

1                   **Chemistry, Manufacturing, and**  
2                   **Control (CMC) Information for**  
3                   **Human Gene Therapy Investigational**  
4                   **New Drug Applications (INDs)**

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7                   **Draft Guidance for Industry**

11                   **This guidance document is for comment purposes only.**

17                   Submit one set of either electronic or written comments on this draft guidance by the date  
18                   provided in the *Federal Register* notice announcing the availability of the draft guidance.  
19                   Submit electronic comments to <https://www.regulations.gov>. Submit written comments to the  
20                   Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm.  
21                   1061, Rockville, MD 20852. You should identify all comments with the docket number listed in  
22                   the notice of availability that publishes in the *Federal Register*.

24                   Additional copies of this guidance are available from the Office of Communication, Outreach  
25                   and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring,  
26                   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  
27                   from the Internet at  
28                   <https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.

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

37                   **U.S. Department of Health and Human Services**  
38                   **Food and Drug Administration**  
39                   **Center for Biologics Evaluation and Research**  
40                   **July 2018**

# Contains Nonbinding Recommendations

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1 **Chemistry, Manufacturing, and Control (CMC) Information for**  
2 **Human Gene Therapy Investigational New Drug Applications**  
3 **(INDs)**  
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6 **Draft Guidance for Industry**  
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9 *This draft guidance, when finalized, will represent the current thinking of the Food and Drug*  
10 *Administration (FDA or Agency) on this topic. It does not establish any rights for any person*  
11 *and is not binding on FDA or the public. You can use an alternative approach if it satisfies the*  
12 *requirements of the applicable statutes and regulations. To discuss an alternative approach,*  
13 *contact the FDA staff responsible for this guidance as listed on the title page.*

14  
15  
16 **I. INTRODUCTION**  
17

18 Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the  
19 biological properties of living cells for therapeutic use. We, the FDA, are providing you,  
20 sponsors of a human gene therapy Investigational New Drug Application (IND),  
21 recommendations regarding chemistry, manufacturing, and control (CMC) information to be  
22 submitted in an IND. The purpose of this draft guidance is to inform sponsors how to provide  
23 sufficient CMC information required to assure product safety, identity, quality, purity, and  
24 strength (including potency) of the investigational product (21 CFR 312.23(a)(7)(i)). This  
25 guidance applies to human gene therapy products and to combination products<sup>1</sup> that contain a  
26 human gene therapy in combination with a drug or device.  
27

28 This draft guidance, when finalized, will supersede the document entitled “Guidance for FDA  
29 Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control  
30 (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs),”  
31 dated April 2008 (April 2008 guidance) (Ref. 1). The field of gene therapy has progressed  
32 rapidly since we issued the April 2008 guidance. Therefore, we are updating that guidance to  
33 provide you with current FDA recommendations regarding the CMC content of a gene therapy  
34 IND. This guidance is organized to follow the structure of the FDA guidance on the Common  
35 Technical Document (CTD). Information on the CTD can be found in the “Guidance for  
36 Industry: M4Q: The CTD – Quality,” dated August 2001 (Ref. 2). For information on the  
37 submission of an electronic CTD (eCTD), please see the FDA website  
38 <https://www.fda.gov/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/ElectronicSubmissions/ucm153574.htm>.  
39

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<sup>1</sup> Combination products are comprised of any combination of a drug and a device; a device and a biological product; a biological product and a drug; or a drug, a device, and a biological product; see 21 CFR 3.2(e) for the complete definition of combination product. Combination products are assigned to a lead center for review; see 21 CFR 3.4.

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40  
41 FDA’s guidance documents, including this guidance, do not establish legally enforceable  
42 responsibilities. Instead, guidance describes the FDA’s current thinking on a topic and should be  
43 viewed only as recommendations unless specific regulatory or statutory requirements are cited.  
44 The use of the word *should* in FDA’s guidance means that something is suggested or  
45 recommended but not required.

46  
47

## 48 **II. BACKGROUND**

49

50 Human gene therapy products are defined as all products that mediate their effects by  
51 transcription or translation of transferred genetic material or by specifically altering host (human)  
52 genetic sequences. Some examples of gene therapy products include nucleic acids, genetically  
53 modified microorganisms (e.g., viruses, bacteria, fungi), engineered site-specific nucleases used  
54 for human genome editing,<sup>2</sup> and ex vivo genetically modified human cells. Gene therapy  
55 products meet the definition of “biological product” in section 351(i) of the Public Health  
56 Service (PHS) Act (42 U.S.C. 262(i)) when such products are applicable to the prevention,  
57 treatment, or cure of a disease or condition of human beings.

58

59 The FDA requires all sponsors of investigational new drug products (DPs), including  
60 investigational gene therapy products, to describe the CMC information for the drug substance  
61 (DS) (21 CFR 312.23(a)(7)(iv)(a)) and the DP (21 CFR 312.23(a)(7)(iv)(b)). FDA may place  
62 the IND on clinical hold if the IND does not contain sufficient CMC information to assess the  
63 risks to subjects in the proposed studies (21 CFR 312.42(b)(1)(iv)).

64

65 The CMC information submitted in an IND is a commitment to perform manufacturing and  
66 testing of the investigational product, as stated. We acknowledge that manufacturing changes  
67 may be necessary as product development proceeds, and you should submit information  
68 amendments to supplement the initial information submitted for the CMC processes (21 CFR  
69 312.23(a)(7)(iii)). The CMC information submitted in the original IND for a Phase 1 study may  
70 be limited, and therefore, the effect of manufacturing changes, even minor changes, on product  
71 safety and quality may not be known. Thus, if a manufacturing change could affect product  
72 safety, identity, quality, purity, potency, or stability, you should submit the manufacturing  
73 change prior to implementation (21 CFR 312.23(a)(7)(iii)).

74

75 We recently published a guidance document, entitled “Providing Regulatory Submissions in  
76 Electronic Format – Certain Human Pharmaceutical Product Applications and Related  
77 Submissions Using the eCTD Specifications; Guidance for Industry,” dated April 2017,  
78 addressing the electronic submission of certain applications in the CTD format (eCTD) (Ref. 3).  
79 Beginning May 5, 2017, all New Drug Applications (NDAs), Abbreviated New Drug  
80 Applications (ANDAs), Biologics License Applications (BLAs), and Master Files must be  
81 submitted in eCTD, and commercial IND submissions must be submitted in eCTD, beginning

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<sup>2</sup> Human Genome Editing: Science, Ethics, and Governance. The National Academies Press; 2017.  
<https://www.nap.edu/read/24623/chapter/1#xvii>

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82 May 5, 2018 (Ref. 3). Excluded from the eCTD requirement are INDs for devices under section  
83 351 of the PHS Act and products that are not intended to be distributed commercially.  
84 Investigator-sponsored INDs and expanded access INDs (e.g., emergency use INDs and  
85 treatment INDs) are also excluded from the eCTD requirement. In preparation for meeting these  
86 requirements, we recommend that sponsors begin to organize and categorize their CMC  
87 information, according to the CTD format.

88  
89 You are not required to complete all CTD sections in your original IND submission. The  
90 amount of CMC information to be submitted in your IND depends on the phase of investigation  
91 (21 CFR 312.23(a)(7)(i)) and the scope of the clinical investigation proposed. The emphasis for  
92 CMC review in all phases of development is product safety and manufacturing control. We  
93 expect that sponsors may need to make modifications to previously submitted information as  
94 clinical development proceeds and additional product knowledge and manufacturing experience  
95 is collected.

96  
97 We are providing detailed recommendations for submitting CMC information in Module 3 of  
98 your IND. We have structured these recommendations to follow the outline of the FDA  
99 “Guidance for Industry: M4Q: The CTD – Quality,” dated August 2001 (Ref. 2). We are also  
100 providing general recommendations regarding administrative and quality summary information  
101 for Modules 1 and 2, respectively, of the CTD structure.

### 102 103 104 **III. ADMINISTRATIVE INFORMATION (MODULE 1 OF THE CTD)**

#### 105 106 **A. Administrative Documents**

107  
108 Administrative documents (e.g., application forms, such as Form FDA 1571, cover  
109 letters, reviewer guides, and cross-reference authorization letters), claims of categorical  
110 exclusion, and labeling information should be included in Module 1 of CTD submissions.  
111 The cover letter of your submission should include a brief explanation of your  
112 submission and its contents. When amendments are submitted to the IND for  
113 manufacturing changes, your cover letter should clearly describe the purpose of the  
114 amendment and highlight proposed changes. For amendments containing numerous or  
115 significant changes, we recommend that you include a “Reviewer’s Guide,” as described  
116 in FDA’s “eCTD Technical Conformance Guide: Technical Specifications Document,”  
117 dated November 2017 (Ref. 4), and that you allow sufficient lead time (e.g., 30 days) for  
118 FDA review before release of a new lot of clinical trial material.

#### 119 120 **B. Labels**

121  
122 Your IND must contain a copy of all labels and labeling to be provided to each  
123 investigator in the clinical study (21 CFR 312.23(a)(7)(iv)(d)). We recommend that you  
124 include sample labels in Module 1 of the CTD. Please note that IND products must bear  
125 a label with the statement, “Caution: New Drug--Limited by Federal (or United States)  
126 law to investigational use” (21 CFR 312.6). For products derived from autologous

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127 donors and other situations described in 21 CFR 1271.90(a) for which a donor eligibility  
128 determination is not required, you must include the required labeling in  
129 21 CFR 1271.90(c), as applicable. For example, for cells intended for autologous use,  
130 you must label the product “FOR AUTOLOGOUS USE ONLY” (21 CFR 1271.90(c)(1))  
131 and “NOT EVALUATED FOR INFECTIOUS SUBSTANCES” if donor testing and  
132 screening is not performed (21 CFR 1271.90(c)(2)).

### 133 **C. Environmental Analysis**

134 Your IND must contain either an environmental analysis or a claim for categorical  
135 exclusion (21 CFR 312.23(a)(7)(iv)(e)). Please note that, under ordinary circumstances,  
136 most INDs are eligible for categorical exclusion under 21 CFR 25.31(e) (Ref. 5). This  
137 information can be submitted in Module 1 of the CTD.  
138

### 139 **D. Previously Submitted Information**

140 For INDs, you generally are not required to resubmit information that you have  
141 previously submitted to the Agency, and you may incorporate such information by  
142 reference. You may submit a written statement in your IND that appropriately identifies  
143 previously submitted information (21 CFR 312.23(b)). We recommend you describe the  
144 information that you are referencing and identify where that information is located in the  
145 previously submitted file.  
146

147 You may also reference information previously submitted by another individual if proper  
148 authorization has been granted. Proper authorization may be granted with a Letter of  
149 Authorization (LOA) from the individual who submitted the information  
150 (21 CFR 312.23(b)). We recommend that the LOA include a description of the  
151 information being cross-referenced (e.g., reagent, container, vector manufacturing  
152 process) and identify where that information is located (e.g., file name, reference number,  
153 volume, page number). Please note that this LOA only allows you to cross-reference the  
154 information outlined in the LOA and submitted by the author of the LOA. The LOA  
155 does not provide you permission to cross-reference information that was submitted by  
156 another individual and cross-referenced by the author of the LOA. In other words, you  
157 may not cross-reference information that is cross-referenced by the author of the LOA.  
158 You are required to submit an LOA for all information submitted by another individual  
159 (21 CFR 312.23(b)).  
160

161 In addition to including LOAs in Module 1 of the CTD, you should list these files in the  
162 IND cover sheet (i.e., Form FDA 1571) of each IND submission. If the LOA is absent or  
163 inadequate or the information in the cross-referenced file is inadequate for the purpose  
164 cited, we will notify you that the information in the cross-referenced file is not sufficient  
165 to support your IND. In the event a cross-referenced IND is placed on clinical hold or is  
166 withdrawn, your IND may also be placed on clinical hold if critical cross-referenced  
167 information is no longer available or adequate.  
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### **IV. SUMMARY OF QUALITY INFORMATION (MODULE 2 OF THE CTD)**

#### **A. General Information**

Your IND should contain a general introduction to the gene therapy product under investigation, including a description of its active ingredient(s), mode of action, and proposed clinical use. This summary should include an overview of the manufacturing process, controls in place to ensure product quality, and general information regarding the qualification of components and starting materials. You should describe the composition of the DS and DP. You should indicate if the DS is formulated into a DP for administration or if the DS is used for ex vivo genetic modification of cells.

Your summary should also include a description of critical quality attributes (CQAs) that are relevant to the safety and biological activity of the product as they are understood at the time of submission. For additional information regarding establishing CQAs, please see Guidance for Industry: “Q8(R2) Pharmaceutical Development,” dated November 2009 (Ref. 6), and “Q11 Development and Manufacture of Drug Substances,” dated November 2012 (Ref. 7). A CQA is defined as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. CQAs apply to DS and DP as well as to excipients and in-process materials. Information to support a CQA and results from specific studies or published literature may be included in Module 3 of the CTD “Pharmaceutical Development” (section 3.2.P.2) (Ref. 2) or linked to the relevant nonclinical or clinical sections of the application in the CTD.

As product development progresses, CQAs may be used to define DS and DP specifications. Understanding and defining product characteristics that are relevant to the clinical performance of the gene therapy may be challenging, particularly during early stages of product development. Therefore, we recommend that you evaluate a number of product characteristics during early clinical development to help you identify and understand the CQAs of your product. This will also help ensure your ability to assess manufacturing process controls, manufacturing consistency, and product stability as product development advances. This is especially important for sponsors of gene therapy products who are pursuing expedited product development programs (Ref. 8).

#### **B. Drug Substance and Drug Product**

Your IND must contain a description of the DS (21 CFR 312.23(a)(7)(iv)(a)) and DP (21 CFR 312.23(a)(7)(iv)(b)), including the physical, chemical, or biological characteristics, manufacturing controls, and testing information, to ensure the DS and DP meet acceptable limits for identity, strength (potency), quality, and purity. For the purpose of this guidance, a DS is defined as an active ingredient that is intended to furnish biological activity or other direct effect in the diagnosis, cure, mitigation,

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216 treatment, or prevention of disease or to affect the structure or any function of the human  
217 body. Further, a DP is defined as the finished dosage form that contains the DS,  
218 generally, but not necessarily in association with one or more other ingredients (e.g.,  
219 excipients).

220  
221 We recognize that distinguishing a DS from a DP may be difficult for some gene therapy  
222 products, due to the complex nature of the manufacturing processes. Some gene therapy  
223 products may not have defined DS. Others may consist of two or more different DSs that  
224 are combined to make the DP. This guidance does not recommend how sponsors should  
225 distinguish the DS and DP. However, we do recommend that you provide an explanation  
226 to support your DS/DP distinction in the summary information in Module 2 of CTD  
227 submissions and that you submit the required information for each DS and DP, as  
228 outlined in Module 3 of the CTD (Ref. 2).

229  
230 When the manufacturing process includes more than one DS, we recommend that you  
231 provide separate DS sections for each active ingredient of the final product. The CTD  
232 DS sections should follow the format and numbering scheme recommended in Module 3  
233 of FDA “Guidance for Industry: M4Q: The CTD – Quality,” dated August 2001 (Ref.  
234 2), and the sections should be distinguished from one another by including the DS name  
235 and manufacturer in the heading (e.g., section 3.2.S.1 General Information [name,  
236 manufacturer]).

237  
238 A summary of the available stability data for the DS and the DP, recommended storage  
239 conditions, and tentative expiry date, if applicable, should also be included in this section.  
240 Information on stability protocols and stability data should be included in the appropriate  
241 sections of Module 3.

### 242 243 **C. Combination Products**

244  
245 For submissions in which the gene therapy is a component of a combination product, as  
246 defined in 21 CFR 3.2(e), we recommend that you briefly describe the combination  
247 product in the summary of your product and briefly state the regulatory status of each  
248 component. To clearly delineate the different components of a combination product, you  
249 should include manufacturing and engineering information for the gene therapy and drug  
250 or device in separate entries of the CTD submission, as described in the FDA “eCTD  
251 Technical Conformance Guide: Technical Specifications Document,” dated November  
252 2017 (Ref. 4).

### 253 254 **D. Product Handling at the Clinical Site**

255  
256 Proper control of the finished DP is critical to your investigational studies. Therefore,  
257 your IND should also include a description of how the product will be shipped to,  
258 received, and handled at the clinical site to ensure safety, product quality, and stability.  
259 Your IND should also include information on shipping conditions, storage conditions,  
260 expiration date/time (if applicable), and chain of custody from the manufacturer to the



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261 site of administration in the summary information of the CTD. Your summary in Module  
262 2 should also include information for product handling at the clinical site prior to  
263 administration (such as thawing, washing, or the addition of diluent or adjuvant, loading  
264 into a delivery device, and transport to the bedside) and summary information on product  
265 stability prior to and during administration (e.g., in-device hold times and temperatures).  
266

267 Details regarding product stability after preparation for delivery and delivery device  
268 compatibility data should be included in Module 3 (sections 3.2.P.8 and 3.2.P.2.6,  
269 respectively) of the CTD (Ref. 2). Instructions for drug handling and preparation for  
270 administration at the clinical site (e.g., Pharmacy Manual or Instructions for Use) should  
271 be provided in the “Clinical Study Reports” section of your IND (section 5.3 of the FDA  
272 “M4E(R2): The CTD – Efficacy; Guidance for Industry,” dated July 2017 (Ref. 9)).  
273 Detailed information about the delivery device may be included in “Regional  
274 Information” (section 3.2.R of the CTD) (Ref. 2).  
275  
276

### 277 **V. MANUFACTURING PROCESS AND CONTROL INFORMATION (MODULE 3** 278 **OF THE CTD)**

279 The headings and text below include CTD section numbers in parentheses for reference (Ref. 2).  
280

#### 281 **A. Drug Substance (3.2.S)**

##### 282 1. General Information (3.2.S.1)

##### 283 a. Nomenclature (3.2.S.1.1)

284 You should provide the name of the DS(s). If the name of the DS has  
285 changed during clinical development, you should provide the names used  
286 to identify the DS at all stages of development. If the United States  
287 Adopted Name (USAN) Council has given it a nonproprietary name, you  
288 may provide it here.  
289

##### 290 b. Structure (3.2.S.1.2)

291 You should submit information on the molecular structure (including  
292 genetic sequence) and/or cellular components of the DS. The genetic  
293 sequence can be represented in a schematic diagram that includes a map of  
294 relevant regulatory elements (e.g., promoter/enhancer, introns, poly(A)  
295 signal), restriction enzyme sites, and functional components (e.g.,  
296 transgene, selection markers). Please note that you should also submit  
297 information on your sequence analysis and the annotated sequence data in  
298 your IND. We recommend that your sequence data, including any data  
299 collected to support the genetic stability of your vector, be submitted in  
300 “Elucidation of Structure and other Characteristics” (section 3.2.S.3.1 of  
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306 the CTD). More information on our recommendations for sequence  
307 analysis is described in “Control of Materials (3.2.S.2.3)” (section  
308 V.A.2.c. of this guidance).

309  
310 Some examples of additional information for structure and structural  
311 elements of different gene therapy products are outlined below:

- 312  
313 • For viral vectors, you should include a description of the  
314 composition of the viral capsid and envelope structures, as  
315 appropriate, and any modifications to these structures (e.g.,  
316 modifications to antibody binding sites or tropism-changing  
317 elements). We recommend that you include biophysical  
318 characteristics (e.g., molecular weight, particle size) and  
319 biochemical characteristics (e.g., glycosylation sites). You should  
320 also describe the nature of the genome of viral vectors, whether  
321 single-stranded, double-stranded, or self-complementary, DNA or  
322 RNA, and copy number of genomes per particle.
- 323  
324 • For bacterial vectors, you should include defining physical and  
325 biochemical properties, growth characteristics, genetic markers  
326 (e.g., auxotrophic or attenuating mutations, antibiotic resistance)  
327 and the location (e.g., on plasmid, episome, or chromosome) and  
328 description of any inserted foreign genes and regulatory elements.  
329 For additional details on microbial vectors, please see the FDA’s  
330 Guidance for Industry “Recommendations for Microbial Vectors  
331 used for Gene Therapy,” dated September 2016 (Ref. 10).
- 332  
333 • For ex vivo genetically modified cells, you should describe the  
334 expected major and minor cell populations as well as the vector  
335 that contains the transgene cassette that is transferred into the cell.  
336 For cells that have been genetically modified using genome  
337 editing, you should describe the gene(s) that are altered and how  
338 the change(s) was made (i.e., the gene editing technology used).

339  
340 c. General Properties (3.2.S.1.3)

341  
342 You should provide a section in the IND that describes the composition  
343 and properties of the DS, including the biological activity and proposed  
344 mechanisms of action.

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350 2. Drug Substance Manufacture (3.2.S.2)

351  
352 a. Manufacturer(s) (3.2.S.2.1)

353  
354 You must provide the name and address of each manufacturer, including  
355 contract manufacturer(s), involved in the manufacture, testing, and storage  
356 of the DS (21 CFR 312.23(a)(7)(iv)(a)). You should indicate the  
357 responsibility of each manufacturer. Your IND should contain complete  
358 information on the DS manufacturer, regardless of whether the process is  
359 performed by you or by a contract manufacturing organization (CMO).  
360 As the sponsor of the IND, you are ultimately responsible for the safety of  
361 subjects in the clinical investigation (21 CFR 312.3); therefore, we  
362 recommend that you and the CMO understand and document your  
363 respective responsibilities for ensuring product quality. Additional  
364 information on quality agreements can be found in FDA’s Guidance for  
365 Industry, “Contract Manufacturing Arrangements for Drugs: Quality  
366 Agreements,” dated November 2016 (Ref. 11).

367  
368 b. Description of Manufacturing Process and Process Controls  
369 (3.2.S.2.2)

370  
371 Your description of the DS manufacturing process and process controls  
372 should include all of the following, as applicable: cell culture;  
373 transduction; cell expansion; harvest(s); purification; filling; and storage  
374 and shipping conditions. Your description should also accurately  
375 represent your process and process controls. Changes and updates to this  
376 information should be submitted as an amendment to the IND prior to  
377 implementation (21 CFR 312.23(a)(7)(iii)), as indicated in section II.  
378 Background of this guidance.

379  
380 i. Batch and Scale

381  
382 A description of how you define each manufacturing run (i.e.,  
383 batch, lot, other) should be submitted with an explanation of the  
384 batch (or lot<sup>3</sup>) numbering system. You should clearly state  
385 whether any pooling of harvests or intermediates occurs during  
386 manufacturing. If pooling is necessary during production, we  
387 recommend that you control the storage conditions (e.g., time,  
388 temperature, container) for each pool and that you describe the  
389 testing that is performed prior to pooling to ensure the quality of  
390 each pool.  
391

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<sup>3</sup> For purpose of this guidance, batch and lot are used interchangeably.

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392 We also recommend that you provide an explanation for how the  
393 batch scale is defined (e.g., bioreactor volume, cell processing  
394 capacity) and how the DS is quantified (e.g., vector genomes,  
395 transducing units, infectious particles, mass, number of gene  
396 modified cells). When known, please include the yield expected  
397 per batch.

#### ii. Manufacturing Process

400  
401 The description of your manufacturing process should include a  
402 flow diagram(s) and a detailed narrative. Your description should  
403 clearly identify any process controls and in-process testing (e.g.,  
404 titer, bioburden, viability, impurities) as well as acceptable  
405 operating parameters (e.g., process times, temperature ranges, cell  
406 passage number, pH, CO<sub>2</sub>, dissolved O<sub>2</sub>, glucose level).

407  
408 We recommend the evaluation of operating parameters on a  
409 periodic basis to ensure process control and allow for trending and  
410 statistical analyses if deemed appropriate to monitor process  
411 consistency. You should clearly describe any environmental  
412 controls as well as tracking and segregation procedures that are in  
413 place to prevent cross-contamination throughout the manufacturing  
414 process.

#### iii. Cell Culture

415  
416 The description of all cell culture conditions should contain  
417 sufficient detail to make understandable any of the process steps  
418 that apply, process timing, culture conditions, hold times and  
419 transfer steps, and materials used (e.g., media components,  
420 bags/flasks). You should describe whether the cell culture system  
421 is open or closed and any aseptic processing steps. If extensive  
422 culture times are needed, you should outline the in-process controls  
423 you have in place to monitor cell quality (e.g., viability, bioburden,  
424 pH, dissolved O<sub>2</sub>). Expectations for media components and cell  
425 bank qualification are outlined in this guidance under “Control of  
426 Materials (3.2.S.2.3)” (section V.A.2.c. of this guidance).

#### iv. Vector Production

427  
428  
429  
430 For the manufacture of gene therapy vectors (e.g., viral vectors,  
431 bacterial plasmids, mRNA), you should provide a description of all  
432 production and purification procedures. Production procedures  
433 should include a description of the cell substrate, cell culture and  
434 expansion steps, transfection or infection procedures, harvest steps,  
435  
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437 hold times, vector purification (e.g., centrifugation, column  
438 purification, density gradients), concentration or buffer exchange  
439 steps, and the reagents/components used during these processes.  
440 You should outline any in-process testing to ensure vector quality  
441 as appropriate (e.g., titer, impurities).  
442

443 You should describe whether the DS will be formulated into the  
444 DP for direct administration or whether it will be formulated for ex  
445 vivo genetic modification of cells, as outlined in section IV.B. As  
446 an active ingredient, the same level of control should be applied to  
447 each DS, and each DS should be manufactured under appropriate  
448 Good Manufacturing Practice (GMP) conditions. For more  
449 information on your Quality Unit and GMP manufacturing, see  
450 “Process Validation and/or Evaluation (3.2.S.2.5)” (section  
451 V.A.2.e. of this guidance).  
452

#### v. Genetically Modified Cell Production

453  
454  
455 If your product consists of genetically modified cells, your cell  
456 processing description should contain sufficient detail to make  
457 understandable any of the following process steps that apply:  
458 source material (e.g., autologous or allogeneic cells); collection of  
459 cellular source material (e.g., leukapheresis, biopsy); storage at the  
460 collection site; shipping to and handling at the manufacturing  
461 facility; cell selection, isolation, or enrichment steps (including  
462 methods, devices, reagents); cell expansion conditions; hold times  
463 and transfer steps; and cell harvest, purification, if any, and  
464 materials used.  
465

466 You should also provide a complete description of all procedures  
467 used for gene modification (such as transfection, infection or  
468 electroporation of vectors, or genome editing components) and any  
469 additional culture, cell selection, or treatments after modification.  
470

#### vi. Irradiated Cells

471  
472  
473 If your product contains or is processed with irradiated cells, you  
474 should provide documentation for the calibration of the irradiator  
475 source and provide supporting data to demonstrate that the  
476 irradiated cells are rendered replication-incompetent, while still  
477 maintaining their desired characteristics.  
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#### vii. Filling, Storage, and Transportation (Shipping)

You should provide a detailed description and identify any associated process controls for formulation, filling, storage, and shipping of the DS, if applicable. You should also describe the container used for storage and shipping of the DS. We recommend that you describe procedures that are in place to ensure appropriate storage and transport (as needed).

#### c. Control of Materials (3.2.S.2.3)

You must provide a list of all materials used in manufacturing (21 CFR 312.23(a)(7)(iv)(b)) and a description of the quality and control of these materials. This information may be provided in tabular format and include the identity of the material, the supplier, the quality (e.g., clinical-grade, FDA-approved), the source of material (e.g., animal, human, insect), and the stage at which each material is used in the manufacturing process (e.g., culture media, vector purification). This includes information on components, such as cells, cell and viral banking systems, and reagents, as described in more detail below; it also includes raw materials and equipment, such as culture bags, culture flasks, chromatography matrices, and tubing, that come into contact with the product.

You should provide documentation that the materials used for manufacturing meet standards appropriate for their intended use (e.g., test results, certificates of analysis (COAs), package inserts). COAs for materials can be provided in “Facilities and Equipment” (section 3.2.A.1 of the CTD) and hyperlinked to relevant sections of your IND. We recommend that you use FDA-approved or cleared or other clinical-grade materials, when they are available. If the material is not FDA-approved or cleared (or in the absence of recognized standards), additional information on the manufacturing and/or testing may be needed to evaluate the safety and quality of the material. The extent of testing will depend on the specific material and the manner in which it is used in the manufacturing process.

#### i. Reagents

For purpose of this guidance, reagents (or ancillary materials) are those materials used for manufacturing (e.g., cell growth, differentiation, selection, purification, or other critical manufacturing steps) that are not intended to be part of the final product. Examples include fetal bovine serum, digestive enzymes (e.g., trypsin, collagenase, DNase/RNase, restriction

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527 endonucleases), growth factors, cytokines, monoclonal antibodies,  
528 antibody-coated beads, antibiotics, media, media components, and  
529 detergents. These reagents can affect the safety, potency, and  
530 purity of the final product, especially by introducing adventitious  
531 agents or other impurities.

532  
533 For biologically sourced reagents, including human, bovine, and  
534 porcine-derived materials, we recommend that you refer to the  
535 FDA Guidance for Industry: “Characterization and Qualification  
536 of Cell Substrates and Other Biological Materials Used in the  
537 Production of Viral Vaccines for Infectious Disease Indications,”  
538 dated February 2010 (Ref. 12). Animal-derived materials increase  
539 the risk of introducing adventitious agents. Certain animal-derived  
540 materials, such as sera, are complex mixtures that are difficult to  
541 standardize, and such materials may have significant batch-to-  
542 batch variations that may affect the reproducibility of your  
543 manufacturing process or the quality of your final product. We  
544 recommend that you use non-animal-derived reagents whenever  
545 possible (for example, serum-free tissue culture media and  
546 recombinant proteases).

547  
548 ii. Bovine

549  
550 We recommend that you provide information on any bovine  
551 material used in manufacturing, including the source of the  
552 material; information on the location where the herd was born,  
553 raised, and slaughtered; and any other information relevant to the  
554 risk of transmissible spongiform encephalopathy (TSE). If serum  
555 is used, we recommend that it be  $\gamma$ -irradiated to reduce the risk of  
556 adventitious agents.

557  
558 Bovine materials used in production of reagents, which are, in turn,  
559 used in manufacturing a product, should also be identified, and the  
560 source and qualification of bovine material should be documented.

561  
562 You should provide COAs for all bovine material lots used in the  
563 manufacture and establishment of cell and virus banks to document  
564 that these materials are compliant with the requirements for the  
565 ingredients of animal origin used for production of biologics  
566 described in 9 CFR 113.53.

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#### iii. Porcine

You should provide COAs for all porcine material lots used in manufacture and establishment of cell and virus banks to document that these materials are compliant with the requirements for the ingredients of animal origin used for production of biologics described in 9 CFR 113.53. In addition, porcine reagents should be tested for porcine circovirus (PCV) 1 and 2 and porcine parvovirus.

#### iv. Murine or Monoclonal Antibodies

Monoclonal antibodies used in manufacturing that have product contact should be tested as per the recommendations described in the FDA “Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use,” dated February 1997 (Ref. 13). Alternatively, you may provide a letter of authorization to cross-reference this information in a different regulatory submission (IND or MF). You should also consider that many monoclonal antibodies and recombinant proteins (such as cytokines) used during the manufacture of gene therapy products may be purified by affinity chromatography, using antibodies generated from mouse hybridomas. This may introduce the risk of contamination with adventitious agents from rodents.

#### v. Human Source

If human albumin is used, you should use FDA-approved products and have procedures in place to ensure that no recalled lots were used during manufacture or preparation of the product.

If human AB serum is used (e.g., for ex vivo genetically modified cells), you should ensure the serum is processed from blood or plasma collected at FDA licensed facilities. Source Plasma, which is often used to make human AB serum, must be collected as described in 21 CFR Part 640, Subpart G. Source Plasma is not tested as extensively as blood products intended for infusion, and we recommend that you ensure the AB serum used in your manufacturing does not have the potential to transmit infectious disease. For example, if your serum is derived from Source Plasma, you may reduce the risk of infectious disease by conducting additional testing for relevant transfusion-transmitted infections. Alternatively, including viral inactivation or clearance steps in the production of AB serum from Source Plasma may be an acceptable alternative.



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617  
618 For all other reagents that are human-derived, you should identify  
619 whether the reagent is a licensed product (e.g., HSA, IL-2) or is  
620 clinical or research grade and provide a COA or information  
621 regarding testing of the donor or reagent.

622  
623 vi. Cells - Autologous and Allogeneic Cells or Tissue

624  
625 For autologous or allogeneic cells or tissue, you should provide a  
626 detailed description of the cell source, the collection procedure,  
627 and any related handling, culturing, storage, and testing that is  
628 performed prior to use in manufacture. Your description should  
629 include the following information:

- 630
- 631 • materials used for collection (including devices, reagents,  
632 tubing, and containers);
  - 633
  - 634 • method of cell collection (i.e., standard blood draw or  
635 apheresis);
  - 636
  - 637 • enrichment steps, if performed;
  - 638
  - 639 • labeling and tracking of collected samples;
  - 640
  - 641 • hold times; and
  - 642
  - 643 • transportation conditions to the manufacturing facility.
  - 644

645 As an example, for cells collected by leukapheresis: you should  
646 provide a detailed description of the collection device(s); operating  
647 parameters; volumes or number of cells to be collected; and how  
648 the collected material is labeled, stored, tracked, and transported to  
649 the manufacturing facility.

650  
651 For multi-center clinical trials, establishing standardized  
652 procedures for cell collection and handling across all collection  
653 sites is critical to assuring the quality and safety of the final  
654 product as well as ensuring control of the manufacturing process.  
655 In your IND, you should include a list of collection sites, their  
656 FDA Establishment Identifier, and any accreditations for  
657 compliance with established standards (e.g., Foundation for the  
658 Accreditation of Cellular Therapy (FACT)), if applicable.

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#### A. Autologous Cells

You are not required to make a donor eligibility determination or to perform donor screening on autologous cells or tissues (21 CFR 1271.90(a)(1)). However, you should determine whether your manufacturing procedures increase the risk to the patient by further propagation of pathogenic agents that may be present in the donor. You should also describe precautions to prevent the spread of viruses or other adventitious agents to persons other than the autologous recipient (Ref. 14).

#### B. Allogeneic Cells

For allogeneic cells or tissues, you must perform donor screening and testing, as required in 21 CFR Part 1271, Subpart C, except for those cells and tissues that meet the exceptions in 21 CFR 1271.90(a). Donors of all types of cells and tissues must be screened for risk factors and clinical evidence of relevant communicable disease agents and diseases, including: human immunodeficiency virus (HIV); hepatitis B virus (HBV); hepatitis C virus (HCV); human TSE, including Creutzfeldt-Jakob disease; and *Treponema pallidum* (syphilis) (21 CFR 1271.75). In addition, donors of viable leukocyte-rich cells or tissues should be screened for human T-lymphotropic virus (HTLV). You must also test a specimen of donor cells or tissue for evidence of infection due to relevant communicable disease agents, including: HIV-1; HIV-2; HBV; HCV; syphilis; and if the material is leukocyte-rich cells or tissue, HTLV-1, HTLV-2, and cytomegalovirus (21 CFR 1271.85). For donor eligibility testing, you must use appropriate FDA-licensed, approved, or cleared donor screening tests (21 CFR 1271.80(c)). You should also refer to recent Center for Biologics Evaluation and Research (CBER) guidance documents on donor eligibility for additional information on testing for emerging relevant communicable disease agents and diseases (e.g., West Nile virus (WNV), Zika virus). If cord blood or other maternally-derived tissue is used, you must perform screening and testing on the birth mothers, as described in 21 CFR 1271.80(a).

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706 Allogeneic cells from a single donor or source tissue may  
707 sometimes be expanded and stored for greater consistency  
708 and control in manufacturing. In these situations, we  
709 generally recommend that you qualify allogeneic master  
710 and working cell banks in the same way as cell banks used  
711 for production of viral vectors (see “Banking Systems,”  
712 below), provided that you have sufficient material for this  
713 testing. In these situations, we are most concerned about  
714 the introduction of adventitious agents (e.g., viruses,  
715 bacteria, mycoplasma) during the bank manufacturing  
716 process, especially from any bovine or porcine materials,  
717 animal feeder cells, other animal-derived reagents, or  
718 human AB serum, if used. If your allogeneic cell bank is  
719 small, we may recommend abbreviated cell bank  
720 qualification. In this case, please consult with the Quality  
721 Reviewer of your file for more information on appropriate  
722 qualification of small scale allogeneic cell banks.  
723

#### vii. Banking Systems (Starting Materials)

724  
725  
726 A banking system improves control and consistency in the  
727 manufacturing of many biologics. Banking assures an adequate  
728 supply of equivalent, well-characterized material for production  
729 over the expected lifetime of production. For these reasons,  
730 banked materials are a common starting point for many routine  
731 production applications. We outline our current thinking for the  
732 qualification of different banking systems below, including banks  
733 of cell substrates for production of viral vectors, banks of  
734 bacterial/microbial cells, and banks of viral vectors. We  
735 recommend that you provide a summary of the testing and COAs  
736 in this section. Information on bank qualification and adventitious  
737 agent testing should also be included in your comprehensive  
738 “Adventitious Agents Safety Evaluation” (section 3.2.A.2 of the  
739 CTD).  
740

#### viii. Master Cell Banks Used as Substrates for Production of Viral Vectors

741  
742  
743  
744 Prior to selecting a cell line for viral vector manufacturing, you  
745 should carefully consider characteristics of the cells that may  
746 impact the safety of the final product (such as presence of  
747 tumorigenic sequences). This is especially important when the  
748 viral vector co-packages non-vector sequences, such as adeno-  
749 associated virus (AAV) (see “Impurities (3.2.S.3.2)” section  
750 V.A.3.b. of this guidance). We also recommend that you consider

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751 cell attributes that can affect production capacity (e.g., growth  
752 characteristics, vector production capacity), prior to generation of a  
753 cell bank.

754  
755 In your IND, you should provide a description of the history and  
756 detailed derivation of the source material for the cell bank. Your  
757 description should include information on cell source (including  
758 species of origin); how the bank was generated (e.g., from a single  
759 colony isolate or through limiting dilution); testing performed to  
760 characterize the bank; and if applicable, materials used to  
761 genetically modify the source material (i.e., packaging cell line).

762  
763 When a cell substrate has been genetically modified (for example,  
764 to provide viral proteins to allow virus replication or packaging),  
765 you should provide a description of the materials used for the  
766 genetic modification, including information on the quality and  
767 control of the vectors used to introduce the genetic changes.  
768 Materials used to manufacture process intermediates should be  
769 sufficiently characterized to ensure safety and purity of the final  
770 gene therapy product. For more information regarding plasmid  
771 intermediates that are used for further manufacture, please see  
772 “Control of Critical Steps and Intermediates (3.2.S.2.4)” (section  
773 V.A.2.d. of this guidance).

774  
775 For the banked material, itself, we recommend that you provide  
776 information on how the cell banks are stored and maintained as  
777 well as detailed information on qualification to adequately  
778 establish the safety, identity, purity, and stability of the cells used  
779 in your manufacturing process. Additional sources of information  
780 regarding qualification of cell substrates can be found in the FDA  
781 guidance “Q5D Quality of Biotechnological/Biological Products:  
782 Derivation and Characterization of Cell Substrates Used for  
783 Production of Biotechnological/Biological Products” (63 FR  
784 50244, September 21, 1998) (Ref. 15) and FDA’s Guidance for  
785 Industry: “Characterization and Qualification of Cell Substrates  
786 and Other Biological Materials Used in the Production of Viral  
787 Vaccines for Infectious Disease Indications,” dated February 2010  
788 (Ref. 12).

789  
790 Cell bank qualification includes tests to:

- 791
- 792 • Ensure absence of microbial contamination, including  
793 sterility, mycoplasma (and spiroplasma for insect cells),  
794 and in vivo and in vitro testing for adventitious viral agents.  
795 For cell lines used for production of vectors, we

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796 recommend that you test for retroviral contamination, using  
797 reverse transcriptase (RT) assays and transmission electron  
798 microscopic (TEM) analysis. The presence of an  
799 adventitious viral agent in your bank should be vigorously  
800 investigated, and re-derivation of the bank should be  
801 considered.

802  
803 - For additional information on the analytical  
804 methods used for cell bank qualification, please see  
805 “Analytical Procedures (3.2.S.4.2)” (section  
806 V.A.4.b. of this guidance).

807  
808 - For cell lines that have been exposed to bovine or  
809 porcine components (e.g., serum, serum  
810 components, trypsin), appropriate testing would  
811 include testing for bovine or porcine adventitious  
812 agents. See further discussion on bovine and  
813 porcine reagents, above.

814  
815 • Ensure absence of species-specific pathogens.

816  
817 - For human cells, this may include testing for  
818 cytomegalovirus (CMV), HIV-1 & 2, HTLV-1 &-2,  
819 human herpesvirus-6 and -8 (HHV-6 & -8), JC  
820 virus, BK virus, Epstein-Barr virus (EBV), human  
821 parvovirus B19, HBV, human papillomavirus  
822 (HPV), and HCV, as appropriate.

823  
824 - For other animal or insect cells, we recommend  
825 tests for species-specific viruses, as appropriate.  
826 For instance, for Vero cells, we recommend testing  
827 for simian polyomavirus SV40 and simian  
828 retrovirus.

829  
830 - For insect cells, you may evaluate the presence of  
831 arboviruses in a susceptible cell line, such as baby  
832 hamster kidney (BHK21) cells. Insect cell lines  
833 with known viral contamination should be avoided.

834  
835 • Identify cells. Identify your cells through tests that  
836 distinguish them from other cell lines used in your facility.  
837 For cell lines that you have purchased from a type  
838 collection or received from another investigator, we  
839 recommend master cell bank (MCB) testing to confirm the

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- 840 purity of the cells by genetic analysis (i.e., short tandem  
841 repeat analysis or other profiling analysis).<sup>4</sup>  
842  
843 • Establish stability of the cell bank. Stability can be  
844 assessed by measuring viability of cells over time after  
845 cryopreservation. We also recommend a one-time test of  
846 end of production cells (EOP) or mock production cells of  
847 similar passage history, to be tested for their suitability to  
848 produce your vector. For stable retroviral vector producer  
849 cells, we recommend that you test the genetic stability of  
850 the gene insert in the EOP cells.  
851  
852 • Assess the ability of new cell lines to form tumors. We  
853 recommend that you perform tumorigenicity tests for cell  
854 lines that have not been previously characterized for their  
855 potential to form tumors. This test would not be necessary  
856 for cells known to form tumors; please see additional  
857 information on testing for process-related impurities under  
858 “Drug Substance Characterization (3.2.S.3)” (section  
859 V.A.3.b.i. of this guidance).

#### ix. Working Cell Banks

860  
861  
862 A Working Cell Bank (WCB) may be derived from one or more  
863 vials of the MCB. The information needed to document  
864 qualification and characterization for a WCB is less extensive than  
865 that needed for the MCB. WCB testing should include but is not  
866 limited to sterility, mycoplasma, identity, and in vitro adventitious  
867 agent tests. For additional information on the analytical methods  
868 used for WCB qualification, please see “Analytical Procedures  
869 (3.2.S.4.2)” (section V.A.4.b. of this guidance).  
870

#### x. Bacterial or Microbial Master Cell Banks

871  
872  
873 For all bacterial or microbial (e.g., yeast) MCBs, you should  
874 describe the genotype and source of the microbial cells. Bacterial  
875 MCBs are frequently used as a starting material to generate  
876 plasmid DNA, which can be used as a vector for gene transfer or as  
877 a manufacturing intermediate for other gene therapy products, such  
878 as the AAV or lentiviral vectors. Microbial MCBs also may be  
879 used to generate a microbial vector for gene therapy. You should  
880

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<sup>4</sup> Reid Y, Storts D, Riss T, Minor L. Authentication of Human Cell Lines by STR DNA Profiling Analysis. In: Sittampalam GS, Coussens NP, Brimacombe K. et al., editors. Assay Guidance Manual. Bethesda (MD): Eli Lilly & Company and the [National Center for Advancing Translational Sciences](https://www.ncbi.nlm.nih.gov/books/NBK144066/); 2004. <https://www.ncbi.nlm.nih.gov/books/NBK144066/>.

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881 provide a detailed description of the history and derivation of the  
882 materials used to generate the cell bank, including information on  
883 how plasmid vectors were designed and constructed. For the bank  
884 material, itself, you should provide information on how the  
885 material was generated and how the bank is stored and maintained  
886 as well as detailed information on qualification of the bank  
887 (including cell bank COAs) to adequately establish the safety,  
888 identity, purity, and stability of the microbial cell preparation used  
889 in the manufacturing process.

890  
891 For bacterial cell banks used to manufacture a DNA plasmid, we  
892 recommend MCB testing include:

- 893 • Bacterial host strain identity;
- 894 • Plasmid presence, confirmed by bacterial growth on  
895 selective medium, restriction digest, or DNA sequencing;
- 896 • Bacterial cell count;
- 897 • Bacterial host strain purity (no inappropriate organisms,  
898 negative for bacteriophage);
- 899 • Plasmid identity by restriction enzyme (RE) analysis;
- 900 • Full plasmid sequencing. We recommend that you fully  
901 sequence plasmid vectors and submit an annotated  
902 sequence for the vector, as described in more detail in the  
903 section below on viral vector banks; and
- 904 • Transgene expression and/or activity.

905  
906 For microbial cell banks used to manufacture a microbial vector,  
907 our recommendations for MCB testing are outlined in the  
908 Guidance for Industry, “Recommendations for Microbial Vectors  
909 used for Gene Therapy,” dated September 2016 (Ref. 10).

#### 910 xi. Master Viral Banks

911  
912  
913 Viral banks may be expanded for viral vector production, or they  
914 may be used as helper viruses for manufacturing non-replicating  
915 vectors (e.g., AAV or gutless adenovirus). You should provide a  
916 detailed description of the history and derivation of the source or  
917  
918  
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925 seed materials for these banks. You should describe how the seed  
926 stock was generated and what cells and animal-derived materials  
927 were used in the derivation process.

928  
929 A gene map of the final vector and vector intermediates is useful  
930 when describing the history and derivation of recombinant viral  
931 vectors. We recommend that you state whether the seed material  
932 was plaque-purified, purified by limiting dilution, or rescued from  
933 DNA or RNA clones and how many times it was passaged, during  
934 expansion.

935  
936 For the banked material, itself, you should describe the  
937 manufacturing process and the conditions under which the banked  
938 material was generated, for example, in a research laboratory or a  
939 GMP facility. We recommend that you list animal-derived  
940 materials used in the generation of the bank and state whether the  
941 master virus bank (MVB) is expected to represent a single clone or  
942 a distribution of viral variants or sequences.

943  
944 We also recommend that you provide information on how the bank  
945 is stored and maintained as well as detailed information on the  
946 qualification of the bank to adequately establish the safety,  
947 identity, purity, and stability of the virus preparation used in the  
948 manufacturing process. If a COA is available, it should be  
949 submitted to the IND. For additional information on the analytical  
950 methods used for MVB qualification, please see “Analytical  
951 Procedures (3.2.S.4.2)” (section V.A.4.b. of this guidance).

952  
953 Viral vector bank qualification includes tests to:

- 954  
955 • Ensure absence of contamination, including sterility,  
956 mycoplasma, and in vivo and in vitro testing for  
957 adventitious viral agents.
- 958  
959 • Ensure absence of specific pathogens that may originate  
960 from the cell substrate, such as human viruses if the cell  
961 line used to produce the MVB is of human origin, or  
962 pathogens specific to the origin of the production cell line  
963 (e.g., murine, non-human primate, avian, insect).
- 964  
965 • Ensure absence of replication competent virus in  
966 replication incompetent vectors.
- 967  
968 • Ensure viral titer or concentration.
- 969



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- Ensure sensitivity to anti-viral drugs, as applicable, for example, herpes simplex virus (HSV) sensitivity to ganciclovir.
  - Ensure transgene activity, if appropriate.
  - Identify the viral vector and therapeutic transgene (e.g., Southern blot or restriction endonuclease analysis), as needed.
  - Ensure the correct genetic sequence. We recommend that you fully sequence all vectors that are 40 kb or smaller, analyze the sequence, and submit an annotated sequence of the entire vector. You should provide an evaluation of the significance of all discrepancies between the expected sequence and the experimentally determined sequence and an evaluation of the significance of any unexpected sequence elements, including open reading frames. We have the following recommendations, regarding sequence analysis:
    - We recommend that viral vectors be sequenced from the MVB, when possible.
    - For integrating viral vectors, we recommend that you perform DNA sequencing on the integrated vector. The material for sequencing can be collected from the producer cell line or, in the case of vectors generated by transient transfection, from material collected from cells that you have transduced after isolation of a vector lot.
    - For other situations in which no MVB exists, sequencing should be performed from the DS or DP. For example, AAV vectors are typically made by plasmid transfection, and the AAV vector is harvested directly from transfected cells to produce a DS. In this situation, we recommend that you sequence one or more lots (either material from DS or DP) to confirm that the vector sequence is stable, during manufacturing.
    - For viral vectors greater than 40 kb, you should summarize the extent and results of sequence analysis that you have performed, including any

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1015 testing performed by restriction endonuclease  
1016 analysis. You should perform sequence analysis of  
1017 the gene insert, flanking regions, and any regions of  
1018 the vector that are modified or could be susceptible  
1019 to recombination. The entire vector sequence will  
1020 be necessary to confirm identity for licensure.

1021  
1022 xii. Working Viral Banks

1023  
1024 A working viral bank (WVB) may be derived from one or more  
1025 vials of the MVB, and the information needed to document  
1026 qualification and characterization of the WVB is less extensive  
1027 than that needed for the MVB. You should describe the process  
1028 used to generate the WVB and whether animal-derived materials  
1029 were used. Testing for WVB should include but is not limited to  
1030 sterility, mycoplasma, identity, and in vitro adventitious agent  
1031 tests.

1032  
1033 d. Control of Critical Steps and Intermediates (3.2.S.2.4)

1034  
1035 You should describe the control of critical steps and intermediates in the  
1036 manufacturing process. Critical control steps include those outlined in the  
1037 “Description of Manufacturing Process and Process Controls” (section  
1038 3.2.S.2.2 of the CTD and section V.A.2.b. of this guidance). We  
1039 recommend that you also consider any steps in which in-process tests with  
1040 acceptance criteria are performed as critical control steps.

1041  
1042 You should provide information on the quality and control of  
1043 intermediates. Manufacturing intermediates should be defined by the  
1044 manufacturer. Intermediates may include material from collection or hold  
1045 steps, such as temporary storage of bulk harvest, concentration steps, or  
1046 purification intermediates (e.g., column fractions or eluate). The duration  
1047 of production steps and hold times should be controlled and recorded to  
1048 facilitate the establishment of process limits and to allow for future  
1049 validation of each step and hold time within the proposed limits in support  
1050 of a license application.

1051  
1052 Intermediates in gene therapy manufacturing may also include DNA  
1053 plasmids that are used in the manufacture of other gene therapy products,  
1054 such as AAV or lentiviral vectors. We recommend that DNA plasmid  
1055 intermediates be derived from qualified banks, as described in more detail  
1056 above and in “Control of Materials (3.2.S.2.3)” (section V.A.2.c. of this  
1057 guidance). In addition, we recommend that you provide information on  
1058 the plasmid manufacturing procedures, reagents, and plasmid  
1059 specifications for use. In general, we recommend that this testing include

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1060 assays to ensure the identity, purity, potency, and safety of the final  
1061 product. For a DNA plasmid, this may include sterility, endotoxin, purity  
1062 (including percent of supercoiled form and residual cell DNA, RNA, and  
1063 protein levels), and identity testing (restriction digest and sequencing if  
1064 sequencing was not performed on the bacterial bank). A COA  
1065 documenting plasmid quality testing should be included in the IND.  
1066

1067 e. Process Validation and/or Evaluation (3.2.S.2.5)  
1068

1069 Process validation studies are generally or typically not required for early  
1070 stage manufacturing, and thus, most original IND submissions will not  
1071 include process performance qualification. We recommend that you use  
1072 early stage manufacturing experience to evaluate the need for process  
1073 improvements and to support process validation studies in the future.  
1074

1075 INDs at all stages of development should have established written  
1076 standard operating procedures (SOPs) to ensure proper manufacturing  
1077 control and oversight. Manufacturing oversight is usually performed by a  
1078 dedicated Quality Unit, the duties of which include implementing  
1079 procedures to prevent microbial contamination, cross-contamination, and  
1080 product mix-ups. Your Quality Unit should have procedures in place to  
1081 investigate lot failures, out-of-specification results, and ways to implement  
1082 corrective actions. Your IND should include a description of your Quality  
1083 Unit, including the manner in which quality control testing and oversight  
1084 are separated from the manufacturing unit.  
1085

1086 Additional information on quality systems and process validation can be  
1087 found in the following FDA guidance documents: “Guidance for Industry:  
1088 CGMP for Phase 1 Investigational Drugs,” dated July 2008 (Ref. 16);  
1089 “Quality Systems Approach to Pharmaceutical CGMP Regulations,” dated  
1090 September 2006 (Ref. 17); and “Process Validation: General Principles  
1091 and Practices,” dated January 2011 (Ref. 18). The application of current  
1092 good manufacturing practices (CGMPs) is required under section  
1093 501(a)(2)(B) of the Federal Food, Drug, and Cosmetic Act at all stages of  
1094 clinical investigation. However, the CGMP regulations (21 CFR Part 211)  
1095 are not required for the manufacture of most investigational new drugs  
1096 under Phase 1 INDs (See Ref. 16).  
1097

1098 f. Manufacturing Process Development (3.2.S.2.6)  
1099

1100 You should provide a description and discussion of the developmental  
1101 history of the manufacturing process described in “Description of  
1102 Manufacturing Process and Process Controls” (section 3.2.S.2.2 of the  
1103 CTD).  
1104

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1105 For early stage INDs, there may be differences between the manufacturing  
1106 and testing of the toxicology lots and the material you plan to use in the  
1107 clinical studies. For later stage INDs, there may be changes to the  
1108 manufacturing process as part of process development or optimization. In  
1109 both situations, we recommend that you describe how manufacturing  
1110 differences are expected to impact product performance. If you make  
1111 significant manufacturing changes, then comparability studies may be  
1112 necessary to determine the impact of these changes on the identity, purity,  
1113 potency, and safety of the product. The extent of comparability testing  
1114 will depend on the manufacturing change, the ability of analytical methods  
1115 to detect changes in the product, and the stage of clinical development.  
1116 For first-in-human studies, any differences between toxicology lots and  
1117 clinical lots should be assessed for their impact on product safety. For  
1118 later phase studies, especially those designed to measure product efficacy,  
1119 differences in clinical lots should be assessed for their impact on product  
1120 safety and activity.

1121  
1122 Please note that it is important to retain samples of the DS and  
1123 manufacturing intermediates, when possible, in the event that  
1124 comparability studies are necessary during future product development.

### 3. Drug Substance Characterization (3.2.S.3)

#### a. Elucidation of Structure and Other Characteristics (3.2.S.3.1)

1130 We recommend that you include annotated sequence data for your vector  
1131 in the original IND submission. In addition, we recommend that you  
1132 provide any further information confirming the primary, secondary, or  
1133 higher order structure; post-translational modifications; and/or distribution  
1134 of cell types for the DS if it has not already been described in “Structure”  
1135 (section 3.2.S.1.2 of the CTD).

#### b. Impurities (3.2.S.3.2)

1137 We recommend that your manufacturing process be designed to remove  
1138 process- and product-related impurities and that you have tests in place to  
1139 measure levels of residual impurities. You should describe your test  
1140 procedures in the IND with appropriate limits. Your initial specification  
1141 for impurities may be refined with additional manufacturing experience.  
1142 We recommend that you measure impurities throughout product  
1143 development, as this will help ensure product safety, contribute to your  
1144 understanding of the manufacturing process, and provide a baseline for  
1145 potential manufacturing changes in the future.

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1150 i. Process-Related Impurities

1151  
1152 We recommend testing for process-related impurities. These  
1153 include but are not limited to residual cell substrate proteins,  
1154 extraneous nucleic acid sequences, helper virus contaminants (i.e.,  
1155 infectious virus, viral DNA, viral proteins), and reagents used  
1156 during manufacture, such as cytokines, growth factors, antibodies,  
1157 selection beads, serum, and solvents.

1158  
1159 A common process-related impurity for many vector preparations  
1160 is residual nucleic acid, such as cell substrate DNA, which can co-  
1161 purify with the vector. Some vectors, including AAV, can also  
1162 package (i.e., inside the viral capsid) a large amount of plasmid  
1163 DNA sequences (used during transfection) as well as cellular  
1164 DNA. The presence of these impurities may have adverse effects  
1165 on product quality and safety. We recommend that you optimize  
1166 your manufacturing process to reduce non-vector DNA  
1167 contamination in your product. Additionally, you should monitor  
1168 and control the amount of extraneous nucleic acid sequences in  
1169 your product.

1170  
1171 Since some cell substrates also harbor tumorigenic genetic  
1172 sequences or retroviral sequences that may be capable of  
1173 transmitting infection, we recommend that you take steps to  
1174 minimize the biological activity of any residual DNA associated  
1175 with your vector. This can be accomplished by reducing the size  
1176 of the DNA to below the size of a functional gene and by  
1177 decreasing the amount of residual DNA. We recommend that you  
1178 limit the amount of residual DNA for continuous non-tumorigenic  
1179 cells to less than 10 ng/dose and the DNA size to below  
1180 approximately 200 base pairs.

1181  
1182 If you are using cells that are tumor-derived (e.g., HeLa) or with  
1183 tumorigenic phenotypes (e.g., 293, also known as HEK293T) or  
1184 other characteristics that give rise to special concerns, more  
1185 stringent limitation of residual DNA quantities may be needed to  
1186 assure product safety. In addition to controlling host cell DNA  
1187 content and size, as described above, you should also control the  
1188 level of relevant transforming sequences in your product with  
1189 acceptance criteria that limit patient exposure. For example,  
1190 products made in 293 cells should be tested for adenovirus E1 and  
1191 SV40 Large T antigen sequences. Your tests should be  
1192 appropriately controlled and of sufficient sensitivity and specificity  
1193 to determine the level of these sequences in your product.

1194

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1195 Some vectors, including AAV, can package a large amount of non-  
1196 vector DNA (e.g., plasmid DNA, helper virus sequences, cellular  
1197 DNA), and it may not be possible to remove or reduce this DNA  
1198 from the product to a level sufficient to assure safety. Therefore,  
1199 we strongly recommend that the cell lines and helper sequences  
1200 used to make viral vectors that package non-vector DNA, such as  
1201 AAV, be carefully chosen to reduce the risks of the product.  
1202

#### ii. Product-Related Impurities

1203  
1204  
1205 Typical product-related impurities for viral vectors may include  
1206 defective interfering particles, non-infectious particles, empty  
1207 capsid particles, or replicating recombinant virus contaminants.  
1208 These impurities should be measured and may be reported as a  
1209 ratio, for example, full:empty particles or virus particles:infectious  
1210 units.  
1211

1212 For ex vivo genetically modified cells, product-related impurities  
1213 include non-target cells, which may be present after selection or  
1214 enrichment, and unmodified target cells, which may be present  
1215 after the ex vivo modification step. We recommend that you  
1216 evaluate the nature and number of non-target cells and measure the  
1217 percentage of cells that have been genetically modified. As you  
1218 develop a greater understanding of the cellular phenotypes present  
1219 in your product during clinical development, you may also  
1220 consider adding impurity tests for specific cell populations in order  
1221 to establish greater manufacturing control.  
1222

#### 4. Control of Drug Substance (3.2.S.4)

##### a. Specification (3.2.S.4.1)

1223  
1224  
1225 You should list DS specifications in your original IND submission.  
1226  
1227 Specifications are defined as a list of tests, references to analytical  
1228 procedures, and appropriate acceptance criteria used to assess quality.  
1229 Acceptance criteria should be established and justified, based on data  
1230 obtained from lots used in preclinical and/or clinical studies, data from lots  
1231 used for demonstration of manufacturing consistency, data from stability  
1232 studies, and relevant development data.  
1233  
1234

1235 For products in the early stages of clinical development, very few  
1236 specifications are finalized, and some tests may still be under  
1237 development. However, the testing plan submitted in your IND should be  
1238 adequate to describe the physical, chemical, or biological characteristics of

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1239 the DS necessary to ensure that the DS meets acceptable limits for  
1240 identity, strength (potency), quality, and purity  
1241 (21 CFR 312.23(a)(7)(iv)(a)).  
1242

1243 Your IND should include specifications with established acceptance  
1244 criteria for safety testing at Phase 1. Safety testing includes tests to ensure  
1245 freedom from extraneous material, adventitious agents, microbial  
1246 contamination, and replication competent virus. Information on some  
1247 common safety test methods is provided in more detail in the following  
1248 section (see “Analytical Procedures (3.2.S.4.2),” section V.A.4.b. of this  
1249 guidance). To maximize the sensitivity of safety testing, it is important  
1250 that you perform each test at the stage of production at which  
1251 contamination is most likely to be detected. For example, tests for  
1252 mycoplasma or adventitious viruses (in vivo or in vitro) should be  
1253 performed on cell culture harvest material (cells and supernatant) prior to  
1254 further processing, e.g., prior to clarification, filtration, purification, and  
1255 inactivation.  
1256

1257 Your IND should also include specifications for measuring an appropriate  
1258 dose level (i.e., strength or potency) at Phase 1. Assays used to determine  
1259 dose (e.g., vector genome titer by quantitative polymerase chain reaction  
1260 (qPCR), transducing units, plaque-forming units, transduced cells) should  
1261 be well-qualified prior to initiating dose escalation studies. Information  
1262 on how to qualify your dose determining assay is provided in “Validation  
1263 of Analytical Procedures (3.2.S.4.3)” (section V.A.4.c. of this guidance).  
1264

1265 Additional testing will depend on the type of gene therapy product and the  
1266 phase of clinical development. These tests may include assays to assess  
1267 product characteristics, such as identity, purity (including endotoxin and  
1268 contaminants, such as residual host cell DNA, bovine serum albumin  
1269 (BSA), DNase), and potency/strength. For additional information on  
1270 potency tests, please refer to the FDA’s Guidance for Industry “Potency  
1271 Tests for Cellular and Gene Therapy Products,” dated January 2011 (Ref.  
1272 19).  
1273

1274 Please note that not all testing listed in this section of the guidance is  
1275 required for release of both the DS and DP. In some cases, repeat testing  
1276 may be good practice; however, redundant testing may not always be  
1277 feasible or practical. In this case, we recommend that you provide a  
1278 rationale to support the selection of testing performed for release of either  
1279 DS or DP.  
1280

1281 We provide some additional comments regarding tests for product  
1282 characterization and impurities under “Specifications (3.2.P.5.1)” (section  
1283 V.B.5.a. of this guidance).

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b. Analytical Procedures (3.2.S.4.2)

You should provide a description of all the analytical procedures used during manufacturing to assess your manufacturing process and product quality. In your original IND submission, your descriptions should have sufficient detail so that we can understand and evaluate the adequacy of your procedures. We recommend that you develop detailed SOPs for how your analytical procedures are conducted at early stages of product development as a part of your quality system. We acknowledge that, during product development, analytical methods may be modified to improve control and suitability. However, assay control is necessary during all phases of clinical development to ensure product quality and safety and to allow for comparability studies, following manufacturing changes.

Documentation submitted in support of your analytical procedures should describe in detail how a procedure is performed and should specify any reference standards, equipment, and controls to be used. Submission of information, such as individual SOPs or batch records, will generally not be necessary, provided descriptions of your analytical procedures are sufficiently detailed in your IND. Contractor test reports are acceptable, provided there is adequate description of the analytical procedure, test sensitivity, specificity, and controls.

i. Safety Testing

Safety testing on the DS should include microbiological testing, such as bioburden (or sterility, as appropriate), mycoplasma, and adventitious viral agent testing, to ensure product quality. Guidelines and/or procedures for many safety tests have been described in detail, elsewhere (e.g., bioburden,<sup>5</sup> sterility,<sup>6</sup> mycoplasma (Ref. 20), adventitious agent testing, and tests for specific pathogens (Ref. 12)). Analytical procedures different than those outlined in the United States Pharmacopeia (USP), FDA guidance, or Code of Federal Regulations (CFR) may be acceptable under IND if you provide adequate information on your test specificity, sensitivity, and robustness. Examples of

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<sup>5</sup> USP<61> describes membrane filtration, plate count, and most probable number methods that can be done to quantitatively determine the bioburden of non-sterile DPs. Although 21 CFR 211.110(a)(6) does not specify a test method, it requires that bioburden in-process testing be conducted pursuant to written procedures during the manufacturing process of DPs.

<sup>6</sup> Sterility testing may be performed on the DS when it cannot be performed on the DP, as outlined in the final rule: Amendments to Sterility Test Requirements for Biological Products (May 3, 2012; 77 FR 26162 at 26165). Sterility tests are described in 21 CFR 610.12 and USP<71> Sterility Tests.



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1322 alternative methods, which may be needed for live cells, include  
1323 rapid sterility tests, rapid mycoplasma tests (including PCR-based  
1324 tests), and rapid endotoxin tests. We recommend that you plan to  
1325 demonstrate equal or greater assurance of your test methodology,  
1326 compared to a compendial method, prior to licensure, as required  
1327 under 21 CFR 610.9. We provide some additional comments  
1328 regarding these tests under “Specifications (3.2.P.5.1)”  
1329 (section V.B.5.a. of this guidance) as well as comments regarding  
1330 replication competent virus and wild-type oncolytic virus testing,  
1331 below.

1332  
1333 ii. Replication Competent Virus

1334  
1335 For many gene therapy viral vectors, we recommend specific  
1336 testing, due to the potential for these vectors to recombine or revert  
1337 to a parental or wild-type (WT) phenotype at a low frequency.  
1338 Tests for replication-competent, parental, or wild-type viruses that  
1339 may be generated during production (e.g., replication-competent  
1340 adenovirus (RCA) and replication-competent retrovirus (RCR))  
1341 should be performed on material collected at the appropriate stage  
1342 of the manufacturing process. For example, we recommend testing  
1343 banked material for the presence of replication-competent viruses  
1344 and as a specification for in-process or release testing of DS or DP,  
1345 as appropriate (please see further details, below, within this  
1346 section).

1347  
1348 A. Replication-Competent Retrovirus (RCR) Testing

1349  
1350 Retroviral-based products (including lentivirus and foamy  
1351 virus-based products) used for most gene therapy  
1352 applications are designed to be replication defective. To  
1353 ensure the absence of RCR, you should perform testing for  
1354 RCR at multiple points, during production of a retroviral  
1355 vector. For further information on retroviral testing, refer  
1356 to “Guidance for Industry: Supplemental Guidance on  
1357 Testing for Replication Competent Retrovirus in Retroviral  
1358 Vector Based Gene Therapy Products and During Follow-  
1359 up of Patients in Clinical Trials Using Retroviral Vectors,”  
1360 dated November 2006 (Ref. 21). This guidance will be  
1361 superseded by “Testing of Retroviral Vector-Based Human  
1362 Gene Therapy Products for Replication Competent  
1363 Retrovirus During Product Manufacture and Patient  
1364 Follow-up; Draft Guidance for Industry,” dated July 2018  
1365 (Ref. 22), when finalized.  
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#### B. Replication-Competent Adenovirus (RCA) Testing

The adenoviral-based products used for most gene therapy applications are designed to be replication defective. A notable exception is oncolytic adenoviruses (see “Wild-Type Oncolytic Virus Testing” in section V.A.4.b.ii.D. of this guidance). RCA may be generated at a low frequency as a result of homologous recombination between viral vector sequences and viral sequences present in the cell substrate, during manufacturing. Therefore, for most adenoviral-based products, we recommend that you qualify your MVB for RCA and test either the DS or DP of each production lot for RCA. We recommend a maximum level of 1 RCA in  $3 \times 10^{10}$  viral particles.

#### C. Replication-Competent AAV (rcAAV) Testing

Preparations of AAV vectors can be contaminated with helper virus-dependent rcAAV, also referred to as wild-type AAV or pseudo wild-type AAV. These rcAAV are generated through homologous or non-homologous recombination events between AAV elements present on the vector and AAV rep and cap sequences that are present, during manufacture. While wild-type AAV has no known associated pathology and cannot replicate without helper virus, expression of cap or rep genes in infected cells can result in unintended immune responses, which can reduce effectiveness and may have unintended safety risks.

Therefore, we recommend that you test for rcAAV, which could potentially replicate in the presence of helper virus, and report these results. A number of methods have been published for evaluating the level of rcAAV, including amplification of AAV in the presence of helper virus, followed by PCR for rep/inverted terminal repeats (ITR) junctions, and PCR for rep and cap sequences, following DNase digestion of the vector preparation. We do not recommend a specific method for determining rcAAV in this guidance. You should describe your test method and assay sensitivity in the IND.

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#### D. Wild-Type Oncolytic Virus Testing

Most oncolytic viruses used in gene therapy applications not only carry transgenes but also have been attenuated or adapted from a parental virus strain to grow selectively in cancer cells. It may be possible for these attenuated or adapted viruses to either recombine or revert to a parental (or WT) genotype, during manufacture. Therefore, we recommend that you conduct tests to determine whether the parental virus sequences are present in your product. In addition, we recommend that you select production cells that do not contain viral sequences that may allow homologous recombination with the product. For example, we do not recommend 293 cell substrates for the manufacture of E1-modified oncolytic adenoviruses, due to the potential for homologous recombination with E1 sequences in the 293 cells.

#### c. Validation of Analytical Procedures (3.2.S.4.3)

Validation of analytical procedures is usually not required for original IND submissions for Phase 1 studies; however, you should demonstrate that test methods are appropriately controlled. In general, scientifically sound principles for assay performance should be applied (i.e., tests should be specific, sensitive, and reproducible and include appropriate controls or standards). We recommend that you use compendial methods when appropriate and qualify safety-related tests prior to initiation of clinical trials.

To ensure safety of gene therapy products, you should also qualify the assays used to determine dose (e.g., vector genome titer by qPCR, transducing units, plaque forming units) prior to initiating dose escalation studies. In your original IND submission, you should provide a detailed description of the qualification protocol (e.g., samples; standards; positive/negative controls; reference lots; and controls evaluated, such as operators, reagents, equipment, dates) and data supporting the accuracy, reproducibility, sensitivity, and specificity of the method. Also critical to ensuring safety is the ability to compare the dose used for preclinical evaluations to the dose to be used for clinical studies. One way to ensure that the doses compare is to use the same qualified method to quantitate preclinical and clinical lots. If it is not possible to use the same qualified method, we recommend that you retain sufficient quantities of preclinical material to enable side by side testing with the clinical material, using the

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1456 same qualified method. In addition, you should validate tests used to  
1457 determine dose prior to initiating clinical studies to demonstrate efficacy  
1458 or support licensure.

1459  
1460 Assays used to measure RCR and RCA should also meet our current  
1461 recommendations for sensitivity at an early stage of development (see  
1462 descriptions “RCR Testing” and “RCA Testing” (section V.A.4.b.ii.A. and  
1463 B. of this guidance). We recommend that you include relevant positive  
1464 and negative controls when conducting these tests and include positive  
1465 controls spiked in the test article to assess whether there are any inhibitory  
1466 effects of the test article on detection.

1467  
1468 For all analytical procedures, we recommend that you evaluate assay  
1469 performance throughout product development, have a validation plan in  
1470 place during later phase clinical studies, and complete validation before  
1471 BLA submission. For more information on validation of analytical  
1472 methods, please see the FDA’s Guidance for Industry: “Q2B Validation  
1473 of Analytical Procedures: Methodology,” dated November 1996 (Ref.  
1474 23).

1475  
1476 d. Batch Analysis (3.2.S.4.4)

1477  
1478 You should include a table with test results for all of the batches (or lots)  
1479 of DS that you have manufactured. For early stage INDs, this may include  
1480 only toxicology lots or developmental batches and a single manufacturing  
1481 run for clinical grade material. Please note that batches manufactured in  
1482 different ways should be clearly identified in the submission. We  
1483 recommend that you annually update this section of your IND as new  
1484 batches are produced. You should indicate any batches that fail to meet  
1485 release specifications and any action taken to investigate the failure (as  
1486 outlined in “Process Validation and/or Evaluation (3.2.S.2.5)” (section  
1487 V.A.2.e. of this guidance). We recommend that you retain samples of all  
1488 production lots for use in future assay development, validation, or  
1489 comparability studies.

1490  
1491 e. Justification of Specification (3.2.S.4.5)

1492  
1493 You should provide justification for the DS specifications in your IND.  
1494 We recognize that acceptance criteria may be adjusted throughout the  
1495 product development stages, based on both manufacturing and clinical  
1496 experience. For early stage clinical studies, production lots may be more  
1497 variable than those used in later phase investigations.

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1501 For later stage investigational studies in which the primary objective is to  
1502 gather meaningful data about product efficacy, we recommend that  
1503 acceptance criteria be tightened to ensure batches are well-defined and  
1504 consistently manufactured.

1505  
1506 5. Reference Standards or Materials (3.2.S.5)

1507  
1508 You should provide information on the reference standards or reference materials  
1509 used for testing the DS in your original IND submission. We recommend that  
1510 you provide the source and lot number; expiration date; certificates of analyses,  
1511 when available; and/or internally or externally generated evidence of identity and  
1512 purity for each reference standard.

1513  
1514 Three types of reference standards are generally used: 1) certified reference  
1515 standards (e.g., USP compendial standards); 2) commercially supplied reference  
1516 standards obtained from a reputable commercial source; and/or 3) other materials  
1517 of documented purity, custom-synthesized by an analytical laboratory or other  
1518 noncommercial establishment. In some cases, the reference material for an assay  
1519 will be a well-characterized lot of the gene therapy product, itself. In this case,  
1520 we recommend that you reserve and maintain a sufficient amount of material  
1521 (e.g., part of a production lot) to serve as a reference material.

1522  
1523 6. Container Closure System (3.2.S.6)

1524  
1525 You should describe the type(s) of container and closure used for the DS in your  
1526 original IND submission, including the identity of materials used in the  
1527 construction of the container closure system. We recommend that you determine  
1528 whether the containers and closures are compatible with the DS. For an original  
1529 IND submission, compatibility with a gene therapy product may be evaluated  
1530 during stability studies or may be based on historical data and experience, using  
1531 similar products. You should indicate whether the container is an approved or  
1532 cleared device and/or the information is cross-referenced to a master file, as  
1533 described in section III. “Administrative Information” of this guidance.

1534  
1535 7. Stability (3.2.S.7)

1536  
1537 a. Stability Summary and Conclusions (3.2.S.7.1)

1538  
1539 We recommend that you describe in your original IND submission the  
1540 types of stability studies (either conducted or planned) to demonstrate that  
1541 the DS is within acceptable limits. The protocol should describe the  
1542 storage container, formulation, storage conditions, testing frequency, and  
1543 specifications (i.e., test methodologies and acceptance criteria). Please  
1544

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1545 note that stability studies may evolve with product development, and if DS  
1546 is immediately processed into DP, long term DS stability data may not be  
1547 needed.

1548  
1549 Your stability analysis may include measures of product sterility (or  
1550 container integrity), identity, purity, quality, and activity or potency. We  
1551 recommend that you provide justification for the test methods and  
1552 acceptance criteria used in the stability analysis. It is often helpful to  
1553 demonstrate that at least one or more of the test methods in your stability  
1554 analysis are stability-indicating. You may demonstrate a test is stability-  
1555 indicating, using forced degradation studies, accelerated stability studies,  
1556 or another type of experimental system that demonstrates product  
1557 deterioration. Information to help you design your stability studies may be  
1558 found in the following guidance documents: FDA “Guideline for  
1559 Industry: Quality of Biotechnological Products: Stability Testing of  
1560 Biotechnological/Biological Products,” dated July 1996 (Ref. 24); FDA  
1561 “Guidance for Industry: Q1A(R2) Stability Testing of New Drug  
1562 Substances and Products,” dated November 2003 (Ref. 25); and FDA  
1563 “Guidance for Industry: Q1E Evaluation of Stability Data,” dated June  
1564 2004 (Ref. 26).

1565  
1566 b. Post-Approval Stability Protocol and Stability Commitment  
1567 (3.2.S.7.2)

1568  
1569 We do not recommend that you provide a post-approval stability protocol  
1570 and stability commitment in the IND. However, as you progress with  
1571 product development, you may want to consider which stability studies  
1572 would be required to determine an expiry date for the approved product or  
1573 to support post-approval changes to expiry. We recommend the  
1574 discussion of these items at your late phase IND meetings.

1575  
1576 c. Stability Data (3.2.S.7.3)

1577  
1578 We recommend that you provide the results of your stability studies in  
1579 your IND and update this information on a regular basis (e.g., annual  
1580 reports). Information on the qualification of analytical procedures used to  
1581 generate stability data should be included in your original IND  
1582 submission.

### 1583 **B. Drug Product (3.2.P)**

1584  
1585  
1586 1. Drug Product Description and Composition (3.2.P.1)

1587  
1588 You should provide a description of the DP and its composition (21 CFR  
1589 312.23(a)(7)(iv)(b)). This includes a description of the dosage form and a list of

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1590 all of its components (active and inactive), the amount on a per unit basis, the  
1591 function, and a reference to quality standards for each component (e.g.,  
1592 compendial monograph or manufacturers' specifications). If a drug or device will  
1593 be used with your gene therapy as a combination product, we recommend that  
1594 quality information for the drug or device be included in section 3.2.P of the CTD  
1595 with appropriate hyperlinks to section 3.2.R of the CTD, as described in the FDA  
1596 "eCTD Technical Conformance Guide: Technical Specifications Document,"  
1597 dated November 2017 (Ref. 4). If a placebo treatment is used in the clinical trial,  
1598 a separate DP section should be provided for the placebo. In addition, you should  
1599 provide a description of any accompanying reconstitution diluents and a  
1600 description of the container and closure used for the dosage form and  
1601 accompanying reconstitution diluent in a separate DP section, if applicable.  
1602

### 2. Pharmaceutical Development (3.2.P.2)

1603  
1604  
1605 The Pharmaceutical Development section should contain information on the  
1606 development studies conducted to establish that product formulation,  
1607 manufacturing process, container closure system, microbiological attributes, and  
1608 instructions for use are appropriate for the stage of clinical development. The  
1609 studies described here are distinguished from routine control tests conducted,  
1610 according to specifications. Additionally, this section should identify and  
1611 describe the formulation and process attributes (critical parameters) that can  
1612 influence batch reproducibility, product performance, and DP quality. Supportive  
1613 data and results from specific studies or published literature can be included  
1614 within or attached to the Pharmaceutical Development section. Additional  
1615 supportive data can be referenced to the relevant nonclinical or clinical sections of  
1616 the application.  
1617

#### a. Components of the Drug Product (3.2.P.2.1)

##### i. Drug Substance (3.2.P.2.1.1)

1618  
1619  
1620 You should describe the compatibility of the DS with the  
1621 components listed in "Description and Composition of the Drug  
1622 Product" (section 3.2.P.1 of the CTD) and the key characteristics  
1623 of the DS (e.g., concentration, viability, aggregation state, viral  
1624 infectivity) that can influence the performance of the DP.  
1625  
1626

##### ii. Excipients (3.2.P.2.1.2)

1627  
1628  
1629 You should describe in your original IND submission the choice of  
1630 excipients and inactive components of the DP listed in  
1631 "Description and Composition of the Drug Product" (section  
1632 3.2.P.1 of the CTD), their concentration, and the characteristics of  
1633 these excipients that can influence DP performance.  
1634

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b. Drug Product (3.2.P.2.2)

i. Formulation Development (3.2.P.2.2.1)

You should briefly describe the development of the DP formulation, taking into consideration the proposed route of administration and usage in your IND.

We recommend that you describe any other formulations that have been used in clinical or preclinical studies and provide a reference to such studies, if applicable. If formulation changes were needed for stability, device compatibility, or safety concerns, this information can be reported here.

ii. Overages (3.2.P.2.2.2)

In your IND, you should describe whether gene therapy product in excess of your label claim is added during formulation to compensate for degradation during manufacture or a product's shelf life or to extend shelf life. We do not recommend the use of overages, and we recommend that you provide justification for an overage, as described in Guidance for Industry: "Q8(R2) Pharmaceutical Development," dated November 2009 (Ref. 6).

iii. Physicochemical and Biologic Properties (3.2.P.2.2.3)

You should describe the parameters relevant to the performance of the DP in your IND. These parameters include physicochemical or biological properties of the product (e.g., dosing units, genotypic or phenotypic variation, particle number and size, aggregation state, infectivity, specific activity (ratio of infectious to non-infectious particles or full to empty particles), biological activity or potency, and/or immunological activity). Understanding these parameters and how they affect product performance usually occurs over the course of product development. More information on pharmaceutical development and consideration in establishing critical quality attributes during the clinical research phase can be found in Guidance for Industry: "Q8(R2) Pharmaceutical Development," dated November 2009 (Ref. 6).

You should update this section on the physicochemical and biological properties of your product as you gain a better understanding of the CQA, during development.



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c. Manufacturing Process Development (3.2.P.2.3)

You should describe the selection and optimization of the DP manufacturing process (described in “Description of Manufacturing Process and Process Controls,” section 3.2.P.3.3 of the CTD) if development studies have been performed.

d. Container Closure System (3.2.P.2.4)

You should describe the suitability of the container closure system, which you have described in the “Container Closure System” (section 3.2.P.7 of the CTD), for the storage, transportation (shipping), and use of the DP.

We recommend that you consider choice of materials, protection from moisture and light, compatibility with the formulation (including adsorption to the container and leaching), safety of materials, and performance. For more information on container closure systems, refer to FDA’s “Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics,” dated May 1999 (Ref. 27).

In the selection of your container closure system, we also recommend that you consider how lots of your product will be tested for final product release. For gene therapy products that are manufactured in small lot sizes (e.g., autologous cell products or products vialled at very high dose levels), it may be challenging or not possible to dedicate a final container or multiple vials for lot release testing. In this case, we recommend that you consider a final container that can be sampled for release testing or that you consider alternatives to final container testing.

e. Microbiological Attributes (3.2.P.2.5)

We recommend, for live products intended to be sterile, that you provide details on measures taken to ensure aseptic processing, describe the final product microbial testing, and address how the integrity of the container closure system to prevent microbial contamination will be assessed.

f. Compatibility (3.2.P.2.6)

You should discuss the compatibility of the DP with the diluent used for reconstitution or the delivery device, as appropriate.

We recommend that compatibility studies include measures of both product quantity and product activity (e.g., for viral vectors, a measure of physical particles and infectivity to assess both adsorption and

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1725 inactivation). This in-use and in-device stability data should support  
1726 recommended hold times and conditions outlined in the clinical protocol  
1727 for patient administration.

1728  
1729 3. Manufacture (3.2.P.3)

1730  
1731 a. Manufacturers (3.2.P.3.1)

1732  
1733 You should provide the name, address, and responsibility of each  
1734 manufacturer, including contractor manufacturer(s), involved in the  
1735 manufacture and testing of the DP.

1736  
1737 For gene therapy-device combination products, we recommend that you  
1738 list the manufacturing facilities for the device components and describe  
1739 the assembly and testing processes taking place at each site, as described  
1740 in FDA’s eCTD Technical Conformance Guide (Ref. 4). You should also  
1741 identify whether facilities follow the combination product streamlined  
1742 manufacturing approach (as described in FDA’s Guidance for Industry  
1743 and FDA Staff: “Current Good Manufacturing Practice Requirements for  
1744 Combination Products,” dated January 2017 (Ref. 28) and identify the  
1745 specific set of regulations (i.e., 21 CFR Part 211 or Part 820).

1746  
1747 b. Batch Formula (3.2.P.3.2)

1748  
1749 You should provide a batch formula that includes a list of all components  
1750 of the dosage form, their amounts on a per-batch basis, and a reference to  
1751 their quality standards.

1752  
1753 c. Description of Manufacturing Process and Process Controls  
1754 (3.2.P.3.3)

1755  
1756 You should provide a detailed description of the DP manufacturing  
1757 process and identify process controls, intermediate tests, and final product  
1758 controls. Your description should include both flow diagram(s) and  
1759 narrative description(s) as well as packaging, product contact materials,  
1760 and equipment used. This process can include manufacturing steps, such  
1761 as final formulation, filtration, filling and freezing, and process controls  
1762 and release testing. For ex vivo genetically modified cells that are  
1763 administered immediately after manufacturing, an in-process sterility  
1764 testing on sample taken 48 to 72 hours prior to final harvest is one part of  
1765 the sterility testing recommended for product release. Please see  
1766 “Microbiological Attributes (3.2.P.2.5)” (section V.B.2.e. of this  
1767 guidance) for more information on final product sterility testing for fresh  
1768 cells.

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d. Controls of Critical Steps and Intermediates (3.2.P.3.4)

You should describe the control of critical steps and intermediates in the manufacturing process. Critical steps should include those outlined in the “Description of Manufacturing Process and Process Controls” (section 3.2.P.3.3 of the CTD) to ensure control as well as steps in which tests with acceptance criteria are performed. We recommend that you provide justification for acceptance criteria or limits set for these tests. In addition, you should provide information on the quality and control of intermediates of the manufacturing process. Manufacturing intermediates are defined by the manufacturer and may include material from collection steps or hold steps.

e. Process Validation and/or Evaluation (3.2.P.3.5)

Process validation is not required for early stage manufacturing, and thus, most original IND submissions will not include this information. However, we do recommend that early stage INDs have information on methods used to prevent contamination, cross-contamination, and product mix-ups. For more information on functions of the Quality Unit under IND, please see “Process Validation and/or Evaluation (3.2.S.2.5)” (section V.A.2.e. of this guidance).

4. Control of Excipients (3.2.P.4)

a. Specifications (3.2.P.4.1)

You should provide specifications for all excipients listed in “Excipients” (section 3.2.P.2.1.2 of the CTD). For purpose of this guidance, an excipient is any component, in addition to the active ingredient, that is intended to be part of the final product (e.g., human serum albumin or Dimethyl Sulfoxide (DMSO)).

b. Analytical Procedures (3.2.P.4.2)

You should describe your analytical procedures for testing excipients.

c. Validation of Analytical Procedures (3.2.P.4.3)

Validation of analytical procedures is usually not required for original IND submissions. We recommend that you provide any available validation information for the analytical procedures used to test excipients.

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- d. Justification of Specifications (3.2.P.4.4)  
You should provide justification for the proposed excipient specifications.
  - e. Excipients of Human or Animal Origin (3.2.P.4.5)  
For excipients of human or animal origin, you should provide information regarding source, specifications, description of testing performed, and viral safety data. For human serum, we recommend that you submit information documenting donor suitability as well as appropriate infectious disease testing. You should ensure that collection is performed by a licensed blood bank and that testing meets the requirements described in 21 CFR Part 640.
  - f. Novel Excipients (3.2.P.4.6)  
For excipients used for the first time in a DP or used for the first time in a route of administration, you should provide full details of manufacture, characterization, and controls, with cross-references to supporting safety data (nonclinical and/or clinical).
5. Control of Drug Product (3.2.P.5)
- a. Specifications (3.2.P.5.1)  
You should list DP specifications in your original IND submission. Your testing plan should be adequate to describe the physical, chemical, or biological characteristics of the DP necessary to ensure that the DP meets acceptable limits for identity, strength (potency), quality, and purity (21 CFR 312.23(a)(7)(iv)(b)). Product lots that fail to meet specifications should not be used in your clinical investigation without FDA approval. For early phase clinical studies, we recommend that assays be in place to assess safety (which includes tests to ensure freedom from extraneous material, adventitious agents, and microbial contamination) and dose (e.g., vector genomes, vector particles, or genetically modified cells) of the product. Additional information on safety testing and measuring product dose is described in “Specification (3.2.S.4.1)” (section V.A.4.a. of this guidance).  
  
We recommend that product release assays be performed at the manufacturing step at which they are necessary and appropriate. For example, mycoplasma and adventitious agents release testing is recommended on cell culture harvest material, as discussed in “Specification (3.2.S.4.1)” (section V.A.4.a. of this guidance). In

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1860 addition, sterility, endotoxin, and identity testing are recommended on the  
1861 final container product to ensure absence of microbial contamination or to  
1862 detect product mix-ups that might have occurred during the final DP  
1863 manufacturing steps (e.g., buffer exchange, dilution, or finish and fill  
1864 steps). DP specifications should be further refined as a part of product  
1865 development under IND. We recommend that sponsors establish or, in  
1866 some cases, tighten acceptance criteria, based on manufacturing  
1867 experience as clinical development proceeds. Acceptance criteria should  
1868 also be established, based on clinical lots shown to be safe and effective,  
1869 when appropriate. We also recommend that sponsors develop testing to  
1870 assess product potency and have this assay in place prior to pivotal  
1871 studies. For licensure, a complete set of specifications to ensure the safety  
1872 and effectiveness of the product must include the general biological  
1873 products standards, as outlined in 21 CFR Part 610.

1874  
1875 b. Analytical Procedures (3.2.P.5.2)

1876  
1877 You should describe the analytical procedures used for testing the DP. If  
1878 the analytical procedures are the same as those for the DS, you do not  
1879 need to repeat this information unless there is a matrix effect from the DP  
1880 on assay performance. Please reference the appropriate section of your  
1881 IND, where this information can be found (e.g., Drug Substance  
1882 “Analytical Procedures,” section 3.2.S.4.2 of the CTD). We have the  
1883 following additional comments regarding these tests:

1884  
1885 i. Sterility

1886  
1887 We recognize that the compendial sterility test may not be suitable  
1888 for all products. As mentioned in “Analytical Procedures” (section  
1889 3.2.S.4.2 of this guidance), rapid sterility tests may be needed for  
1890 ex vivo genetically modified cells administered fresh or with  
1891 limited hold time between final formulation and patient  
1892 administration.

1893  
1894 For ex vivo genetically modified cells that are administered  
1895 immediately after manufacturing, in-process sterility testing on  
1896 sample taken 48 to 72 hours prior to final harvest is recommended  
1897 for product release. For such products, aside from an in-process  
1898 sterility test, we also recommend that sponsors perform a rapid  
1899 microbial detection test, such as a Gram stain, on the final  
1900 formulated product and a sterility test, compliant with 21 CFR  
1901 610.12, on the final formulated product.

1902  
1903 Under this approach, the release criteria for sterility would be  
1904 based on a negative result of the Gram stain and a no-growth result

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1905 from the 48 to 72 hour in-process sterility test. Although the  
1906 results of the sterility culture performed on the final product will  
1907 not be available for product release, this testing will provide useful  
1908 data. A negative result will provide assurance that an aseptic  
1909 technique was maintained. A positive result will provide  
1910 information for the medical management of the subject and trigger  
1911 an investigation of the cause of the sterility failure. The sterility  
1912 culture on the final formulated product should be continued for the  
1913 full duration (usually 14 days) to obtain the final sterility test  
1914 result, even after the product has been administered to the patient.  
1915

1916 In all cases where product release is prior to obtaining results from  
1917 a full 14-day sterility test, the investigational plan should address  
1918 the actions to be taken in the event that the 14-day sterility test is  
1919 determined to be positive after the product is administered to a  
1920 subject. You should report the sterility failure to both the clinical  
1921 investigator and FDA. We recommend that you include results of  
1922 investigation of cause and any corrective actions in an information  
1923 amendment submitted to your IND within 30 calendar days after  
1924 initial receipt of the positive culture test result (21 CFR 312.31).  
1925

1926 In the case of a positive microbial test result, the clinical  
1927 investigator should evaluate the subject for any signs of infection  
1928 that may be attributable to the product sterility failure. If the  
1929 patient experiences any serious and unexpected adverse drug event  
1930 that could be from administration of the non-sterile gene therapy  
1931 product, then you must report this information to FDA in an IND  
1932 safety report no more than 15 calendar days after your initial  
1933 receipt of the information (21 CFR 312.32). If you determine that  
1934 an investigational drug presents an unreasonable and significant  
1935 risk to subjects of a positive microbial test result or for any other  
1936 reason, you must discontinue those investigations that present the  
1937 risk and notify FDA, all Institutional Review Boards, and all  
1938 investigators (21 CFR 312.56(d)).  
1939

1940 In addition, please be aware that a product may sometimes  
1941 interfere with the results of sterility testing. For example, a  
1942 product component or manufacturing impurities (e.g., antibiotics)  
1943 may have mycotoxic or anti-bacterial properties. Therefore, we  
1944 recommend that you assess the validity of the sterility assay using  
1945 the bacteriostasis and fungistasis testing, as described in USP <71>  
1946 Sterility Tests.  
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If you freeze DP before use, we recommend that you perform sterility testing on the product prior to cryopreservation so that results will be available before the product is administered to a patient. However, if the product undergoes manipulation after thawing (e.g., washing, culturing), particularly if procedures are performed in an open system, you may need to repeat sterility testing.

We recommend that you incorporate the results of in-process sterility testing into your acceptance criteria for final product specifications.

ii. Identity

We recommend that identity assays uniquely identify a product and distinguish it from other products in the same facility. This test is performed on the final labeled product to verify its contents (21 CFR 610.14). Sometimes, a single test is not sufficient to distinguish clearly among products, and therefore, it is good practice to use different types of test methods (e.g., vector genome restriction digest and protein capsid analysis).

If the final product is ex vivo genetically modified cells, we recommend that identity testing include an assay to measure the presence of vector (i.e., expression assay, restriction digest) or genetic change and an assay specific for the cellular composition of the final product (e.g., cell surface markers).

iii. Purity

Product purity is defined as the relative freedom from extraneous matter in the finished product, whether or not it is harmful to the recipient or deleterious to the product (21 CFR 600.3). Purity testing includes assays for pyrogenicity or endotoxin and residual manufacturing impurities, as outlined under “Impurities (3.2.S.3.2)” (section V.A.3.b. of this guidance) of drug substance, which include but are not limited to proteins; DNA; cell debris; reagents/components used during manufacture, such as cytokines, growth factors, antibodies, and serum; and in the case of ex vivo genetically modified cells, any unintended cellular populations.

Although the rabbit pyrogen test method is the current required method for testing licensed biological products for pyrogenic substances (21 CFR 610.13), we generally accept alternative test methods, such as the Limulus Amebocyte Lysate (LAL), under

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1994 IND. For any parenteral drug, except those administered  
1995 intrathecally, we recommend that the upper limit of acceptance  
1996 criterion for endotoxin be 5 EU/kg body weight/hour. For  
1997 intrathecally-administered drugs, we recommend an upper limit of  
1998 acceptance be set at 0.2 EU/kg body weight/hour.  
1999

2000 iv. Potency

2001

2002 You should describe and justify in your IND all assays that you  
2003 will use to measure potency. A potency assay is not required to  
2004 initiate early phase clinical studies, but we recommend that you  
2005 have a well-qualified assay to determine dose, as described below  
2006 and in “Validation of Analytical Procedures (3.2.S.4.3)” (section  
2007 V.A.4.c. of this guidance). For additional information on potency  
2008 assays, please see FDA’s “Guidance for Industry: Potency Tests  
2009 for Cellular and Gene Therapy Products,” dated January 2011  
2010 (Ref. 19).  
2011

2012 v. Viability

2013

2014 You should establish minimum release criteria for viability, where  
2015 appropriate. For ex vivo genetically modified cells, we  
2016 recommend a minimum acceptable viability of at least 70 percent.  
2017 If this level cannot be achieved, we recommend that you submit  
2018 data in support of a lower viability specification, demonstrating,  
2019 for example, that dead cells and cell debris do not affect the safe  
2020 administration of the product and/or the therapeutic effect.  
2021

2022 vi. Cell Number or Dose

2023

2024 Your dose-determining assay is an important part of your product  
2025 specifications. For additional information on your dose-  
2026 determining assay, please see “Specification (3.2.S.4.1)” (section  
2027 V.A.4.a. of this guidance). If your final product is a genetically  
2028 modified cell therapy, you should have an acceptance criterion for  
2029 the minimum number of modified cells in a product lot. We  
2030 recommend that the product dose be based on the total number of  
2031 genetically modified cells.  
2032

2033 c. Validation of Analytical Procedures (3.2.P.5.3)

2034

2035 Validation of analytical procedures is usually not required for original  
2036 IND submissions, but we do recommend that you qualify certain safety-  
2037 related or dose-related assays, even at an early stage of development (see  
2038



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2039 “Validation of Analytical Procedures (3.2.S.4.3),” section V.A.4.c. of this  
2040 guidance). If they are the same as those listed for DS testing, you do not  
2041 need to repeat them but should reference that section of your IND.  
2042

2043 d. Batch Analyses (3.2.P.5.4)

2044  
2045 You should provide final product COA(s) or a description of batches and  
2046 results of batch analyses for the DP.  
2047

2048 e. Characterization of Impurities (3.2.P.5.5)

2049  
2050 You should provide information on characterization of impurities if not  
2051 previously provided in “Impurities” (section 3.2.S.3.2 of the CTD).  
2052

2053 f. Justification of Specifications (3.2.P.5.6)

2054  
2055 You should provide justification for the DP specifications. See  
2056 “Justification of Specification (3.2.S.4.5)” (section V.A.4.e. of this  
2057 guidance) for additional details.  
2058

2059 6. Reference Standards or Materials (3.2.P.6)

2060  
2061 You should provide information on the reference standards or reference materials  
2062 used in testing the DP if not previously provided in “Reference Standards or  
2063 Materials” (section 3.2.S.5 of the CTD).  
2064

2065 7. Container Closure System (3.2.P.7)

2066  
2067 You should provide a description of the container closure systems, including  
2068 identity of materials of construction or each primary packaging component and its  
2069 specification. You should also provide information on how the container is  
2070 sterilized.  
2071

2072 Please see “Container Closure System (3.2.P.2.4)” (section V.B.5.d. of this  
2073 guidance) for more information and recommendations, regarding the suitability of  
2074 different final product containers.  
2075

2076 If the final container is an FDA-cleared device, we recommend that you reference  
2077 the 510(k) number for the device in your submission. For device combination  
2078 products, we recommend that you include a table of contents for the combination  
2079 product (with reference links to other files) in this section, as described in FDA’s  
2080 eCTD Technical Conformance Guide (Ref. 4).  
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8. Stability (3.2.P.8)
    - a. Stability Summary and Conclusion (3.2.P.8.1)

You should summarize the types of studies conducted, protocols used, and the results of the studies. Your summary should include, for example, conclusions regarding storage conditions and shelf life as well as in-use and in-device storage conditions.

If a short-term clinical investigation is proposed, or if a continuous manufacturing process with limited product hold times is used, stability data submitted may be correspondingly limited. For early stage INDs, stability data for the gene therapy may not be available to support the entire duration of the proposed clinical investigation. Therefore, we recommend that you submit a prospective plan to collect stability information and update this information to the IND in a timely manner (e.g., in an annual IND update).
    - b. Post-Approval Stability Protocol and Stability Commitment (3.2.P.8.2)

We do not recommend that you provide a post-approval stability protocol and stability commitment in your IND submission. However, as product development continues, we recommend that you consult with your Quality Reviewer to determine the type of studies that will be necessary to support product expiration dates for commercial manufacturing.
    - c. Stability Data (3.2.P.8.3)

You should provide results of the stability studies in your IND in an appropriate format (e.g., tabular, graphic, narrative). Information on the analytical procedures used to generate the data should also be included, and this may be referenced to other sections of your submission (e.g., “Analytical Procedures,” section 3.2.P.5.2 of the CTD).
  - C. **Appendices (3.2.A)**
    1. Facilities and Equipment (3.2.A.1)

You should provide a diagram, illustrating the manufacturing flow of the manufacturing areas, information on all developmental or approved products manipulated in this area, a summary of product contact equipment, and information on procedures and design features of the facility, to prevent contamination or cross-contamination.

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2128 A description of the Quality Unit and the quality control (QC) and quality  
2129 assurance (QA) responsibilities may be included in this section.

2130  
2131 COAs for all raw materials and reagents described in your IND may be put in this  
2132 section.

2133  
2134 2. Adventitious Agents Safety Evaluation (3.2.A.2)

2135  
2136 You should provide information assessing the risk of potential contamination with  
2137 adventitious agents. For non-viral adventitious agents, we recommend that you  
2138 provide detailed information on the avoidance and control of transmissible  
2139 spongiform encephalopathy agents, bacteria, mycoplasma, and fungi. This  
2140 information can include certification and/or testing of components and control of  
2141 the production process. For viral adventitious agents, we recommend that you  
2142 provide information on viral safety studies. Study reports and data to support  
2143 qualification of your manufacturing components (such as adventitious agents test  
2144 reports for banked materials) may be submitted as a part of this appendix. These  
2145 studies should demonstrate that the materials used in production are considered  
2146 safe and that the approaches used to test, evaluate, and eliminate potential risks,  
2147 during manufacture, are suitable.

2148  
2149 Data collected (i.e., study reports) for adventitious agent testing can be placed in  
2150 this section.

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