4160-01-P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Parts 600, 610, and 680

[Docket No. FDA-2011-N-0080]

Amendments to Sterility Test Requirements for Biological Products

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending the sterility test requirements for biological products. This rule provides manufacturers of biological products greater flexibility, as appropriate, and encourages use of the most appropriate and state-of-the-art test methods for assuring the safety of biological products. FDA is taking this action as part of its ongoing efforts to comprehensively review and, as necessary, revise its regulations related to biological products.

DATES: This rule is effective [INSERT DATE 30 DAYS AFTER DATE OF PUBLICATION IN THE FEDERAL REGISTER].

FOR FURTHER INFORMATION CONTACT:

Paul E. Levine, Jr.,

Center for Biologics Evaluation and Research (HFM-17),

Food and Drug Administration,

1401 Rockville Pike, suite 200N,

Rockville, MD 20852-1448,

301-827-6210.

SUPPLEMENTARY INFORMATION:

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I. Background

This rule revises the sterility requirements for most biological products under title 21 of the Code of Federal Regulations (CFR), subchapter F, parts 600 through 680 (21 CFR parts 600 through 680)[1] and is intended to promote improvement and innovation in the development of sterility test methods by allowing manufacturers the flexibility needed for sterility testing of some novel products that may be introduced to the market, enhancing sterility testing of

[1] The sterility test provisions of this regulation do not apply to Whole Blood, Cryoprecipitated Antihemophilic Factor (AHF), Platelets, Red Blood Cells, Plasma, Source Plasma, Smallpox Vaccine, Reagent Red Blood Cells, Anti-Human Globulin, or Blood Grouping Reagents. The provisions also do not apply in cases where the Director of the Center for Biologics Evaluation and Research (CBER) or the Director of the Center for Drug Evaluation and Research (CDER), as appropriate, exempts a product from the requirements because the Director finds the manufacturer's data adequate to establish that the mode of administration, the method of preparation, or the special nature of the product precludes or does not require a sterility test or that the sterility of the lot is not necessary to assure the safety, purity, and potency of the product. (See 21 CFR 610.12(g)(4).)

currently approved products, and encouraging manufacturers to utilize scientific and technological advances in sterility test methods as they become available.

In the Federal Register of June 21, 2011 (76 FR 36019), FDA published a proposed rule that proposed revisions to update requirements for sterility testing of biological products. As described in the preamble of the proposed rule (76 FR 36019 at 36019 to 36020), any product that purports to be sterile should be free of viable contaminating microorganisms to assure product safety (§ 600.3(q) (21 CFR 600.3(q)). Absolute sterility of a lot cannot be practically demonstrated without complete destruction of every finished article in that lot (USP, Chapter 1211). Therefore, sterility assurance is accomplished primarily by validation of the sterilization process or of aseptic processing under current good manufacturing practice (CGMP), and is supported by sterility testing using validated and verified test methods (see e.g., USP Chapter 71, European Pharmacopeia 2.6.1.).

In the Federal Register of November 20, 1973 (38 FR 32048), we reorganized and republished the biologics regulations, which included regulations governing sterility testing, as parts 600 through 680.

Over the years, FDA has amended the biologics regulations, as necessary, to clarify and update the sterility test requirements. On March 11, 1976 (41 FR 10427) and March 2, 1979 (44 FR 11754), we updated § 610.12 (21 CFR 610.12) to clarify the procedures for repeat testing. On December 15, 1986 (51 FR 44903), we clarified and updated certain requirements for sterility testing to ensure the reliability of the growth-promoting qualities of the sterility test culture media and to provide greater consistency with the test methods of USP XXI. Finally, on September 15, 1997 (62 FR 48174), we incorporated by reference into § 610.12(f) the 1995 edition of the USP concerning the procedures for the membrane filtration test method.

Prior to this final rule, § 610.12 required that the sterility of most licensed biological products[2] be demonstrated through the performance of tests prescribed in § 610.12(a) and (b). Specifically, § 610.12 provided that the sterility of each lot of each product, with the exception of certain products[3], be demonstrated by the performance of prescribed sterility tests for both bulk and final container material, unless different sterility tests were prescribed in the license (see § 610.12(g)(1)) or the manufacturer submitted adequate data[4] establishing that the mode of administration, the method of preparation, or the special nature of the product precluded or did not require a sterility test, or that the sterility of the lot was not necessary to assure the safety, purity, and potency of the product (§ 610.12(g)(4)(ii)).

The regulation also specified the test method and culture media to be used. For example, the prescribed sterility test methods relied upon culture media (either Fluid Thioglycollate Medium or Soybean-Casein Digest Medium) to detect growth of microorganisms (§ 610.12(a)(1) and (a)(2)). Moreover, § 610.12 specified criteria, such as incubation conditions (time and temperature) to be used during testing, suitable test organisms for the evaluation of the growth-promoting qualities of the culture media, storage and maintenance of test organism cultures, and storage and condition of media.

Since we last clarified and updated our regulations governing sterility testing, advances in technology in recent years have allowed the development of new sterility test methods that yield accurate and reliable test results in less time and with less operator intervention than the currently prescribed methods. Some examples of novel methods include the Adenosine Triphosphate bioluminescence, chemiluminescence, and carbon dioxide head space

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[2] See list of exemptions in § 610.12(g)(4).
[3] Whole Blood, Cryoprecipitated AHF, Platelets, Red Blood Cells, Plasma, Source Plasma, Smallpox Vaccine, Reagent Red Blood Cells, Anti-Human Globulin, or Blood Grouping Reagents (§ 610.12(g)(4)(i)).
[4] In such an instance, the Director of CBER or CDER, as appropriate, would determine the adequacy of the data (§ 610.12(g)(4)(ii)).

measurement. Manufacturers may benefit from using such sterility test methods with rapid and advanced detection capabilities.

Accordingly, we have amended § 610.12 to promote improvement and innovation in the development of sterility test methods, to address the challenges of novel products that may be introduced to the market in the future, and to potentially enhance sterility testing of currently approved products. This final rule provides manufacturers the flexibility to take advantage of methods as they become available, provided that these methods meet certain criteria.

II. Summary of the Final Rule

FDA is adopting as final, without material change, the proposed requirements for sterility testing. Specifically, this final rule:

- Eliminates specified sterility test methods, culture media formulae (or formulation), and culture media test requirements;
- Eliminates specified membrane filtration procedure requirements for certain products;
- Eliminates specified sterility test requirements for most bulk material;
- Modifies the repeat sterility test requirements, so that repeat tests will occur only once for each lot. These repeat tests are limited to situations when the quality control unit conclusively determines, after conducting an investigation upon detection of viable microbial contamination during the initial test of the lot, that the contamination is the result of laboratory error or faulty materials used in conducting the sterility test;
- Replaces the storage and maintenance requirements for cultures of test organisms used to determine the "growth-promoting qualities" of culture media with: (1) Validation requirements specifying that any sterility test used is able to consistently detect the presence of viable contaminating microorganisms and (2) verification of

"growth-promoting properties" or microorganism-detection capabilities of test and test components;

- Replaces the sample size or amount requirement with a requirement that the sample be appropriate to the material being tested;
- Replaces the Interpretation of test results section under § 610.12(c) with a requirement that manufacturers establish, implement, and follow written procedures for sterility testing that describe, at a minimum, the test method used, the method of sampling, and the written specifications for acceptance or rejection of each lot;
- Simplifies and clarifies the Exceptions section under § 610.12(h); and
- Identifies the Director of CDER as one of the two Center directors authorized to grant an exemption under the exception provision at § 610.12(h)(2). In the proposed rule, the Center for Devices and Radiological Health was erroneously identified in this exception, instead of the Center for Drug Evaluation and Research.
- Revises the definition of the term "sterility" under § 600.3(q); and
- Eliminates certain exceptions for allergenic products related to sterility testing under § 680.3(c).

III. Comments on the Proposed Rule and FDA's Responses

We received 17 letters of comments on the proposed rule. These comments were received from biologics manufacturers, industry associations, and other interested persons. A summary of the comments received and our responses follow. We first respond to general comments and then respond to comments on the specific topics set forth in the preamble of the proposed rule.

To make it easier to identify the comments and our responses, the word "Comment," in parentheses, will appear before the comment's description, and the word "Response," in parentheses, will appear before our response. We have also numbered each comment to help distinguish between different comments. The number assigned to each comment is purely for organizational purposes and does not signify the comment's value or importance or the order in which it was received. Certain comments were grouped together because the subject matter of the comments was similar.

A. General Comments and FDA's Response

(Comment 1) Thirteen of the letters of comments supported the proposed rule. Many of the comments agreed that the proposed amendments would provide manufacturers of biological products greater flexibility and would promote improvement and innovation in the development of sterility test methods. Several comments agreed that the proposed amendments would allow manufacturers to use the most appropriate and state-of-the-art test methods for assuring the safety of biological products. Several comments applauded FDA's effort to amend sterility test requirements to permit the use of new methods and systems in assessing microbiological contamination in sterile products. Another comment was pleased to see FDA's commitment to advancing the principles of innovation in product development for public health.

(Response) FDA acknowledges and appreciates the supportive comments. As stated previously, the rule provides needed flexibility and encourages manufacturers to benefit from scientific and technological advances in sterility test methods as they become available.

(Comment 2) One comment noted an error in the reference to the European Pharmacopeia 2.6.2. provided in the first paragraph in section I of the preamble to the proposed

rule. The comment pointed out that European Pharmacopeia 2.6.2. is the chapter for Mycobacteria testing.

(Response) We agree with this comment. The reference should have been to European Pharmacopeia 2.6.1. Sterility testing.

(Comment 3) One comment concurred with the preamble statement that "* * * sterility assurance is accomplished primarily by validation of the sterilization process or by the aseptic processing procedures under CGMP, and is supported by sterility testing using validated and verified test methods," (76 FR 36019 at 36019). However, the commenter went on to state that "* * * the regulations would be better suited by ensuring that the aseptic manufacturing processes follow strict GMP, further leveraging the requirements for aseptic environments, media fill programs, and strict oversight of the aseptic process as opposed to the perceived assurance that sterility testing of samples provides. This is best illustrated through existing verbiage in § 211.113(b) (21 CFR 211.113(b)) but should be further expanded upon to provide improved guidance to industry and investigators."

(Response) We acknowledge that product sterility testing does not provide absolute assurance of product sterility. However, we believe validation of aseptic processes,[5] using process simulations or media fills, together with operational controls and product sterility testing, provide a sufficient level of assurance that products purported to be sterile are in fact sterile. Therefore, we do not agree that additional requirements are necessary because the existing CGMP requirements under parts 210 and 211 (21 CFR parts 210 and 211) and the other applicable regulations in parts 600 through 680 already address the concerns raised by the

[5] See the applicable requirements in parts 210, 211, and 600 through 680, and FDA's guidance document entitled "Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing--Current Good Manufacturing Practice," dated September 2004.

commenter. We believe this final rule, together with the other applicable regulations and Agency guidance, provide manufacturers appropriate latitude to determine how to achieve the level of control necessary for compliance.

(Comment 4) One comment expressed a concern that an environmental requirement is not part of the proposed rule. The commenter stated, "Environmental conditions are important to avoid cross-contamination" and proposed the addition of the following wording described in European Pharmacopeia 2.6.1. "The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any microorganisms which are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls."

(Response) In discussing "environmental conditions," we understand the comment to mean environmental controls. We have considered the issue, including the points raised in this comment and have decided not to adopt the suggested language or revise the rule in light of the suggested language because the concerns expressed by the commenter are currently addressed in the CGMP requirements in parts 210 and 211 and the applicable regulations in parts 600 through 680. In addition, manufacturers may turn to relevant Agency guidance documents for additional guidance. Furthermore, as the commenter states, the proposed wording regarding environmental controls under which the sterility test is to be performed is already described in European Pharmacopeia 2.6.1., and USP Chapter 71, both of which are additional, valuable resources for manufacturers.

(Comment 5) One comment noted that while § 610.12 addresses aspects of sterility, the current theme of the section is specific to sterility testing. The commenter therefore suggested either renaming the title of § 610.12 as "Sterility Test," or broadening § 610.12 so that the regulation addresses all critical elements in the content area of sterility.

(Response) We decline to adopt either recommended change because we believe that the current title of § 610.12 remains appropriate and that the suggested title change is unnecessary. In response to the comment expressing a desire to broaden § 610.12 to address all critical elements in the content area of sterility, FDA notes that this comment is outside the scope of this final rule.

B. Comments and FDA's Response on Specific Topics From the Proposed Rule

The following are comments and FDA's responses, as identified by the specific topic in the proposed rule to which the comment and FDA's response applies.

1. When Is Sterility Testing Required?

For the reasons discussed in the preamble to the proposed rule (76 FR 36019 at 36020 to 36021), we proposed amending § 610.12 to eliminate the sterility test requirement for most bulk materials. We have determined that, in most cases, for purposes of sterility testing, the most appropriate test material is the final container material. We recognize that due to the nature of some biological products, testing the final container material may not always be feasible or appropriate. Thus, as finalized, § 610.12 requires that prior to release, manufacturers of biological products must perform sterility testing of each lot of each biological product's final container material or other material (e.g., bulk material or active pharmaceutical ingredient (API), in-process material, stock concentrate material), as appropriate, and as approved in the biologics license application (BLA) or BLA supplement. For example, as discussed in the

preamble to the proposed rule (76 FR 36019 at 36021), certain allergenic and cell and gene therapy products may need to be tested for sterility at an in-process stage or some other stage of the manufacturing process (e.g., intermediate, API, bulk drug substance) instead of the final container material because the final container material may interfere with the sterility test. Likewise, as discussed in the preamble to the proposed rule, some cell therapy products and cell-based gene therapy products may need to be tested for sterility at an in-process stage or some other stage of manufacturing process because low production volumes may result in an insufficient final container material sample for sterility testing or a short product shelf-life may necessitate administration of the final product to a patient before sterility test results on the final container material are available.

(Comment 6) Three comments were particularly supportive of FDA's proposal to eliminate the sterility test requirements for bulk material. One comment noted this change will be particularly helpful for cellular therapy products.

(Response) We appreciate the supportive comments. We agree that the elimination of specified sterility test requirements for most bulk materials will provide manufacturers with greater flexibility and in most cases, for purposes of sterility testing, the most appropriate test material is the final container (76 FR 36019 at 36021). We also acknowledge that due to the nature of some biological products, this change could result in the need for some manufacturers to modify their testing procedures to eliminate testing for bulk materials. However, we note that these modifications to eliminate testing for bulk materials would be made following existing change control procedures and a submission to FDA to report the change would not be required.

If it is determined that sterility testing needs to be performed on material other than the final product, due to the nature of the final product, we would expect the manufacturer, as

required under §§ 601.2 and 601.12, to include in its BLA or BLA supplement: (1) A description of the details of the sterility test method used, including the procedure for testing the alternate material instead of the final container material; and (2) the scientific rationale for selecting the specific test material instead of the final container material.

As discussed in the preamble to the proposed rule (76 FR 36019 at 36021), a manufacturer who desires to utilize an alternate sterility test method other than the one approved in its BLA must submit a BLA supplement in accordance with § 601.12(b).

(Comment 7) One comment asserted that upon finalization of the rule, a manufacturer who desires to utilize an alternative sterility test other than the one approved in its BLA should be permitted to submit the change to FDA in its annual report in accordance with § 601.12(d), as opposed to a prior approval supplement to an approved application under § 601.12(b).

(Response) We consider changes that may affect the sterility assurance level of a product to have substantial potential to affect the safety, purity, or potency of a product and have consistently identified this change as one that requires prior approval. Therefore, a manufacturer who desires to utilize an alternate sterility test method other than the one approved in its BLA must submit a prior approval supplement to an approved application in accordance with § 601.12(b). We note that approval of the supplement will be based on the determination that the data submitted with the request establishes a regulatory basis for approval.

2. What Are the Sterility Test Requirements?

a. Test methods--We proposed amending § 610.12 to eliminate references to specific test methods and culture media for sterility testing and to instead require that the sterility test be appropriate to the material being tested such that the material does not interfere with or otherwise hinder the test. As discussed in the preamble to the proposed rule (76 FR 36019 at 36021), we

believe this revision recognizes current practices and provides manufacturers the flexibility to take advantage of suitable modern sterility test methods and keep pace with advances in science and technology.

As also discussed in the preamble to the proposed rule (76 FR 36019 at 36021), because we are expanding potentially acceptable sterility test methods to include non-culture-based methods in addition to culture-based methods, we also have removed the definition of "a lot of culture medium." Previously, § 610.12(e)(2)(i) defined this term as "* * * that quantity of uniform material identified as having been thoroughly mixed in a single vessel, dispensed into a group of vessels of the same composition and design, sterilized in a single autoclave run, and identified in a manner to distinguish one lot from another." Although we have deleted this term from § 610.12, we believe (as stated in the preamble to the proposed rule) that this concept is captured by the definition of "lot" in § 600.3(x). We note that this change is also consistent with our understanding that prepared culture media may be purchased, in which case a lot may be predetermined by the vendor.

(Comment 8) Two comments opposed the elimination of the specified sterility test methods and culture media because eliminating the specific requirements may lead to different interpretations by industry, as well as FDA investigators. One comment stated that the current text on acceptable culture media, reference organisms, and incubation temperatures for sterility testing represents essential guidance for industry. The comments suggested that either the current regulations be retained in addition to the proposed amendments or retained as guidance.

(Response) We reiterate that the purpose of this rule is to provide manufacturers of biological products greater flexibility and to encourage use of the most appropriate and state-of-the-art test methods for assuring the safety of biological products. Accordingly, at this time, we

decline to retain the current specified sterility test methods, culture media, reference organisms, and incubation temperatures in regulation or guidance. Furthermore, we disagree that this rule may lead to inconsistent interpretations by industry and FDA staff because sterility test methods for biological products are approved in the manufacturer's BLA or BLA supplement, and hence, the data submitted with the request are reviewed in a consistent manner in accordance with review management procedures. Therefore, we believe the commenters' concerns about inconsistencies in interpretation are unfounded.

(Comment 9) One commenter expressed concern about the applicability of the proposed changes in the global regulatory market in that the use of approved alternative sterility methods would not be globally applicable in the absence of compendial harmonization. The commenter inquired whether FDA has plans to harmonize the use of alternative sterility methods with the three main global compendia.

(Response) We do not agree that the final rule and the use of a suitable modern sterility test method will interfere with the global regulatory market. The purpose of the rule is to provide for greater flexibility and to encourage use of the most appropriate and state-of-the-art test methods for assuring the safety of biological products. We believe this final rule will foster the adoption of novel methods and that alignment with global pharmacopeial methods will occur over time. With respect to FDA's future plans to harmonize the use of alternative sterility methods with the three main global compendia, we note that any such discussion is outside the scope of this rule.

(Comment 10) One comment proposed adding a reference in the regulations to a compendial method and allowing for the implementation of alternative methods. The commenter expressed concern that, in the global marketplace, implementation of a novel method

different from USP Chapter 71 would not be harmonized with other compendia and might pose risks to approval of marketing authorizations if new tests are not recognized or accepted by foreign health authorities.

(Response) We do not agree with the comment and note that incorporating such a reference would be inconsistent with the intent of this rule. We reiterate that we do not agree that this final rule will interfere with the global marketplace. Rather, we believe that facilitating flexibility and encouraging the use of the most appropriate and state-of-the-art test methods will foster the adoption of novel method technologies and that alignment with pharmacopeia methods will occur over time. Furthermore, as we have explained in the preamble to the proposed rule, FDA considers established USP compendial sterility test methods to already have been validated using an established validation protocol; therefore their accuracy, specificity, and reproducibility need not be reestablished to fulfill the validation requirements under the final rule. Only a manufacturer who desires to utilize an alternative method other than the one approved in its BLA must submit a BLA supplement in accordance with § 601.12(b). This rule does not require manufacturers to utilize an alternative method other than the one approved in their BLA.

(Comment 11) One comment stated that the absence of references to standards such as USP Chapter 71 within § 610.12 may lead to confusion and suggested that a general disclaimer that FDA is not endorsing any particular standard or the provision of specific examples within the regulation may provide an important point of reference for compliance. Two comments stated that USP Chapter 71 and European Pharmacopeia 2.6.1. should be listed within § 610.12 as a baseline or standard for sterility testing. Two other comments recommended referring to the USP Chapter 71 as the "referee" method instead of referring to it as an example.

(Response) The concerns expressed in the comments are unfounded. We reiterate that we consider the current sterility test methods in a manufacturer's BLA or BLA supplement to already have been validated. In contrast, newer methods (for example, non-culture-based methods that have not been validated according to an established protocol) or those that deviate from the official compendial sterility test methods will require validation.

Moreover, the final rule requires that a novel method be validated in accordance with an established protocol to demonstrate that the test is capable of consistently detecting the presence of viable microorganisms. We believe methods validation is a well recognized activity and can be performed without comparison to a "referee" test method.

Furthermore, we note that there is no single "referee" test method that would work for all products and that some novel methods cannot be easily compared to culture-based methods such as USP Chapter 71 because these testing methods do not measure microbial growth. Therefore, we believe that it is neither necessary nor appropriate to add a reference to a standard or "baseline" in this final rule.

(Comment 12) We received two comments regarding growth-promotion testing. One comment asserted that the proposal to eliminate the requirements to test culture media with specific test organisms, to eliminate the number of organisms that must be used to demonstrate growth-promoting qualities of culture media, and to eliminate specific incubation conditions and visual examination requirements may lead to different interpretations on which organisms can and should be used. The comment proposed that a reference to a "referee" method be added to the regulation including requirements for growth promotion and the strains and number of organisms to be used. The other comment supported the elimination of the list of specified

organisms, while also stating that providing a list of organisms for manufacturers to consider would be a benefit to facilities that do not have the necessary expertise or staffing.

(Response) Because we are providing manufacturers the flexibility to use sterility test methods that are either culture-based or non-culture-based, which may necessitate different verification activities, we decline to retain the existing requirements for specified sterility test reference organisms. For similar reasons, we do not believe a reference to a "referee" method is necessary or appropriate and we decline to adopt the recommended change.

Instead of specifying the number and type of test organisms, under § 610.12(b) of the final rule, we require that: (1) The sterility test must be appropriate to the material being tested such that the material does not interfere with or otherwise hinder the test; (2) the sterility test must be validated to demonstrate that the test is capable of reliably and consistently detecting the presence of viable contaminating microorganisms; and (3) the sterility test and test components must be verified to demonstrate that the test method can consistently detect the presence of viable contaminating microorganisms.

Due to the variety of currently available and potential future sterility test methods, we have eliminated specified incubation conditions (time and temperature) and visual examination requirements previously prescribed in § 610.12. Since we are allowing any validated sterility test method that is appropriate to the material being tested, rather than specifying the test and the media used, we have also eliminated the Fluid Thioglycollate Medium incubation temperatures previously prescribed in § 610.12(a)(1)(ii) for the final container material containing a mercurial preservative.

(Comment 13) One comment recommended that, with respect to validation, a definition for the terms "reliably" and "consistently" be added to the regulation for greater utility in

understanding expectations when validating a method. The commenter offered, for example, "* * * that a validated method, though performing consistently and reliably, may still not be centered on the true value of the specific parameter being tested. Consequently, when this method would be used during testing the results may be in a statistical state of control, but not necessarily statistically capable of measuring the true value." The commenter asked FDA to consider "* * * that the use of the terms 'reliably and consistently' may infer that the validation of a test for non-sterility does not require proof of performance at least equivalent to the USP referee method." The comment therefore asked that § 610.12(b)(2) be revised to require that the sterility test be validated to demonstrate an equivalent or superior detection of viable contaminating microorganisms compared to the USP compendial or like method.

(Response) FDA has considered the issues raised by these comments and has determined that making the suggested changes would be inconsistent with the intent of this rule. With respect to the comment that the rule should be revised to require that the sterility test be validated to demonstrate an equivalent or superior detection of viable contaminating microorganisms compared to the USP compendial or like method, we reiterate that some novel methods cannot be easily compared to culture-based methods such as USP Chapter 71 because they do not measure microbial growth. Moreover, we note that the final rule requires that a novel method be validated in accordance with an established protocol to demonstrate that the test is capable of consistently detecting the presence of viable microorganisms. With respect to the comment that the terms "reliably" and "consistently" should be defined, we note that these terms are already well understood in the industry.

b. Validation--As discussed in the preamble to the proposed rule (76 FR 36019 at 36021 to 36022), the International Conference on Harmonisation (ICH) publication entitled "Validation

of Analytical Procedures: Text and Methodology Q2(R1)" dated November 2005, states that "The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose."[6] Similarly, USP General Chapter 1223, "Validation of Alternative Microbiological Methods," states "Validation of a microbiological method is the process by which it is experimentally established that the performance characteristics of the method meet the requirements for the intended application." For sterility testing, this means that the test can consistently detect the presence of viable contaminating microorganisms.

We have eliminated the prescribed sterility test methods found in § 610.12 and instead will allow the use of sterility test methods that are validated in accordance with established protocols to be capable of consistently detecting the presence of viable contaminating microorganisms. If an established USP compendial sterility test method is used, a manufacturer must verify that this established method is suitable for application to the specific product (see §§ 211.165(e) and 211.194(a)); however, FDA considers established USP compendial sterility test methods to already have been validated using an established validation protocol, so their accuracy, specificity, and reproducibility need not be reestablished to fulfill the validation requirement under the final rule. In contrast, novel methods and any methods that deviate from the USP compendial sterility test methods require the detailed validation discussed in this document and elsewhere in this preamble.

We again note that § 610.12 requires the use of a material sample that does not interfere with or otherwise hinder the sterility test from detecting viable contaminating microorganisms.

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[6] This guideline for industry was previously named "Text on Validation of Analytical Procedures" (ICH-Q2A), dated March 1995 (approved by the Steering Committee in October 1994). An accompanying guideline entitled "Validation of Analytical Procedures: Methodology (Q2B)," dated November 6, 1996, was subsequently developed and approved by the Steering Committee in November 1996. The parent guideline is now renamed "Validation of Analytical Procedures: Text and Methodology Q2(R1)" and was revised in November 2005. At that time, the guideline on methodology (Q2B) was incorporated into the parent guideline.

This requirement is crucial because the material itself or substances added to the material during formulation may make some sterility tests inappropriate for use. A validated sterility test method is a critical element in assuring the safety, purity, and potency of the product. USP General Chapter 1223, as well as the ICH guideline referenced earlier entitled "Text on Validation of Analytical Procedures," dated March 1995 (ICH-Q2A), provide general descriptions of typical validation parameters, how they are determined, and which subset of each parameter is required to demonstrate validity, based on the method's intended use. Validation of each test method should be performed on a case-by-case basis to ensure that the parameters are appropriate for the method's intended use. In the context of reviewing sterility test methods as part of BLAs and BLA supplements, FDA may decide, as appropriate, to encourage the use of the compendial method as a benchmark or starting point for validation of novel methods and certain other methods.

(Comment 14) One comment requested clarification regarding validation of novel methods and any methods that deviate from the USP. This commenter stated that to validate novel test methods, "the sponsor not only has to test the matrix effects", but also has to validate the new method against the USP compendial method. The commenter also stated that this would impede the use of innovative technologies and increase the risk and cost to the sponsor. In addition, the commenter recommended that duplicative testing requirements be avoided and that the manufacturer of the technology or a third party be allowed to perform the validation of new methods.

(Response) The commenter misinterpreted the validation requirements under the proposed (and final) rule. The revisions we are adopting in the final rule do not require duplicative validation of novel methods against the USP compendial method or testing under a

separate validation procedure. Instead, novel methods and any methods that deviate from the USP compendial sterility test methods will require a single, detailed validation study to be conducted, which may include the use of the compendial method as a benchmark or starting point. We disagree that such validation will impede the use of innovative technologies and will increase the risk and cost to the sponsor. Instead, we believe that, as discussed elsewhere in this document and in the preamble to the proposed rule, that this final rule will encourage the use of innovative technology.

(Comment 15) One comment referenced the preamble statement that "* * * FDA may decide, as appropriate, to encourage the use of the compendial method as a benchmark or starting point for validation of novel methods and certain other methods." (76 FR 36019 at 36022) and suggested that the use of the compendial method as a benchmark or starting point should be more strongly encouraged.

(Response) While FDA may decide, as appropriate, to encourage the use of the compendial method as a benchmark or starting point for validation of some novel or other methods, we also may decide not to encourage such use for some (for example, non-culture-based) methods that cannot easily be compared to culture-based methods such as the USP compendial method. Therefore, we disagree that the use of the compendial method as a benchmark or starting point should be more strongly encouraged or required.

(Comment 16) We received two comments in response to our request in the proposed rule for comments on whether the proposed requirements are sufficient to ensure adequate validation of novel sterility test methods or whether additional criteria or guidance is needed. One comment recommended that any guidance to accompany the final rule be developed to include such things as a list of organisms for manufacturers to consider in the development of

their validation and verification plans, including examples of when verification is required. One comment suggested that such additional guidance include information related to a determination of the panel of relevant organisms in the sample matrix used in challenging the sterility test during validation.

(Response) We appreciate the interest in additional guidance for validation of novel sterility test methods and will consider the need to develop future guidance in accordance with the good guidance practices set out in 21 CFR 10.115.

As discussed in the preamble to the proposed rule, it is important to consider validation principles, such as limit of detection, specificity, ruggedness, and robustness, while developing the validation protocol and performing validation studies. These terms are defined as follows:

- The "limit of detection" reflects the lowest number of microorganisms that can be detected by the method in a sample matrix. This is necessary to define what is considered contaminated.
- "Specificity" is the ability of the test method to detect a range of organisms necessary for the method to be suitable for its intended use. This is demonstrated by challenging the sterility test with a panel of relevant organisms in the sample matrix.
- "Ruggedness" is the degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions, such as different analysts, different instruments, and different reagent lots.
- "Robustness" is the capacity of the test method to remain unaffected by small, but deliberate, variations in method parameters, such as changes in reagent concentration or incubation temperatures.

(Comment 17) One comment stated that for the detailed validation of a novel method, the validation principles should be restricted to the limit of detection, specificity, and robustness (i.e., to not include ruggedness).

(Response) We agree that the validation principles of limit of detection, specificity, and robustness are important to consider when developing protocols and performing validation studies. However, we understand the comment to suggest excluding ruggedness. We view ruggedness as an important validation principle to be considered, and we do not agree with excluding it from the scope of this rule. We note that the final rule does not include prescriptive details on how to conduct validation studies; it simply codifies our longstanding policy that the sterility test must be validated to demonstrate that the test is capable of reliably and consistently detecting the presence of viable contaminating microorganisms.

(Comment 18) One comment objected to the requirement in existing § 211.160(b) as to the establishment of sampling plans because "* * * it is not practical or feasible to develop a scientifically sound sampling plan to ensure a product conforms to standards of sterility." The comment recommended as a solution to either remove the requirement for scientific sampling plans with respect to sterility testing or to provide a clarification of "scientifically sound" versus "appropriate."

(Response) The suggested revisions go beyond the scope of the proposed changes to the sterility test requirements. Furthermore, § 211.160(b) is an existing current good manufacturing practice requirement for finished pharmaceuticals, which states that laboratory controls must include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of

identity, strength, quality, and purity. We consider such laboratory controls to be needed for both culture-based and non-culture-based sterility test methods. As stated in the preamble to the proposed rule (76 FR 36019 at 36022), the manufacturer must establish and document the test method's accuracy, sensitivity, specificity, and reproducibility (§ 211.165(e)), as specified in the BLA or BLA supplement (§§ 601.2, 601.12). For sterility tests, FDA believes that a validation protocol that would meet these standards would, at a minimum, include samples of the material to be marketed and incorporate appropriate viable contaminating microorganisms to demonstrate the sterility test's growth-promoting properties or the method's detection system capabilities, depending on the type of test method used. In addition, validation protocols for culture-based methods should include both aerobic and anaerobic microorganisms when selecting test organisms and include microorganisms that grow at differing rates so that manufacturers can establish that the test media are capable of supporting the growth of a wide range of microorganisms.

When utilizing culture-based methods, where appropriate, validation protocols should require that challenge organisms be added directly to the product prior to membrane filtration or direct inoculation. If this is not possible due to inhibition by the product, then validation protocols should require that the challenge organism be added to the final portion of sterile diluent used to rinse the filter, if a membrane filtration test method is used, or directly to the media containing the product if a direct inoculation test method is used.

For non-culture-based methods, the feasibility of identifying microorganisms from a contaminated sample should be evaluated during validation. If a method does not have the capability to identify microorganisms to the species level, the validation protocol should require that an additional method for species identification be utilized for investigation of detected

contaminants. The test organisms selected should reflect organisms that could be found in the product, process, or manufacturing environment.

(Comment 19) Two comments sought clarification of the following statement in the preamble to the proposed rule: "When utilizing culture-based methods, validation protocols should require that challenge organisms be added directly to the product prior to membrane filtration or direct inoculation. If this is not possible due to inhibition by the product, then validation protocols should require that the challenge organism be added to the final portion of sterile diluent used to rinse the filter if a membrane filtration test method is used, or directly to the media containing the product if a direct inoculation test method is used." (76 FR 36019 at 36022)

One commenter stated that this language is inconsistent with the harmonized compendial method suitability test which states, "After transferring the content of a container or containers to be tested to the membrane, add an inoculum of small number of viable microorganisms (not more that 100 colony-forming units) to the final portion of sterile diluents used to rinse the filter." Another comment sought clarification of the suggested limits for the density of the inoculum of challenge organisms added directly to the product.

(Response) The intent of these statements was to clarify that for certain biological products utilizing culture-based methods, method suitability testing necessitates adding the challenge organism directly to the product prior to membrane filtration or direct inoculation. Therefore, we are now clarifying that when utilizing culture-based methods, where appropriate, validation protocols should require that challenge organisms be added directly to the product before membrane filtration or direct inoculation. If this is not possible due to inhibition by the product, then validation protocols should require that the challenge organism be added to the

final portion of sterile diluent used to rinse the filter if a membrane filtration test method is used or directly to the media containing the product if a direct inoculation test method is used.

(Comment 20) One comment addressed the selection of organisms to be used. The comment suggested that with respect to validation protocols, for consistency, the wording regarding the selection of organisms should specifically include wild-type isolates that have been recovered from the controlled manufacturing environment and past contaminants of the product or any of its sterile components. The comment also suggested that this requirement should extend beyond culture-based methods. Further, the comment suggested that the statement in the preamble that "'The test organisms selected should reflect organisms that could be found in the product, process, or manufacturing environment (emphasis added)[76 FR 36019 at 36022],' should be tightened to require use of strains actually isolated from the product, process, or manufacturing environment, as the word 'reflect' probably implies use of relevant species that might be sourced from culture collections rather than explicitly requiring use of wild-type strains (plant isolates)."

(Response) Our intention with respect to this statement was to include those organisms recovered both from the controlled manufacturing environment and from the product. Furthermore, the preamble statement was intended to refer to validation protocols in general, where appropriate, to both culture-based and non-culture-based test methods.

The validation study design should contain the appropriate controls to evaluate the product sample's potential to generate false-positive and false-negative results. Validation of the sterility test should be performed on all new products, and repeated whenever there are changes in the test method or production method that could potentially inhibit or enhance detection of viable contaminating microorganisms.

(Comment 21) One comment recommended the addition of "or production method" to the statement in the preamble so that it would now read, "Validation of the sterility test should be performed on all new products, and repeated whenever there are changes in the test method or production method that could potentially inhibit or enhance detection of viable contaminating microorganisms." (See original statement 76 FR 36019 at 36022.) The commenter stated that the additional language is appropriate because the production process may influence the matrix of the test article, which may in turn influence the sterility test verification.

(Response) We agree that changes in the production method or manufacturing process could affect the results of testing conducted on the product. Therefore, we agree that validation of the sterility test should be performed on all new products and repeated whenever there are changes in the test method or production method that could potentially inhibit or enhance detection of viable contaminating microorganisms.

c. Verification--As stated in the proposed rule (76 FR 36019 at 36022), verification is the confirmation that specified requirements have been fulfilled as determined by examination and provision of objective evidence. While validation of a sterility test method is the initial process of demonstrating that the procedure is suitable to detect viable contaminating microorganisms, verification occurs over the lifetime of the sterility test method and is the process of confirming that the sterility test and test components continue to be capable of consistently detecting viable contaminating microorganisms in the samples analyzed. This verification activity may be necessary on a periodic basis or each time a sample is tested, depending upon the test method used. Under § 610.12(e) of the final rule, we require that the sterility test and test components be verified, as appropriate, to demonstrate that they can continue to consistently detect viable contaminating microorganisms.

(Comment 22) One comment maintained that the section of the preamble to the proposed rule regarding verification was not totally clear and should be reworded to explain the intended purpose. Specifically, the comment suggested, in order to clarify the goal of verification, adding the following sentence, "The intended purpose of the verification is to confirm that all the reagents utilized in the sterility test are qualified." The commenter also noted that validation is to be done using the product to be tested and proposed adding the phrase "in the product to be tested" to the following statement in the preamble "While validation of a sterility test method is the initial process of demonstrating that the procedure is suitable to detect viable contaminating microorganisms, verification occurs over the lifetime of the sterility test method and is the process of confirming that the sterility test and test components continue to be capable of consistently detecting viable contaminating microorganisms in the samples analyzed." (76 FR 36019 at 36022 to 36023)

(Response) To the extent that the commenter is arguing that our explanation is unclear, we disagree. As stated in the preamble to the proposed rule at section III.E (76 FR 36019 at 36022 to 36023), we believe that in order to verify the sterility test, verification activities are necessary to demonstrate that sterility test methods can continue to reliably and consistently detect viable contaminating microorganisms and that verification is the process of confirming that the sterility test and test components continue to be capable of consistently detecting viable contaminating microorganisms in the samples analyzed. In addition, we acknowledge that method suitability testing using the product is an important part of a validation protocol for a sterility test method.

3. What Information Is Needed in Written Procedures for Sterility Testing?

We have finalized, as proposed, the replacement of the requirements found in current

§ 610.12(c) entitled Interpretation of test results, with the requirements that manufacturers must establish, implement, and follow written procedures for sterility testing. Written procedures are essential to ensure consistency in sampling, testing, and interpretation of results and to provide prospective acceptance criteria for the sterility test. Written procedures should include all steps to be followed in the sterility test method for initial and repeat tests and be detailed, clear, and unambiguous. Under the current good manufacturing practice regulations, manufacturers are required to document that a drug product satisfactorily conforms to final specifications for the drug product (§ 211.165(a)). As such, scientifically sound and appropriate specifications, standards, sampling plans, and test procedures must be designed and written to ensure that materials conform to appropriate standards of sterility; and written procedures must include a description of the sampling method and the number of units per batch to be tested (see § 211.165(c)).

Under the final rule, manufacturers may use either culture-based or non-culture-based sterility test methods to evaluate material for sterility. There are marked differences between culture-based and non-culture-based sterility tests. Section 610.12(c) provides the minimum critical considerations that must be included in the written procedures for culture-based and non-culture-based sterility tests.

For culture-based sterility test methods, the written procedures must include, at a minimum, a description of the composition of the culture media, growth-promotion test requirements, and incubation conditions (time and temperature). For non-culture-based sterility test methods, the written procedures must include the composition of test components, test parameters, including the acceptance criteria, and the controls used to verify the test method's ability to consistently detect the presence of viable contaminating microorganisms.

4. What Is an Appropriate Sample for Sterility Testing?

Selection of an appropriate sample of a lot is critical for purposes of sterility testing. Under § 610.12(d) as finalized, due to the variety of products covered under § 610.12, the regulation requires that the sample be appropriate to the material being tested.

(Comment 23) Five comments requested clarification of the proposed requirement that the sample be "appropriate to the material being tested," with respect to the size or volume of the final product lot. The comments asserted that the example provided in the preamble of the proposed rule, "For example, a final product lot size of 100,000 units would necessitate a greater number of samples to be evaluated than a final product lot size of 5,000 units," (76 FR 36019 at 36023), conflicts with USP Chapter 71 regarding the minimum number of articles to be tested in relation to the number of articles in the batch.

(Response) We acknowledge that the example provided in the preamble of the proposed rule erroneously compared a final product lot size of 100,000 units to one of 5,000 units. We had intended to compare a final product lot size of 100,000 to one of 500 units. We recognize that this error may have caused confusion among some readers, and that the example was inconsistent with the USP Chapter 71 methods for the minimum number of articles to be tested in relation to the number of articles in the batch. It was not our intent to suggest that established USP compendial sterility test methods, including the minimal number of articles to be tested in relation to the number of articles in the batch, were unacceptable under the new requirements in § 610.12(d).

In order to clarify the new requirement that the sample be "appropriate to the material being tested," we reiterate that in selecting an appropriate sample size, § 610.12(d) requires that the following minimal criteria be considered:

- The size or volume of the final product lot. For example, a final product lot size of 100,000 units would necessitate a greater number of samples to be evaluated than a final product lot size of 500 units;
- The duration of manufacturing of the drug product.[7] For example, it is important that samples be taken at different points of manufacture, which, at a minimum, should include the beginning, middle, and end of manufacturing, in an effort to provide evidence of sterility of the drug product throughout the duration of the manufacturing process;[8]
- The final container configuration and size. We believe this will ensure appropriate representation of the lot;
- The quantities or concentrations of inhibitors, neutralizers, and preservatives, if present, in the test material;
- For a culture-based test method, the volume of test material that results in a dilution of the product that was determined not to be bacteriostatic or fungistatic; and
- For a non-culture-based test method, the volume of test material that results in a dilution of the product that does not inhibit or otherwise hinder the detection of viable contaminating microorganisms.

(Comment 24) Two comments stated that the proposed changes related to sample size are vague and leave too much room for interpretation by industry as well as investigators or auditors when determining an appropriate sample size.

(Response) We disagree that requiring the sample to be appropriate to the material being tested is vague and leaves too much open to interpretation. Our intent in requiring that the

[7] See § 210.3(b)(4) for the definition of the term "drug product."
[8] See § 211.160(b) for general requirements for laboratory controls.

sample be "appropriate to the material being tested," with consideration of a list of minimal criteria, is to provide manufacturers flexibility to retain their existing procedures for sterility testing using culture-based methods, or to take advantage of modern methods as they become available, provided that these modern methods meet certain criteria, as described in our response to Comment 23. In addition, as noted previously, sterility test methods are approved by FDA in either a manufacturer's BLA or BLA supplement, thereby alleviating concern that the final rule leaves too much room for interpretation.

(Comment 25) One comment asked FDA to clarify whether the quantities or concentrations of inhibitors, neutralizers, and preservatives, if present in the test material, have an impact on sample size and selection. The comment also asked about the relationship between the impact of preservatives and any increase in the sample size.

(Response) In selecting an appropriate sample size, § 610.12(d) requires consideration of certain minimal criteria, including the quantities or concentrations of inhibitors, neutralizers, and preservatives, if present in the test material. The consideration of the quantities or concentrations of inhibitors, neutralizers, and preservatives, if present in the test material, will depend upon the product and the test method utilized. This provides both manufacturers of future innovative products, as well as manufacturers of currently approved products, the flexibility to take advantage of modern methods or to retain the sterility testing method as approved in the BLA or BLA supplement.

5. What Is Required to Verify the Sterility Test?

As discussed in the preamble to the proposed rule (76 FR 36019 at 36023), verification activities are necessary to demonstrate that sterility test methods can continue to reliably and consistently detect viable contaminating microorganisms. The degree of verification that is

necessary depends upon the sterility test method employed. Depending upon the sterility test method, verification of each individual test might be appropriate. On the other hand, some sterility test methods may only need verification activities performed on the selected culture media or test organisms. Under § 610.12(e), a manufacturer must perform verification activities appropriate for the sterility test method chosen, as set forth in the final rule.

(Comment 26) In the proposed rule (76 FR 36019 at 36020, footnote 6), we proposed to refer to "growth-promoting properties" rather than "growth-promoting qualities" and requested comments on which term is most appropriate. We received two comments in response to our request. Both comments support the use of "growth-promoting properties" and agree that "growth-promoting properties" reflects more accurate and current terminology.

(Response) We appreciate and agree with these comments and have retained the term "growth-promoting properties" in the final rule.

(Comment 27) Two comments requested clarification of the requirements for verification of culture-based test methods. One comment asked if, for culture-based test methods, all media must undergo growth-promotion testing over their shelf-life, and if validation were performed for three lots, whether it is acceptable to perform growth-promotion testing on the media only when it is initially received. One comment acknowledged that each media lot would have to be tested for growth-promotion at least at the beginning and the end of its use; however, the comment sought clarification whether companies would be expected to keep performing the test at regular intervals.

(Response) For culture-based methods, it is important that each lot of all culture media undergo growth-promotion testing at regular intervals over the shelf-life of the media, not just when the media is initially received. The final rule requires that the sterility test and test

components be verified, as appropriate, to demonstrate that they can continue to consistently detect viable contaminating microorganisms. The degree of verification depends upon the sterility test method employed.

For culture-based test methods, studies must be conducted to demonstrate that the performance of the test organisms and culture media are suitable to consistently detect the presence of viable contaminating microorganisms, including tests for each lot of culture media to verify its growth-promoting properties over the shelf-life of the media and not only at the beginning and end of use. Growth-promotion testing is important to demonstrate that the culture media are capable of supporting the growth of microorganisms.

(Comment 28) One comment recommended that with the proposal to remove the definition of a lot of culture medium currently defined in § 610.12(e)(2)(i), revisions to the rule should clearly state that each delivery of each vendor lot of media be "QC tested" by the end user to verify its ability to detect viable microorganisms. The comment states, "It must be made clear that the vendor cannot be totally in control of the product once it has been shipped from the distribution centre." Further, the comment states it is the user's responsibility to test each delivery of each vendor lot to ensure that undetected mistreatment of the testing product during its shipment and delivery to the end-user has not caused deterioration in its efficacy.

(Response) We agree that the user of the culture media must verify that each lot can continue to consistently detect viable contaminating microorganisms. For the reasons noted previously, we do not believe the suggested changes are needed because the rule, as proposed and now finalized, already reflects this requirement.

(Comment 29) One comment stated that usually validation data provided by the media suppliers are used to cover the shelf-life of the media and proposed adding the following text "or

media supplier validation data must be available" after the text "over the shelf-life of the media" in proposed § 610.12(e)(1) to capture the fact that the supplier of the media may also supply this parameter.

(Response) We do not agree that reliance on media supplier validation data alone, in lieu of testing by the manufacturer, would be acceptable. Under § 610.12(e)(1) of the final rule, for culture-based test methods, manufacturers must conduct tests to demonstrate that the performance of the test organisms and culture media are suitable to consistently detect the presence of viable contaminating microorganisms, including tests for each lot of culture media to verify its growth-promoting properties over the shelf-life of the media. Therefore, reliance on media supplier validation data alone, in lieu of testing by the manufacturer, would not be acceptable.

6. Can a Sterility Test Be Repeated?

For the reasons discussed in the preamble to the proposed rule (76 FR 36019 at 36023 to 36024), we have amended the regulations in § 610.12(b) for repeat testing. Therefore, we have eliminated the reference to repeat testing of bulk material because, under the final rule, sterility testing is no longer required on bulk material in most instances. We also have finalized the proposal to eliminate the use of a second repeat test for final container material to harmonize our regulatory expectations with current scientific understanding of quality manufacturing controls.[9] Under the final rule, consistent with USP Chapter 71, if the initial test indicates the presence of microorganisms, then the product being examined does not comply with the sterility test requirements, unless a thorough investigation by the quality control unit can conclusively ascribe

[9] See also Barr D., A. Celeste, R. Fish, et al., Application of Pharmaceutical CGMPs; FDLI (1997) at p. 146 ("In the case of a clearly identified laboratory error, the retest results substitute for the original test results. * * * If, on the other hand, no laboratory error could be identified in the first test, then there is no scientific basis for discarding the initial out-of-specification results in favor of passing retest results.").

the initial evidence of microbial presence to a laboratory error or faulty materials used in conducting the test.

If the test of the initial sample is conclusively found to be invalid, due to laboratory error or faulty test materials, the sterility test may be repeated one time. If no evidence of microorganisms is found in the repeat test, the product examined complies with the test requirements for sterility. If, however, evidence of microorganisms is found in the repeat test, the product examined does not comply with the test requirements for sterility.

Further, as discussed in the preamble to the proposed rule, both a comparable product that is reflective of the initial sample in terms of sample location and the stage in the manufacturing process from which it was taken, and the same sterility test method must be used for both the initial and repeat tests. This is intended to ensure that the same volume of material is used for the initial test and each repeat test, and that the interpretation of the results is conducted in the same manner.

(Comment 30) One comment supported FDA's proposal to modify the provision for repeat testing to harmonize regulatory expectations with current scientific understanding of quality manufacturing controls by eliminating the use of a second repeat test of final container material and agreed with FDA that the proposed modification of the provision for repeat testing is in accordance with the USP and the European Pharmacopeia. However, the commenter noted that FDA's proposed requirement to take repeat test samples that are reflective of the initial samples may be difficult to fulfill. For instance, the commenter states, "* * * at the time when the sterility test might show a positive result (after a few days), it could be that it is no longer possible to distinguish which vials were filled at which point in time." The comment suggested deleting the requirement in proposed § 610.12(f)(3) that the repeat test must be conducted with

"comparable product that is reflective of the initial sample in terms of sample location and the stage in the manufacturing process from which it was obtained."

(Response) We appreciate the supportive comments. However, we do not agree with the recommended change to § 610.12(f)(3). We believe the final rule is consistent with current scientific understanding of quality manufacturing controls. If a repeat test is conducted, the same test method must be used for both the initial and repeat tests, and the repeat test must be conducted with comparable product that is reflective of the initial sample in terms of sample location and the stage in the manufacturing process from which it was obtained.

As discussed in the preamble to the proposed rule, we appreciate that this final rule could result in the need for some manufacturers to modify their repeat test procedures. We continue to consider these modifications to be minor changes in accordance with § 601.12(d) and to have a minimal potential for an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product. Therefore, such changes must be reported in the annual report within 60 days of the anniversary date of approval of the BLA.

7. What Records Must Be Kept Relating to Sterility Testing?

Previously, § 610.12(h) incorporated by reference the record keeping and maintenance requirements contained in §§ 211.167 and 211.194. We continue to maintain these requirements. As discussed in the preamble to the proposed rule (76 FR 36019 at 36024), this is intended to assure that data derived from sterility tests comply with established specifications. This includes describing the samples received for testing, stating the method used to test the samples, identifying the location of relevant validation or verification data, recording all calculations performed, and stating how the results of tests performed compare to set specifications.

8. Are There Any Exceptions to Sterility Test Requirements?

In the proposed rule we invited comments on whether any of the current exceptions should be removed (76 FR 36019 at 36024). We specifically requested comments on whether to remove the exemption for platelets. Bacterial contamination of platelets is a recognized public health risk, and the blood collection industry has already called for and implemented methods to detect and limit or inactivate bacteria in platelet components. Requiring testing for platelets would be consistent with these industry practices.

(Comment 31) In response to our request for comment, a joint comment from industry groups recommended that FDA continue to except Whole Blood, Cryoprecipitated Antihemophilic Factor (AHF), Platelets, Red Blood Cells, and Plasma from the sterility test requirements in § 610.12. The comment acknowledged that the blood industry has called for and implemented methods to detect and limit or inactivate bacteria in platelet components and that some culture-based methods are in wide use as a quality control tool. However, there are currently no available tests that will ensure the sterility of platelet products. In addition, the joint comment noted that if the current exception for platelets would be removed, manufacturers of blood and blood components would not be able to satisfy the new requirement. Further, the comment recommended that FDA vigorously support applications for pathogen inactivation processes for platelet components. Moreover, the joint comment noted that any sterility test requirement tied to a BLA is too narrow an approach to ensure optimal bacterial testing of platelet products, as any platelet collected or manufactured by a facility that does not have a BLA would not be subject to the sterility test regulation. Accordingly, the joint comment recommended that FDA use a different mechanism to require testing of all platelet products for bacterial contamination when testing becomes technologically feasible.

(Response) We appreciate these comments and we generally agree. We recognize that blood establishments have begun to take steps to test for bacterial contamination in platelet components. We welcome the acknowledgement of the importance of bacterial testing and pathogen inactivation processes for platelet components and believe that appropriate microbial testing of platelet components may be necessary to assure product quality. However, while these technologies are developing, we have retained the exception from this rule for these products. Instead, we will continue to review these issues and available technologies and will take appropriate steps at another time to address microbial testing of blood components.

(Comment 32) One comment recommended adding an exception stating that a manufacturer with parametric release programs is not required to comply with the sterility test requirements. The comment noted that parametric release for articles sterilized with moist heat has been recognized by FDA since 1987, and that many companies have adopted this approach.

(Response) We disagree with the proposed change and decline to add an exception for drug products terminally sterilized by moist heat processes and subject to parametric release because the exception under § 610.12(h) (previously under § 610.12(g)) already provides for an exception for such parametric release programs. As noted in FDA's guidance document entitled "Guidance for Industry: Submission of Documentation in Applications for Parametric Release of Human and Veterinary Drug Products Terminally Sterilized by Moist Heat Processes," dated February 2010, FDA approval of parametric release must be requested either in an original application submission under 21 CFR 314.50 or § 601.2, or in a prior approval supplement under 21 CFR 314.70 or § 601.12.

(Comment 33) Two comments recommended adding other exceptions to the sterility test requirements. One comment recommended adding granulocytes to the exception, and one

comment recommended adding in vitro diagnostic devices regulated as biological products, which do not purport to be sterile.

(Response) We decline to adopt the suggested changes because neither granulocytes nor in vitro diagnostic devices, which do not purport to be sterile, are subject to the sterility test requirements in § 610.12. Therefore, we believe the recommendations are beyond the scope of this rule.

(Comment 34) One comment recommended that the exceptions provision be revised to "specifically include or exclude various biological product types such as Bioequivalent/Biosimilars and combination products."

(Response) We do not believe the suggested change is needed. Biological products must comply with the applicable requirements in parts 600 through 680, in addition to other applicable regulations.

For the reasons discussed in the preamble to the proposed rule (76 FR 36019 at 36024), we have finalized the proposed minor modifications to the current exception in § 610.12(g)(4)(ii), under which the Director of CBER or CDER, as appropriate, determines that data submitted adequately establish that the mode of administration, the method of preparation, or the special nature of the product precludes or does not require a sterility test or that the sterility of the lot is not necessary to assure the safety, purity, and potency of the product. Specifically, the minor modification that we refer to is the "route of administration" rather than the "mode of administration" and to "any other aspect of the product" rather than "the special nature of the product" in finalized § 610.12(h)(2) so as to account for novel products that may be introduced to the market in the future. This exception allows the Director of CBER or CDER, as appropriate, to exempt biological material from the sterility test requirements of this section if,

based upon the scientific evidence presented in the BLA or BLA supplement, the data adequately establish that the route of administration, method of preparation, or any other aspect of the product precludes or does not necessitate a sterility test to assure the safety, purity, and potency of the product. We note that in the proposed rule, the Center for Devices and Radiological Health was erroneously identified in this exception, instead of CDER. In the final rule, we have correctly identified CDER in the exception provision at § 610.12(h)(2).

In addition to comments regarding exceptions as stated in this document, we have also eliminated, as proposed, the current exceptions under § 610.12(g)(1) and (2) because they are no longer necessary given the flexibility now built into the final rule. In addition, we have eliminated, as proposed, the current exceptions in § 610.12(g)(5) through (g)(9) because they are no longer necessary and because the revised rule now requires manufacturers to determine the appropriate sample volume and size for the material being tested and requires that the sterility test be "appropriate to the material being tested." (See 76 FR 36019 at 36024 to 36025 for more information.)

IV. Revisions to Other Regulations

In addition to the revisions to the sterility regulation in § 610.12, we have also revised, as proposed, two other FDA regulations in this final rule. These revisions are as follows:

- Section 600.3(q): Previously, § 600.3(q) defined "sterility" to mean "freedom from viable contaminating microorganisms, as determined by the tests prescribed in § 610.12 of this chapter." As proposed, we have reworded this definition to eliminate the term "prescribed" since § 610.12 no longer prescribes specific test methods. Thus, we have amended § 600.3(q) to define "sterility" as "freedom from viable

contaminating microorganisms, as determined by tests conducted under § 610.12 of this chapter."

- Section 680.3(c) (21 CFR 680.3(c)): As proposed, we have amended § 680.3(c) to eliminate the term "prescribed." Section 680.3(c) now states that "A sterility test shall be performed on each lot of each Allergenic Product, as required by § 610.12 of this chapter." Additionally, we have eliminated § 680.3(c)(1) through (c)(4) because these exceptions are no longer necessary under the revisions to § 610.12. (See 76 FR 36019 at 36025 for more information.)

V. Legal Authority

FDA is issuing this regulation under the biological products provisions of the Public Health Service Act (the PHS Act) (42 U.S.C. 262 and 264) and the drugs and general administrative provisions of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) (sections 201, 301, 501, 502, 503, 505, 510, 701, and 704) (21 U.S.C. 321, 331, 351, 352, 353, 355, 360, 371, and 374). Under these provisions of the PHS Act and the FD&C Act, we have the authority to issue and enforce regulations designed to ensure that biological products are safe, effective, pure, and potent, and to prevent the introduction, transmission, and spread of communicable disease.

VI. Analysis of Impacts

FDA has examined the impacts of the final rule under Executive Order 12866, Executive Order 13563, the Regulatory Flexibility Act (5 U.S.C. 601-612), and the Unfunded Mandates Reform Act of 1996 (Public Law 104-4). Executive Orders 12866 and 13563 direct Agencies to assess all costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential

economic, environmental, public health and safety, and other advantages; distributive impacts; and equity). The Agency believes that this final rule is not a significant regulatory action under Executive Order 12866.

The Regulatory Flexibility Act requires Agencies to analyze regulatory options that would minimize any significant impact of a rule on small entities. While the rule restricts retesting when sterility tests are failed, the change codifies an approach for retesting that is similar to the approach prescribed by the USP. The rule does not otherwise add any new regulatory responsibilities and generally increases flexibility for sterility testing. Therefore, the Agency certifies that the final rule will not have a significant economic impact on a substantial number of small entities.

Section 202(a) of the Unfunded Mandates Reform Act of 1995 requires that Agencies prepare a written statement, which includes an assessment of anticipated costs and benefits, before proposing "any rule that includes any Federal mandate that may result in the expenditure by State, local, and tribal governments, in the aggregate, or by the private sector, of $100,000,000 or more (adjusted annually for inflation) in any one year." The current threshold after adjustment for inflation is $136 million, using the most current (2010) Implicit Price Deflator for the Gross Domestic Product. FDA does not expect this final rule to result in any 1-year expenditure that would meet or exceed this amount.

These amendments would generally provide manufacturers of biological products with more flexibility as to how they evaluate the sterility of their products and reduce the number of evaluations required. The net effect would be to reduce costs.

One part of these amendments might impose some additional costs on manufacturers, however. Under the current regulations, if a biological product fails a sterility test, the test may

be repeated. If the product passes a subsequent test, it is inferred that the first test was flawed and only the latter results are used. Under the new regulations, the test may be repeated only if it is possible to "ascribe definitively" the initial failure to "a laboratory error or faulty materials used in conducting the sterility testing."

This change could increase costs for manufacturers because additional products could be discarded. The size of the increase, if any, would be determined by the number of additional lots discarded, the lot sizes, and the production costs per unit. Some or all of the costs of this change, could, in turn, be mitigated by the reduction in losses associated with the provision of contaminated products.

This change is expected to affect few manufacturers. The method for sterility testing described in USP Chapter 71 already limits the repetition of tests to circumstances similar to those described in these amendments. It is anticipated that, in the absence of these amendments, the majority of manufacturers would limit the repetition of sterility tests in order to comply with USP Chapter 71.

The benefit of limiting retests would be fewer illnesses caused by contaminated biological products. We are unable to quantify the value of the reduction in illnesses because we do not have an estimate of the risk of illness from contaminated biological products or the decline in that risk associated with limiting retests.

VII. Environmental Impact

The Agency has determined under 21 CFR 25.31(h) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

VIII. Federalism

FDA has analyzed this final rule in accordance with the principles set forth in Executive Order 13132. FDA has determined that the rule does not contain policies that have substantial direct effects on the States, on the relationship between the National Government and the States, or on the distribution of power and responsibilities among the various levels of government. Accordingly, the Agency has concluded that the rule does not contain policies that have federalism implications as defined in the Executive order and, consequently, a federalism summary impact statement is not required.

IX. The Paperwork Reduction Act of 1995

This final rule contains collections of information that were submitted for review and approval to the Director of the Office of Management and Budget (OMB), as required by section 3507(d) of the Paperwork Reduction Act of 1995 (44 U.S.C. 3501-3520). The collections of information in §§ 211.165 and 610.12 have been approved and assigned OMB control number 0910-0139.

List of Subjects

21 CFR Part 600

Biologics, Reporting and recordkeeping requirements.

21 CFR Part 610

Biologics, Labeling, Reporting and recordkeeping requirements.

21 CFR Part 680

Biologics, Blood, Reporting and recordkeeping requirements.

Therefore, under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, and under the authority delegated to the Commissioner of Food and Drugs, 21 CFR parts 600, 610, and 680 are amended as follows:

PART 600--BIOLOGICAL PRODUCTS: GENERAL

1. The authority citation for 21 CFR part 600 continues to read as follows:

Authority: 21 U.S.C. 321, 351, 352, 353, 355, 360, 360i, 371, 374; 42 U.S.C. 216, 262, 263, 263a, 264, 300aa-25.

§ 600.3 [Amended]

2. Section 600.3 is amended in paragraph (q) by removing "prescribed in" and by adding in its place the phrase "conducted under".

PART 610--GENERAL BIOLOGICAL PRODUCTS STANDARDS

3. The authority citation for 21 CFR part 610 continues to read as follows:

Authority: 21 U.S.C. 321, 331, 351, 352, 353, 355, 360, 360c, 360d, 360h, 360i, 371, 372, 374, 381; 42 U.S.C. 216, 262, 263, 263a, 264.

4. Section 610.12 is revised to read as follows:

§ 610.12 Sterility.

(a) The test. Except as provided in paragraph (h) of this section, manufacturers of biological products must perform sterility testing of each lot of each biological product's final container material or other material, as appropriate and as approved in the biologics license application or supplement for that product.

(b) Test requirements. (1) The sterility test must be appropriate to the material being tested such that the material does not interfere with or otherwise hinder the test.

(2) The sterility test must be validated to demonstrate that the test is capable of reliably and consistently detecting the presence of viable contaminating microorganisms.

(3) The sterility test and test components must be verified to demonstrate that the test method can consistently detect the presence of viable contaminating microorganisms.

(c) Written procedures. Manufacturers must establish, implement, and follow written procedures for sterility testing that describe, at a minimum, the following:

(1) The sterility test method to be used;

(i) If culture-based test methods are used, include, at a minimum:

(A) Composition of the culture media;

(B) Growth-promotion test requirements; and

(C) Incubation conditions (time and temperature).

(ii) If non-culture-based test methods are used, include, at a minimum:

(A) Composition of test components;

(B) Test parameters, including acceptance criteria; and

(C) Controls used to verify the method's ability to detect the presence of viable contaminating microorganisms.

(2) The method of sampling, including the number, volume, and size of articles to be tested;

(3) Written specifications for the acceptance or rejection of each lot; and

(4) A statement of any other function critical to the particular sterility test method to ensure consistent and accurate results.

(d) The sample. The sample must be appropriate to the material being tested, considering, at a minimum:

(1) The size and volume of the final product lot;

(2) The duration of manufacturing of the drug product;

(3) The final container configuration and size;

(4) The quantity or concentration of inhibitors, neutralizers, and preservatives, if present, in the tested material;

(5) For a culture-based test method, the volume of test material that results in a dilution of the product that is not bacteriostatic or fungistatic; and

(6) For a non-culture-based test method, the volume of test material that results in a dilution of the product that does not inhibit or otherwise hinder the detection of viable contaminating microorganisms.

(e) Verification. (1) For culture-based test methods, studies must be conducted to demonstrate that the performance of the test organisms and culture media are suitable to consistently detect the presence of viable contaminating microorganisms, including tests for each lot of culture media to verify its growth-promoting properties over the shelf-life of the media.

(2) For non-culture-based test methods, within the test itself, appropriate controls must be used to demonstrate the ability of the test method to continue to consistently detect the presence of viable contaminating microorganisms.

(f) Repeat test procedures. (1) If the initial test indicates the presence of microorganisms, the product does not comply with the sterility test requirements unless a thorough investigation by the quality control unit can ascribe definitively the microbial presence to a laboratory error or faulty materials used in conducting the sterility testing.

(2) If the investigation described in paragraph (f)(1) of this section finds that the initial test indicated the presence of microorganisms due to laboratory error or the use of faulty materials, a sterility test may be repeated one time. If no evidence of microorganisms is found in the repeat test, the product examined complies with the sterility test requirements. If evidence of

microorganisms is found in the repeat test, the product examined does not comply with the sterility test requirements.

(3) If a repeat test is conducted, the same test method must be used for both the initial and repeat tests, and the repeat test must be conducted with comparable product that is reflective of the initial sample in terms of sample location and the stage in the manufacturing process from which it was obtained.

(g) Records. The records related to the test requirements of this section must be prepared and maintained as required by §§ 211.167 and 211.194 of this chapter.

(h) Exceptions. Sterility testing must be performed on final container material or other appropriate material as defined in the approved biologics license application or supplement and as described in this section, except as follows:

(1) This section does not require sterility testing for Whole Blood, Cryoprecipitated Antihemophilic Factor, Platelets, Red Blood Cells, Plasma, Source Plasma, Smallpox Vaccine, Reagent Red Blood Cells, Anti-Human Globulin, and Blood Grouping Reagents.

(2) A manufacturer is not required to comply with the sterility test requirements if the Director of the Center for Biologics Evaluation and Research or the Director of the Center for Drug Evaluation and Research, as appropriate, determines that data submitted in the biologics license application or supplement adequately establish that the route of administration, the method of preparation, or any other aspect of the product precludes or does not necessitate a sterility test to assure the safety, purity, and potency of the product.

PART 680--ADDITIONAL STANDARDS FOR MISCELLANEOUS PRODUCTS

5. The authority citation for 21 CFR part 680 continues to read as follows:

Authority: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371; 42 U.S.C. 216, 262, 263, 263a, 264.

6. Section 680.3 is amended by revising paragraph (c) to read as follows:

§ 680.3 Tests.

* * * * *

(c) Sterility. A sterility test shall be performed on each lot of each Allergenic Product as required by § 601.12 of this chapter.

Dated: April 27, 2012.

Leslie Kux,

Assistant Commissioner for Policy.

[FR Doc. 2012-10649 Filed 05/02/2012 at 8:45 am; Publication Date: 05/03/2012]