, proposed use, and intended population for enrollment. For example, live microorganisms contained in LBPs may be isolated from human hosts, genetically modified for use as vectors, and/or engineered to exhibit enhanced functions or new characteristics. Dosage forms and routes of administration

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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.

Regulations applicable to investigational biological products that meet the FD&C Act's definition of "drug" can be found at 21 CFR Part 312. Except as provided under the limited exemptions set out at 21 CFR 312.2(b), these requirements apply to all clinical investigations of products that are subject to section 505 of the FD&C Act or to section 351 of the PHS Act (see 21 CFR 312.2(a)). The intended use of a product plays a central role in how it is regulated. For example, products that contain live microorganisms may be regulated as dietary supplements, conventional foods, or drugs under the FD&C Act, depending on the product's intended use and other factors relevant to the statutory definition of the product category.<sup>5</sup> The intended use of a product in a clinical investigation determines whether IND requirements for clinical investigation of the product are applicable.<sup>6</sup> Note that under section 201(ff)(3)(B)(ii) of the FD&C Act (21 U.S.C. 321(ff)(3)(B)(ii)), if an LBP that meets the other parts of the dietary supplement definition in section 201(ff) is authorized for investigation under an IND, and was marketed as a dietary supplement or conventional food before the IND was authorized, it may continue to be marketed as a dietary supplement despite the existence of the IND.<sup>7</sup> Under section 301(l) of the FD&C Act (21 U.S.C. 331(l)), if substantial clinical investigations of an LBP as a drug or biologic were instituted before the LBP was marketed in food, a food

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<sup>5</sup> See "Draft Guidance for Industry: Complementary and Alternative Medicine Products and Their Regulation by the Food and Drug Administration" dated December 2006 at page 4, issued for public comment and available at <http://www.fda.gov/RegulatoryInformation/Guidances/ucm144657.htm>.

<sup>6</sup> See also "Guidance for Clinical Investigators, Sponsors, and IRBs: Investigational New Drug Applications (INDs) – Determining Whether Human Research Studies Can Be Conducted Without an IND" dated September 2013, available at

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM229175.pdf>. A stay is in effect for parts of this guidance document. See "Investigational New Drug Applications (INDs) – Determining Whether Human Research Studies Can Be Conducted Without an IND; Guidance for Clinical Investigators, Sponsors, and IRBs; Partial Stay and Republication of Guidance" (80 FR 66907, October 30, 2015).

<sup>7</sup> If an LBP was not marketed as a dietary supplement or conventional food before the IND was authorized, the LBP may only be marketed as a dietary supplement if one or more of the following is true: (1) substantial clinical investigations of the LBP have not been instituted; (2) the existence of substantial clinical investigations of the LBP (if such clinical investigations were instituted) has not been made public; or (3) the Secretary has issued a regulation, after notice and comment, finding that the article would be lawful under the FD&C Act (21 U.S.C. 321(ff)(3)(B)(ii)). As of the date of issuance of this guidance, no such regulations have been issued.

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containing the LBP may continue to be marketed only if the existence of such investigations has not been made public, or if one of the exceptions in section 301(l)(2)-(3) of the FD&C Act applies.<sup>8</sup>

FDA's primary goals in reviewing an IND are, in all phases of the investigation (phases 1, 2, and 3), to assure the safety and rights of subjects, and in Phases 2 and 3, to help assure that the quality of the scientific evaluation of drugs is adequate to permit an evaluation of the drug's effectiveness and safety (21 CFR 312.22(a)). The general principles of the IND submission and IND content and format are contained in 21 CFR 312.22 and 312.23, respectively. This guidance describes information that should be provided in an IND in order to meet the requirements under 21 CFR 312.23 for early clinical trials evaluating LBPs.

### **III. ADMINISTRATIVE AND REGULATORY PROCEDURES**

#### **A. Pre-IND Advice and Meetings**

Formal communication with FDA representatives before submission of an IND may be helpful, especially for sponsors that have not previously interacted with CBER, for sponsors who have limited experience with IND submissions, or for sponsors who have new products, technology, or assays under development. The purpose of a "pre-IND meeting" is generally to discuss information required under an IND, such as product characterization, final and in-process testing of the product, any animal data, and the proposed clinical protocol (see 21 CFR 312.23). For information on how to request, schedule, conduct, and document pre-IND meetings, please refer to the FDA document entitled "Guidance for Industry: Formal Meetings Between the FDA and Sponsors or Applicants" dated May 2009 (Ref. 2).

#### **B. Address for Submission of INDs**

An original IND submission, as well as IND amendments, must be submitted in triplicate (21 CFR 312.23(d)). Each submission should be accompanied by a cover letter and must include Form FDA-1571 (21 CFR 312.23(a)(1)). You should sequentially number all pages of the entire submission, including its attachments and appendices. The mailing address for submission of INDs to CBER is (21 CFR 312.140(a)(3)):

Food and Drug Administration  
Center for Biologics Evaluation and Research  
Document Control Center  
10903 New Hampshire Ave.  
Bldg. 71, Rm. G112  
Silver Spring, MD 20993-0002.

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<sup>8</sup> See Footnote 6.

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For information on the submission of electronic INDs, please refer to the following FDA guidance documents: “Guidance for Industry: Providing Regulatory Submissions to CBER in Electronic Format — Investigational New Drug Applications (INDs)” dated March 2002 (Ref. 3), “Providing Regulatory Submissions in Electronic Format — Standardized Study Data; Guidance for Industry” dated December 2014 (Ref. 16), “Providing Regulatory Submissions in Electronic Format — Submissions Under Section 745A(a) of the Federal Food, Drug, and Cosmetic Act; Guidance for Industry” dated December 2014 (Ref. 17) and “Providing Regulatory Submissions in Electronic Format — Certain Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications; Guidance for Industry” dated May 2015 (Ref. 18).

### **C. Drug Master Files**

An IND sponsor who does not manufacture the product proposed for use in a clinical trial may ask the manufacturer of the product to submit a Drug Master File (DMF). A DMF is a submission of information to FDA that may be used to provide confidential information about the methods used in the manufacturing, processing, packaging, or storing of a drug product. DMFs can be used by a commercial entity to allow an IND sponsor to reference the information in the DMF in support of the IND, without disclosure of the contents of the DMF to the IND sponsor. The DMF holder submits to FDA written authorization permitting the IND sponsor to reference the DMF.<sup>9</sup> When submitted in support of an IND reviewed by CBER, original DMF submissions and updates should be sent to CBER (to the same address in section III.B of this document) rather than to the Center for Drug Evaluation and Research. If a DMF will be submitted or substantially updated, the DMF holder should alert the appropriate review division and relevant IND sponsors.

## **IV. CHEMISTRY, MANUFACTURING, AND CONTROL (CMC) INFORMATION**

### **A. Regulatory Considerations**

In 2008, FDA issued a guidance entitled, “Guidance for Industry: CGMP for Phase 1 Investigational Drugs” dated July 2008 (Ref. 4). The approach described in this guidance recognizes that both manufacturing controls and the extent of such manufacturing controls needed to achieve appropriate product quality differ not only between investigational and commercial manufacture, but also among the various phases of clinical trials.

Production of investigational drug and biological products is subject to section 501(a)(2)(B) of the FD&C Act (21 U.S.C. 351(a)(2)(B)) and the IND regulations at 21 CFR Part 312. During Phase 1 studies, emphasis should generally be placed on elements to assure the safety of subjects (see 21 CFR 312.22). This should include

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<sup>9</sup> For more information on DMFs, see the FDA guidance entitled “Guideline for Drug Master Files,” dated September 1989, available at <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm122886.htm>.

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identification and control of the raw materials and the drug substance, stability assurance, and, where appropriate, non-clinical safety assessments (see, e.g., 21 CFR 312.23(a)(8)). Quality control and quality assurance should be refined as product development proceeds. Although in each phase of the investigation, sufficient information is required to assure the proper identification, quality, purity, and strength of the investigational drug, the amount of information necessary to make that assurance will vary with the phase of the investigation, proposed duration, dosage form and amount of information otherwise available (21 CFR 312.23(a)(7)). In preparing your IND, you should refer to the FDA guidance on the content and format of INDs for Phase 1 studies entitled, “Guidance for Industry: Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-derived Products” dated November 1995 (Ref. 5).

Section IV.B (Drug Substance) and section IV.C (Drug Product) of this guidance detail the information that should be included in the CMC section of an IND in order to support proceeding to clinical evaluation of an LBP’s safety in human subjects. The terms “drug substance” and “drug product” are used in this guidance document for consistency with documentation on traditional drug products and biological products regulated under the PHS Act (Ref. 1). The CMC information detailed in the drug substance and drug product sections may be submitted in the IND or in a supporting DMF.

### **B. Drug Substance**

#### 1. Description

A description of the LBP’s drug substance, including its physical, chemical, or biological characteristics, must be included in the IND (21 CFR 312.23(a)(7)(iv)(a)). A description of the drug substance should include the following:

- Biological name and strain designations;
- Original source of cells from which the drug substance was derived;
- Culture/passage history of the strains;
- 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 would not permit a conclusion that the product is adequately safe);
- 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; and
- 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.

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### 2. Characterization

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 (21 CFR 312.23(a)(7)(iv)(a)). 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.” Additionally, you should submit in your IND detailed descriptions of the methods and assays used to develop and initially characterize your LBP, such as the following:

- You should provide identification of the cells used to establish the Master Cell Bank (MCB), as described in section IV.B.4.c. of this guidance. This information should be at the species and strain level. We recommend that you use at least two complementary methods of identification, e.g., biochemical identification and genetic identification.
- You should determine minimum inhibitory or minimum bactericidal concentrations for a panel of antibiotics proposed by the investigator and include your assessment of whether the product strains are sensitive or resistant to each of the antibiotics in the panel.
- You may need to develop an assay to determine whether or to what extent antibiotic resistance is transferable from a product strain to relevant microbial flora.
- When an antibiotic resistance gene has been intentionally introduced in the product strain(s) for selection or maintenance of genetic modifications, you should discuss the need for such a gene and any possible alternative approaches.
- You should provide the methods you used to attenuate an otherwise virulent strain, as well as documentation addressing the stability of such attenuation.
- If the ability of the product strain(s) to cross a mucosal barrier is a critical safety concern, you should use a reproducible assay for translocation, preferably in an appropriate animal model, such as germ-free mice, for oral products.
- If the product’s mechanism(s) of action(s) is known, you should submit data in the IND to support the mechanism(s) of action(s). Investigating the mechanism(s) of action(s) can be valuable for developing quality assurance criteria. For example, if you are able to identify a limited number of genes that may be potency-indicating, we recommend that you investigate the genetic stability of those genes.

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### 3. Manufacturer

The IND submission must contain the name and address of the manufacturer(s) of the drug substance (21 CFR 312.23(a)(7)(iv)(a)). You should include any other pertinent organizational information. You should also provide a comprehensive list of all additional products that are manufactured or manipulated in the same or adjacent areas used to produce the drug substance. In addition, you should indicate whether the production of other products will utilize the same product contact equipment and, if so, how that equipment will be cleaned between operations for the manufacturing of different products. A floor diagram is suggested as the most effective presentation to enable visualization of the production flow and to identify adjacent operations that may create particular concerns, such as contamination by extraneous microorganisms.

### 4. Method of Manufacture

#### a. Raw Materials

You should provide a list of all materials (e.g., culture media, buffers, etc.) used in the manufacture of the drug substance, and their tests and specifications, or reference(s) to official compendia. For purchased materials, you should provide representative certificates of analysis from the supplier(s) and/or manufacturer's acceptance criteria. The source and quality of materials of ruminant origin are of particular importance and you should provide the appropriate documentation.<sup>10</sup>

#### b. Flow Charts

You should provide a flow chart of the manufacturing process for each drug substance. For drug substances prepared from more than a single microbial strain, a common flow chart is acceptable, with indications of where the processing diverges. A flow chart should show the steps in production, equipment and materials used, the room or area where the operation is performed, and a complete list of the in-process controls and tests performed on the product at each step. You should include in-process holding steps and indicate time and temperature limits. Your flow chart should also identify manufacturing steps that

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<sup>10</sup> Many medical products are manufactured with, or otherwise use, ruminant-derived material because this material can provide necessary nutrients for cell growth. Bovine derived materials are frequently used. If a proposed rule published in 2007 entitled "Use of Materials Derived from Cattle in Medical Products Intended for Use in Humans and Drugs Intended for Use in Ruminants" (72 FR 1583, January 12, 2007), is finalized as proposed, drugs for humans could not be manufactured from, or otherwise contain, prohibited cattle materials without written permission from FDA.

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are computer controlled. You may reference other sections of your IND for more detailed process information. You should also document equipment dedicated to specific areas or products.

### c. Cell Bank System

The cell bank system generally consists of two tiers: an MCB; and Working Cell Banks (WCBs) generated from the MCB for product manufacturing (although a WCB may not have been generated prior to Phase 1 studies). You should provide a detailed description of the cell banking procedures you have 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. You should provide a description of the procedures used to avoid extraneous microbial contamination. You should also include a discussion of precautions (e.g., storage of cell banks in multiple freezers or at different sites) taken to prevent any event that could render the cell banks unusable.

You should identify the cells comprising the MCB and provide a complete history and characterization of the MCB (see section IV.B.2 on “Characterization”). 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 of all media components.

You should provide the same information for any WCBs that you establish.

### d. Cell Growth and Harvesting

This section should generally contain descriptions of:

- each step in propagation, from retrieval of the working cell bank to culture harvest (stages of growth);
- the media used at each step (including water quality), with details of their preparation and sterilization;
- the inoculation and growth of initial and sub-cultures, including volumes, time and temperature of incubation(s);
- how transfers are performed;

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- precautions taken and in-process testing conducted to control contamination;
- the nature of the main culture system, including operating conditions and control parameters, (e.g., temperature of incubation, static vs. agitated, aerobic vs. anaerobic, culture vessels vs. fermenter, volume of fermenter, or number and volume of culture vessels);
- the use of antibiotics in the medium and rationale, if applicable; and
- the methods and the criteria used for harvesting and determining yields, and the criteria for pooling more than one harvest, if applicable.

### e. Purification and Downstream Processing

You should provide a description of the methods and materials by which you separate and/or concentrate intermediate forms and the final bulk of whole cells. The description of each step in downstream processing should also include the 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.

### f. In-Process Testing

For all in-process testing indicated in the flow charts discussed above, you should provide a brief description of the sampling procedures and the test methods used. Acceptance limits for the in-process testing may be necessary to ensure that the product can be manufactured reproducibly. For testing performed at significant phases of production, you should specify the criteria for accepting or rejecting an in-process batch.

## 5. Drug Substance Specifications

You should provide the preliminary specifications and tests for each drug substance. These should include, but not be 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. We recommend that you include upper and lower estimates of variability, as well as justification for the choice of such limits. Specifications are likely to be tighter once experience is gained in the manufacturing process and prior to use of the product in pivotal studies supporting licensure.

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We recommend that the drug specifications section include the following:

- The identity of each microbial strain present in the drug substance should be 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. In addition, if one or more genetic loci, either naturally occurring or engineered, have been identified as critical for biological activity, we recommend that you 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). Additional measures of product potency may be applicable, depending on the specific product strain(s) and knowledge of the mechanism(s) of action.
- Purity tests of a LBP may include assessment of endotoxin content, residual antibiotics, and/or the quantification of residual toxic components or contaminants introduced during manufacture.
- Depending on the clinical setting and route of administration, LBPs may need to be devoid of any extraneous organisms, or alternatively should have a low level of extraneous organisms. Tests for microbial bioburden should be in accordance with the US Pharmacopeia (39 USP <61>) Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests (Ref. 6). Depending on the nature of other products campaigned at the manufacturing facility and the proposed use of the product, the inclusion of tests specific for other organisms may be necessary.

### C. Drug Product

The drug product contains the drug substance(s) formulated with other necessary ingredients in the finished dosage. Other necessary ingredients, active or inactive, may include adjuvants, stabilizers, and/or excipients. The other ingredients may also be held separately from the drug product, such as diluents for reconstitution.

#### 1. Composition

Composition includes a list of all components in the drug product, including drug substance(s) and other ingredients (see generally 21 CFR 312.23(a)(7)(iv)(b)). You should specify quantitative composition of a unit dose and batch. This section should also contain a description of the tests and preliminary specifications for all of the drug product ingredients, including the acceptable upper and lower limits of CFUs for the product strain. You should submit certificates of analysis for all ingredients in the drug product, if not included in

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the drug substance section. If the drug product is encapsulated or is packaged within a delivery device<sup>11</sup>, you should also submit detailed information on the source, quality, and testing of such encapsulation or device.

### 2. Manufacture

You must submit the name(s) and address(es) of the manufacturer(s) of the drug product (21 CFR 312.23(a)(7)(iv)(b)). A list of all other products (research and development, clinical, or approved) manufactured in the same rooms also should be provided. In addition, you should indicate whether the production of other products will utilize the same product contact equipment and, if so, how that equipment will be cleaned between operations for the manufacturing of different products.

You should submit a description of the manufacturing process flow of the formulated bulk and finished drug product, including the processing procedures, lyophilization, and packaging. You should provide records documenting production of the drug product, as well as manufacturing instructions for formulation, filling, labeling, and packaging. You must provide the drug product labeling (see 21 CFR 312.23(a)(7)(iv)(d)).

### 3. Drug Product Specification

Information within this section should include, but not be limited to, sampling procedures and a description of all test methods not already described in the Drug Substance section (section IV.B of this document).

Most INDs for LBPs should contain preliminary specifications for the drug product release/acceptability testing, such as:

- Identity (Drug Substance: section IV.B)
- Potency (Drug Substance: section IV.B)
- Potency Assay: During early clinical development, the potency assay may be an assessment of CFU. As development proceeds, an alternate or additional potency assay may be used. Whenever possible, evidence that the selected potency assay correlates with activity or efficacy observed in clinical trials should be provided.
- Purity (Drug Substance: section IV.B);
- Microbial bioburden/contamination (Drug Substance: section IV.B);
- Appearance/visual inspection; and
- Where applicable, additional tests or modifications thereof, such as

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<sup>11</sup> Drug products packaged within delivery devices such as pre-filled syringes may meet the definition of a combination product (21 CFR 3.2(e)). See <http://www.fda.gov/CombinationProducts/AboutCombinationProducts/ucm118332.htm>

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percent viable cells, a determination of particulate matter (39 USP <788>) (Ref. 7), the rabbit pyrogenicity test (39 USP <151>) (Ref. 8), pH testing, and/or residual moisture (21 CFR 610.13(a)(1)).

### 4. Stability

In an IND submission, you must provide stability data to demonstrate that the new drug substance and drug product are within acceptable chemical and physical limits for the planned duration of the clinical investigation (21 CFR 312.23(a)(7)(ii), 312.23(a)(7)(iv)(a)-(b)). For lyophilized products, you should develop and initiate an additional stability protocol that examines the shelf-life after reconstitution. If the product is frozen, you should develop data supporting product stability through a stated number of freeze-thaw cycles.

The stability study protocol should include, but not be limited to: testing for potency; viable cell determination; microbial contamination; pH; and residual moisture, if applicable.

### 5. Placebo

You must include a brief general description of the composition, manufacture, and control of any placebo used in a controlled clinical trial in your IND submission (21 CFR 312.23(a)(7)(iv)(c)).

### 6. Environmental Assessment

You must include a claim for a categorical exclusion with the basis for the exclusion or an environmental assessment (EA), as outlined in 21 CFR 312.23(a)(7)(iv)(e).<sup>12</sup> An EA is likely to be needed for use of virulent organisms, organisms that are ecologically more fit than their wild-type counterparts, or organisms wherein eradication is problematic or difficult to document.

## **D. IND Studies Utilizing Commercially Available Live Biotherapeutic Products (LBPs)**

For certain investigations of commercially marketed conventional foods and dietary supplements, we anticipate that the label on a commercially available LBP will be sufficient to satisfy the purpose of the CMC information under 21 CFR 312.23(a)(7)(iv)(a)-(b) and that noncompliance with 21 CFR 312.23(a)(7)(iv)(a)-(b) would not pose a significant and unreasonable risk to human subjects of the investigation (see 21 CFR 312.10(b)). Specifically, FDA generally

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<sup>12</sup> For more information on EAs, see the FDA guidance entitled “Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications” dated July 1998, available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070561.htm>.

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intends to grant a waiver provided that: 1) the LBP that is proposed for investigational use is lawfully marketed as a conventional food or dietary supplement; 2) the investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of risk) associated with the use of the food or dietary supplement; 3) the investigation is not intended to support a marketing application of the LBP as a drug for human use or a biological product for human use; and 4) the investigation is otherwise conducted in compliance with the requirements for INDs (21 CFR Part 312). For FDA to grant a waiver from the requirements under 21 CFR 312.23(a)(7)(iv)(a)-(b), sponsors must request a waiver from FDA under 21 CFR 312.10(a). We recommend that sponsors submit the following information to the IND to meet the requirements for a waiver request under

21 CFR 312.10(a): (1) information showing the above; (2) a copy of the label of the commercially available food or dietary supplement; and (3) a commitment to record the lot(s) number and date of expiry in the case report form.

### **V. NON-CLINICAL INFORMATION**

You must submit adequate information about pharmacological and toxicological studies of the LBP in laboratory animals, or in vitro, to support a proposed clinical trial evaluating the investigational LBP (21 CFR 312.23(a)(8)). We recommend that you summarize available information and that you include studies you conduct as well as those reported in the relevant literature. Conclusions based on data obtained from animal studies and/or in vitro studies evaluating the clinical formulation or individual ingredients in the clinical formulation, as opposed to similar or unknown formulations, are generally most supportive of a proposed clinical trial. Depending on the product and as appropriate for the proposed clinical study, we recommend that you address: general toxicity; target organs or systems of toxicity; teratogenic, carcinogenic, or mutagenic potential of any ingredient in the product; and relationship of dosage and duration to toxic response and pharmacological activity. Additional FDA guidance documents, prepared under the auspices of the International Conference on Harmonization (ICH) are available at <http://www.fda.gov/RegulatoryInformation/Guidances/ucm122049.htm> (Refs. 9 and 10).

As discussed above, we encourage you to consult the applicable CBER review division, preferably in a pre-IND meeting, regarding the extent and type of non-clinical data necessary to support the proposed clinical investigation (Ref. 2).

### **VI. CLINICAL INFORMATION**

#### **A. Previous Human Experience**

Previous human experience varies from prospectively designed, randomized, controlled clinical trials to case reports. Similarly, varying degrees of product characterization may be reported for the products used in the respective study or studies. Drawing conclusions

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or making assumptions as to the relevance of previous studies may be difficult or impossible without adequate available CMC information.

In general, the relevance of previous human experience to support a proposed study is based upon the similarity of the product(s) under study, as well as study design, objectives and endpoints, the number of individuals exposed, the level and duration of exposure, the type and duration of active monitoring and passive surveillance, and the integrity of study conduct, data collection and subsequent analyses. If you wish to reference a study submitted to the Agency by a person other than you (the sponsor), you must obtain and include in your submission, a signed letter of cross-reference stating that FDA has permission to access this information (21 CFR 312.23(b)). Also, an accounting of the final disposition of all randomized or enrolled study subjects is important in order to provide a context for the study data, since a large number of dropouts, withdrawals, or protocol violations make data analysis and conclusions less convincing. Multiple statistical analyses without appropriate correction and post hoc analyses, including analyses of population subsets, can be useful to generate hypotheses in designing subsequent studies; however, conclusions based solely upon multiple statistical analyses without appropriate correction may be misleading. Finally, safety data based on active adverse event monitoring provides a more meaningful safety profile of a product than data based on passive monitoring and/or reporting of only those events that an individual investigator deems “related” based upon an individual investigator’s judgment.

### **B. Proposed Initial Studies**

Safety data obtained from the administration of an investigational product to healthy volunteers can be important in identifying common product-associated adverse events before proceeding to studies in more vulnerable populations, e.g., children or those with the disease of interest. Proposed studies evaluating treatment effects should include disease definitions and criteria for worsening, improvement, relapse, etc., applicable to the disease and population studied. For useful sources of information on clinical investigations generally, and clinical studies in pediatric or geriatric populations specifically, you should refer to the appropriate FDA guidance documents, including those prepared under the auspices of ICH at <http://www.fda.gov/RegulatoryInformation/Guidances/ucm122049.htm> (Refs. 11 through 15).

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