# Guidance for Industry

## for the Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products

**Center for Drug Evaluation and Research (CDER)**
**Center for Veterinary Medicine (CVM)**

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# GUIDANCE FOR INDUSTRY[1]

# FOR THE SUBMISSION OF

# DOCUMENTATION FOR STERILIZATION PROCESS VALIDATION IN APPLICATIONS FOR HUMAN AND VETERINARY DRUG PRODUCTS

## I. INTRODUCTION

### A. Purpose

This document is intended to provide guidance for the submission of information and data in support of the efficacy of sterilization processes in drug applications for both human and veterinary drugs. The recommendations in the guidance apply to applications for sterile drug products (new drug applications, new animal drug applications, abbreviated new drug applications, abbreviated antibiotic applications, and abbreviated new animal drug applications). These recommendations also apply to previously approved applications when supplements associated with the sterile processing of approved drugs are submitted. Information and data in support of sterility assurance may also be necessary in investigational new drug and investigational new animal drug applications.

In the FEDERAL REGISTER of October 11, 1991 (56 FR 51354), the agency published a proposed rule entitled "Use of Aseptic Processing and Terminal Sterilization in the Preparation of Sterile Pharmaceuticals

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[1]This guidance has been prepared by the Sterility Technical Committee of the Chemistry Manufacturing Controls Coordinating Committee of the Center for Drug Evaluation and Research (CDER), and the Center for Veterinary Medicine (CVM), at the Food and Drug Administration. Although this guidance does not create or confer any rights for or on any person and does not operate to bind FDA or the industry, it does represent the agency's current thinking on sterilization process validation documentation. For additional copies of this guidance, contact the Division of Communications Management, HFD-210, CDER, FDA, 5600 Fishers Lane, Rockville, MD 20857 (Phone: 301-594-1012) Send one self-addressed adhesive label to assist the office in processing your request. An electronic version of this guidance is also available via Internet via World Wide Web (WWW) (connect to the FDA Home Page at WWW.FDA.GOV/CDER and go to the "Regulatory Guidance" section).

for Human and Veterinary Use." This guidance is not a substitution for or a supplement to that proposed rule. Regardless of whether the applicant uses terminal sterilization or aseptic processing to manufacture a drug product that is purported to be sterile, certain information about the validation of that process should be submitted for both of those types of sterilization.

## B. Documenting Sterilization Process Validation

The efficacy of a given sterilization process for a specific drug product is evaluated on the basis of a series of protocols and scientific experiments designed to demonstrate that the sterilization process and associated control procedures can reproducibly deliver a sterile product. Data derived from experiments and control procedures allow conclusions to be drawn about the probability of nonsterile product units (sterility assurance level). Based on the scientific validity of the protocols and methods, as well as on the scientific validity of the results and conclusions, the agency concludes that the efficacy of the sterilization process is validated. Whether a drug product is sterilized by a terminal sterilization process or by an aseptic filling process, the efficacy of the sterilization process may be validated without the manufacture of three production batches. Sterilization process validation data, however, should be generated using procedures and conditions that are fully representative and descriptive of the procedures and conditions proposed for manufacture of the product in the application.

The Center for Drug Evaluation and Research's (CDER's) and the Center for Veterinary Medicine's (CVM's) review of the validation of the sterilization process consists of a scientific evaluation of the studies submitted in the applications. This review is conducted by FDA's review staff, and is part of a cooperative effort between the review staff, compliance staff, and field investigators to ensure the overall state of control of the sterile processing of human and veterinary drug products. Information and data in support of sterility assurance may be provided directly to the application or by specific reference to a drug master file (DMF), a veterinary master file (VMF), or another application. Letters of authorization to refer to the referenced files should be included.

## C. Remarks

This guidance is intended to provide recommendations for the types of information applicants should include in human and animal drug applications. Regulatory requirements for the submission of information and data in various applications are specified in the sections listed below:

1. Human Drugs:

| | |
|---|---|
| Investigational new drug applications | 21 CFR 312.23(a)(7) |
| New drug applications | 21 CFR 314.50 |
| Abbreviated new drug and abbreviated antibiotic drug applications | 21 CFR 314.94 and 314.50 |
| Supplements to NDA's and ANDA's | 21 CFR 314.70 |

2. Animal Drugs:

| | |
|---|---|
| Investigational new animal drug applications | 21 CFR Part 511 |
| New animal drug applications | 21 CFR 514.1 |
| Supplements to NADA's | 21 CFR 514.8 |

## II. INFORMATION FOR TERMINAL MOIST HEAT STERILIZATION PROCESSES

The following types of information should be submitted in support of sterility assurance for products produced using terminal moist heat sterilization. Although the following outline directly addresses moist heat processes, the same types of information would generally pertain to other terminal sterilization processes (e.g., ethylene oxide or radiation). (See section III of this guidance.) The following information should be submitted for each facility to be used in the manufacture of the proposed drug product:

### A. Description of the Process and Product

1. The Drug Product and Container-Closure System

   Descriptions of the drug product and the container-closure system(s) to be sterilized (e.g., size(s), fill volume, or secondary packaging).

2. The Sterilization Process

   A description of the sterilization process used to sterilize the drug product in its final container-closure system, as well as a description of any other sterilization process(es) used to sterilize delivery sets, components, packaging, bulk drug substance or bulk product, and related items. Information and data in support of the efficacy of these processes should also be submitted. (See also sections II.B. and II.C. of this guidance.)

3. The Autoclave Process and Performance Specifications

A description of the autoclave process, including pertinent information such as cycle type (e.g., saturated steam, water immersion, and water spray), cycle parameters and performance specifications including temperature, pressure, time, and minimum and maximum $F_o$. Identify the autoclave(s) to be used for production sterilization, including manufacturer and model.

4. Autoclave Loading Patterns

A description of representative autoclave loading patterns should be provided.

5. Methods and Controls to Monitor Production Cycles

Methods and controls used to monitor routine production cycles (e.g., thermocouples, pilot bottles, and biological indicators) should be described, including the number and location of each as well as acceptance and rejection specifications.

6. Requalification of Production Autoclaves

A description of the program for routine and unscheduled requalification of production autoclaves, including frequency, should be provided.

7. Reprocessing

A description and validation summary of any program that provides for reprocessing (e.g., additional thermal processing) of product should be provided. Please note that the stability program is also affected by additional thermal processing. For further information concerning the stability program, reference is made to the Center for Drug Evaluation and Research "Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics" and to the Center for Veterinary Medicine "Drug Stability Guideline."

## B. Thermal Qualification of the Cycle

1. Heat Distribution and Penetration Studies

Heat distribution and penetration study protocols and data

summaries that demonstrate the uniformity, reproducibility, and conformance to specifications of the production sterilization cycle should be provided. Results from a minimum of three consecutive, successful cycles should be provided to ensure that the results are consistent and meaningful.

2. Thermal Monitors

The number of thermal monitors used and their location in the chamber should be described. A diagram is helpful.

3. The Effects of Loading on Thermal Input

Data should be generated with minimum and maximum load to demonstrate the effects of loading on thermal input to product. Additional studies may be necessary if different fill volumes are used in the same container line. Data summaries are acceptable for these purposes. A summary should consist of, for example, high and low temperatures (range), average temperature during the dwell period, minimum and maximum $F_0$ values, dwell time, run date and time, and identification of the autoclave(s) used. These data should have been generated from studies carried out in production autoclave(s) that will be used for sterilization of the product that is the subject of the application.

4. Information Included in the Batch Record

The batch record supplied with the chemistry, manufacturing, and controls section of the application should identify the validated processes to be used for sterilization and for depyrogenation of any container-closure components. This information can be included in the batch record by reference to the validation protocol or standard operating procedure (SOP). Validation information should be provided as described above.

## C. Microbiological Efficacy of the Cycle

Validation studies that demonstrate the efficacy (lethality) of the production cycle should be provided. A sterility assurance of $10^{-6}$ or better should be demonstrated for any terminal sterilization process. This level of sterility assurance should be demonstrated for all parts of the drug product (including the container and closure, if applicable), which are claimed to be sterile. The specific type of study and the methods used to carry out the study (or studies) are product and process specific

and may vary from manufacturer to manufacturer. In general, the following types of information and data should be provided.

1. Identification and Characterization of Bioburden Organisms

   Describe the methods and results from studies used to identify and characterize bioburden organisms. The amount and type of information supplied may be dependent on the validation strategy chosen. For example, more information may be needed for bioburden-based autoclave processes than for overkill processes. Information concerning the number, type, and resistance of bioburden organisms may be necessary, including those organisms associated with the product solution and the container and closure. It may be necessary to identify the most heat-resistant bioburden organisms.

2. Specifications for Bioburden

   Specifications (alert and action levels) for bioburden should be provided. A description should be included of the program for routinely monitoring bioburden to ensure that validated and established limits are not exceeded (e.g., frequency of analysis and methods used in bioburden screening). The methods provided should be specific.

3. Identification, Resistance, and Stability of Biological Indicators

   Information and data concerning the identification, resistance (D and Z values), and stability of biological indicators used in the biological validation of the cycle should be provided. If biological indicators are purchased from a commercial source, it may be necessary to corroborate the microbial count and resistance, and provide performance specifications.

4. The Resistance of the Biological Indicator Relative to That of Bioburden

   Studies characterizing the resistance of the biological indicator relative to that of bioburden may be necessary. Resistance in or on the product (i.e., in the product solution, or on the surface of container or closure parts or interfaces) should be determined as necessary. If spore carriers are used (e.g., spore strips), the resistance of spores on the carrier relative to that of directly inoculated product should be determined, if necessary.

5. Microbiological Challenge Studies

Microbiological validation studies should be submitted that demonstrate the efficacy of the minimum cycle to provide a sterility assurance of $10^{-6}$ or better to the product under the most difficult to sterilize conditions (e.g., the most difficult to sterilize load with biological indicators at microbiological master sites or in master product or both). Use of a microbiological master product or site should be supported by scientific data. Microbiological master sites or solutions are those sites or solutions in which it is most difficult to kill the biological indicator under sterilization cycles that simulate production conditions.

## D. Microbiological Monitoring of the Environment

Section 211.160 of the Code of Federal Regulations requires, in part, the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to ensure that components, drug product containers, closures, in-process materials, and drug products conform to appropriate quality standards. Therefore, a microbiological monitoring program for production areas along with a bioburden monitoring program for product components and process water should be established. Process water includes autoclave cooling water. Applicants should provide information concerning this program. Frequency, methods used, action levels, and data summaries should be included. A description of the actions taken when specifications are exceeded should be provided.

## E. Container-Closure and Package Integrity

An applicant should provide scientific validation studies (and data) in support of the microbial integrity of the drug packaging components. The following types of information should be included:

1. Simulation of the Stresses from Processing

Experimental designs should simulate the stresses of the sterilization process, handling, and storage of the drug and their effects on the container-closure system. Physical, chemical, and microbiological challenge studies may be necessary.

2. Demonstrate Integrity Following the Maximum Exposure

Container-closure integrity should be demonstrated on product

units that have been exposed to the maximum sterilization cycle(s). If a product is exposed to more than one process, then exposure to the maximum cycle of all processes should be incorporated into the study design.

3. Multiple Barriers

Each barrier that separates areas of the drug product claimed to be sterile should be separately evaluated and validated.

4. The Sensitivity of the Test

The sensitivity of the experimental method used for container-closure integrity testing should be specified and provided.

5. Integrity Over the Product Shelf Life

Microbial integrity of the container-closure system should be demonstrated over the shelf life of the product. (See section V.A. of this guidance.)

## F. Bacterial Endotoxins Test and Method

The bacterial endotoxins test used for the product should be described. The description should include qualification of the laboratory, inhibition and enhancement testing and results, determination of noninhibitory concentration and maximum valid dilution. For further information see the agency guidance entitled "Guideline on Validation of the Limulus Amebocyte Lysate Test As An End-Product Endotoxin Test for Human And Animal Parenteral Drugs, Biological Products, and Medical Devices."

## G. Sterility Testing Methods and Release Criteria

Sterility test methods should be described and should include the protocol for the selection of representative units during production. When test methods differ significantly from compendial test methods, a demonstration of the equivalency to the compendial method should be provided. Testing performed within barrier systems should be described, and information concerning validation of the barrier system may be necessary.

## H. Evidence of Formal, Written Procedures

Section 211.113(b) of the Code of Federal Regulations requires that written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, be established and followed. Such procedures should include validation of any sterilization process. Therefore, evidence should be provided that there are formal, written procedures describing the elements listed above and that these procedures are followed. Such evidence may consist of SOP's, listing of SOP's, and protocols submitted as part of these elements.

## III. OTHER TERMINAL STERILIZATION PROCESSES

Although the information above (sections I.A. through I.G. of this guidance) directly addresses moist heat processes, the same type of information would pertain to other terminal sterilization processes used singly or in combination to sterilize a drug product. The types of information outlined are, in general, also applicable to ethylene oxide and radiation (gamma and electron beam). These other processes should be addressed as each applies to the drug product, sterile packaging and in-process sterilization of components. Examples of such information might include: descriptions of loading configurations; qualification and validation of master load configurations; determination and validation of the efficacy of the minimum cycle to provide sterility assurance at the product master sites; requalification of the cycle; provisions for resterilization; specifications and monitoring program for product bioburden; and container-closure integrity. Specific examples are provided below to demonstrate the application of these concepts to other sterilization processes.

Additional information relating to the effects of the sterilization process on the chemical and physical attributes of the drug substance or drug product may be applicable, and should be supplied to the chemistry, manufacturing, and controls section of the application.

### A. Ethylene Oxide

1. Description of the Sterilizer

   The sterilizer(s) and controlled site(s) for prehumidification and aeration of the product load should be described.

2. Cycle Parameters

The parameters and limits for all phases of the cycle, e.g., prehumidification, gas concentration, vacuum and gas pressure cycles, exposure time and temperature, humidity, degassing, aeration, and determination of residuals should be specified. Specific procedures used to monitor and control routine production cycles to assure that performance is within validated limits should be provided.

3. Microbiological Methods

The microbiological methods (growth medium, incubation temperature, and time interval) for cultivating spores from inoculated samples during validation experiments should be described as well as the microbiological methods used as part of routine production cycles.

4. Stability

The program for monitoring the stability of packaging and the integrity of the container-closure system barrier over the claimed shelf life should be described.

## B. Radiation

1. The Facility and the Process

The radiation facility should be identified. The radiation source, method of exposure (i.e., movement through the irradiator), and the type and location of dosimeters used to monitor routine production loads should be described. If the low dose site is not used for routine monitoring, data that show the dose relationship between the two sites should be provided.

2. The Packaging of the Product

The packaging of the drug product within the shipping carton and within the carrier should be described.

3. Multiple-Dose Mapping Studies

Multiple-dose mapping studies for identification of low and high dose sites and demonstration of uniformity and reproducibility of

the process should be described.

4. Microbiological Methods and Controls

The microbiological methods and controls used to establish, validate, and audit the efficacy of the cycle should be described.

5. Monitoring Stability

The program for monitoring the stability of packaging and the integrity of the container-closure system barrier over the claimed shelf life should be described.

## IV. INFORMATION FOR ASEPTIC FILL MANUFACTURING PROCESSES WHICH SHOULD BE INCLUDED IN DRUG APPLICATIONS

The following types of information should be submitted in support of sterility assurance for products manufactured by aseptic processing.

### A. Buildings and Facilities

A brief description of the manufacturing building and facilities should be provided. The following information should be included:

1. Floor Plan

A floor plan of the areas holding the aseptic filling facilities including preparation and holding areas, filtering and filling areas, and gowning rooms should be included. The air cleanliness class of each area should be identified (e.g., Class 100, Class 10,000, Class 100,000). Isolators or barrier systems should be identified.

2. Location of Equipment

The placement of all critical equipment, including, but not limited to, laminar flow hoods, autoclaves, lyophilizers, and filling heads, should be identified. Equipment within barrier or isolation systems should be noted.

### B. Overall Manufacturing Operation

The overall manufacturing operation including, for example, material flow, filling, capping, and aseptic assembly, should be described. The normal

flow (movement) of product and components from formulation to finished dosage form should be identified and indicated on the floor plan described above. The following information should be considered when describing the overall manufacturing operation:

1. Drug Product Solution Filtration

   The specific bulk drug product solution filtration processes, including tandem filter units, prefilters, and bacterial retentive filters, should be described. A summary should be provided containing information and data concerning the validation of the retention of microbes and compatibility of the filter used for the specific product. Any effects of the filter on the product formulation should be described (e.g., adsorption of preservatives or active drug substance, or extractables).

2. Specifications Concerning Holding Periods

   Section 211.111 of the Code of Federal Regulations requires, in part, when appropriate, the establishment of time limits for completing each phase of production to ensure the quality of the drug product. Therefore, specifications concerning any holding periods between the compounding of the bulk drug product and its filling into final containers should be provided. These specifications should include, for example, holding tanks, times, temperatures, and conditions of storage. Procedures used to protect microbiological quality of the bulk drug during these holding periods should be indicated. Maintenance of the microbiological quality during holding periods may need verification.

3. Critical Operations

   The critical operations that expose product or product contact surfaces to the environment (such as transfer of sterilized containers or closures to the aseptic filling areas) should be described. Any barrier or isolation systems should be described.

## C. Sterilization and Depyrogenation of Containers, Closures, Equipment, and Components

The sterilization and depyrogenation processes used for containers, closures, equipment, components, and barrier systems should be described. A description of the validation of these processes should be provided including, where applicable, heat distribution and penetration

summaries, biological challenge studies (microbiological indicators and endotoxin) and routine monitoring procedures. Validation information for sterilization processes other than moist heat should also be included. Methods and data (including controls) demonstrating distribution and penetration of the sterilant and microbiological efficacy of each process should be submitted. The section of this guidance concerning terminal sterilization contains information that may be of further assistance.

1. Bulk Drug Solution Components That are Sterilized Separately

   If the bulk drug solution is aseptically formulated from components that are sterilized separately, information and data concerning the validation of each of these separate sterilization processes should be provided.

2. Sterilization Information in the Batch Records

   The completed batch record supplied with the chemistry, manufacturing, and controls section of the application should identify the validated processes to be used for sterilization and depyrogenation of any container-closure components. This information may be included in the batch record by reference to the validation protocol or SOP.

## D. Procedures and Specifications for Media Fills

The procedures and specifications used for media fills, and summaries of results for validation using the same container- closure system and filling process that is to be used for the product should be described. The microbiological testing method(s) used should be described. Any procedural differences between the media fill and the production process should be indicated. A summary of recent media fill results, including failures, should be provided. These data should be obtained using the same filling line(s) that are to be used for the drug product. The following are recommended to be included with the data summary for each media fill run described:

1. The filling room

   Identify the aseptic filling area used and relate this to the floor plan provided in section IV.A.1 of this guidance.

2. Container-closure type and size

3. Volume of medium used in each container

4. Type of medium used

5. Number of units filled

6. Number of units incubated

7. Number of units positive

8. Incubation parameters

   The incubation time and temperature for each group of units incubated and specifications for any group of units subjected to two (or more) different temperatures should be specified.

9. Date of each media fill

10. Simulations

    The procedures used to simulate any steps of a normal production fill should be described. This might include, for example, slower line speed, personnel shift changes, equipment failure and repair, mock lyophilization and substitution of vial headspace gas.

11. Microbiological monitoring

    The microbiological monitoring data obtained during the media fill runs should be provided (see section IV.F. of this guidance).

12. Process parameters

    The parameters used for production filling and for media fills (e.g., line speed, fill volume, number of containers filled, or duration of fill) should be compared.

## E. Actions Concerning Product When Media Fills Fail

The disposition of product made before and after a failed media fill should be described. The description should include details of investigations, reviews, and how decisions are made to reject or release product.

## F. Microbiological Monitoring of the Environment

The microbiological monitoring program used during routine production and media fills should be described. The frequency of monitoring, type of monitoring, sites monitored, alert and action level specifications, and precise descriptions of the actions taken when specifications are exceeded should be included.

1. Microbiological Methods

   The microbiological materials and methods used in the environmental monitoring program should be described. Methods may include sample collection, transport, neutralization of sanitizers, incubation, and calculation of results. The following are sources of microbial contamination and their monitoring that should be addressed, including specifications:

   a. Airborne microorganisms

   b. Microorganisms on inanimate surfaces

   c. Microorganisms on personnel

   d. Water systems

   e. Product component bioburden

2. Yeasts, Molds, and Anaerobic Microorganisms

   A description of periodic or routine monitoring methods used for yeasts, molds, and anaerobes should be provided.

3. Exceeded Limits

   A description of the actions taken when specifications are exceeded should be provided.

## G. Container-Closure and Package Integrity

The methods and results demonstrating the integrity of the microbiological barrier of the container-closure system should be summarized. This should include testing for initial validation. The procedures used for the stability protocol also should be described. For initial validation of microbiological integrity of container-closure systems, product sterility testing is not normally considered sufficient. The sensitivity of the experimental method used for container-closure integrity

testing should be specified and provided.

### H. Sterility Testing Methods and Release Criteria

Sterility test methods should be described and should include the protocol for the selection of representative units during production. For a drug product represented to be a drug recognized in an official compendium, when test methods differ significantly from official compendial test methods, a demonstration of the equivalency to the official compendial method should be provided. Testing performed within barrier systems should be discussed, and information concerning validation of the barrier system may be necessary.

### I. Bacterial Endotoxins Test and Method

The bacterial endotoxins test used for the product should be described, if applicable. This description should include qualification of the laboratory, inhibition and enhancement testing and results, determination of noninhibitory concentration and maximum valid dilution. For further information see the agency guidance entitled "Guidance on Validation of the Limulus Amebocyte Lysate Test As An End-Product Endotoxin Test for Human And Animal Parenteral Drugs, Biological Products, and Medical Devices."

### J. Evidence of Formal Written Procedures

Evidence should be provided that there are formal, written procedures describing the above elements and that these procedures are followed. Such evidence may consist of SOP's or a listing of SOP's or protocols submitted as part of the elements listed above.

## V. MAINTENANCE OF MICROBIOLOGICAL CONTROL AND QUALITY: STABILITY CONSIDERATIONS

### A. Container-Closure Integrity

The ability of the container-closure system to maintain the integrity of its microbial barrier, and, hence, the sterility of a drug product throughout its shelf life, should be demonstrated. Reference is made to sections II.E. and IV.G. of this guidance. As previously stated, sterility testing at the initial time point is not considered sufficient to demonstrate the microbial integrity of a container-closure system. Documentation of the sensitivity of the container-closure integrity test should be provided.

### B. Preservative Effectiveness

The efficacy of preservative systems to control bacteria and fungi inadvertently introduced during drug product use should be demonstrated at the minimum concentration specified for drug product release or at the minimum concentration specified for the end of the expiration dating period, whichever is less. Since the efficacy of preservative systems is judged by their effect on microorganisms, microbial challenge assays should be performed. The United States Pharmacopeia (USP) provides a microbial challenge assay under the title "Antimicrobial Preservatives-Effectiveness." For purposes of the stability protocol, the first three production lots should be tested with a microbial challenge assay at the beginning and end of the stability period. Chemical assays to monitor the concentration of preservatives should be performed at all test intervals. For subsequent lots placed on stability, chemical assays may be adequate to demonstrate the presence of specified concentrations of preservatives, and such testing should be carried out according to the approved stability study protocol.

### C. Pyrogen or Endotoxin Testing

For drug products purporting to be pyrogen free, it is recommended that pyrogen or endotoxin tests be carried out at the beginning and end of the stability period as part of the approved stability study protocol.

## VI. ADDITIONAL INFORMATION

Further information concerning content and format of drug applications is available in the form of guidances and other publications. The following documents contain information related to the topics discussed in this guidance:

"Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics" (CDER).

"Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices" (CDER, CVM, CBER, CDRH).

"Guideline on Sterile Drug Products Produced by Aseptic Processing" (CDER).

"Drug Stability Guideline" (CVM).

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