Technical Report No. 3

VALIDATION OF DRY HEAT PROCESSES USED FOR STERILIZATION AND DEPYROGENATION

PARENTERAL DRUG ASSOCIATION, Inc.

PREFACE

THIS IS THE THIRD Technical Report dealing with sterilization validation[1,2]. Specifically, this report presents a review of validation for processes that use dry heat to achieve sterilization and/or depyrogenation.

Task Group 16, under the co-chairmanship of William Frieben and Thomas Lypka, has developed a discussion of various biological and engineering parameters which may be considered in validation.

Sol Motola—1980
R. Michael Enzinger—1981
Chairman
Research Committee

(1) "Validation of Steam Sterilization Cycles," Parenteral Drug Association Inc., Technical Monograph No. 1.
(2) "Validation of Aseptic Filling For Solution Drug Products," Parenteral Drug Association Inc., Technical Monograph No. 2.

CONTENTS

1.0 INTRODUCTION

Dry heat is often the agent of choice for sterilizing items which will tolerate relatively high temperatures, yet which might not be adequately penetrated by steam or are damaged by moist heat. Dry heat is often employed for sterilizing such items as powders, oils, petrolatum jellies, glassware, and stainless steel equipment. Dry heat sterilization processes are generally less complicated than steam processes, although higher temperatures and/or longer exposure times are required because microbial lethality associated with dry heat is much lower than that for saturated steam at the same temperature. However, many of the basic concepts and methods developed for steam sterilization can also be adapted for dry heat sterilization. For example, in comparing the relative sterilizing capabilities of dry heat processes, a unit of lethality similar to the F_0 value is used. Changing conditions of time and temperature may be integrated to equivalent time at 170 °C (assuming a z value of 20 °C) in a manner similar to that used for steam heat.* This term is referred to as the F_H value.

Validation of a dry heat sterilization process can include both physical and biological tests. Sequential procedures could be as follows:

- Verification of equipment installation.
- Verification of basic equipment performance.
- Calibration of critical sensing, monitoring, and controlling equipment.
- Verification of thermodynamic characteristics of the unit.
- Engineering qualification of the process.
- Microbiological validation of the process.
- Review of test data.
- Final certification of the documentation package.

* Although the Task Group has selected a "z" value of 20 °C as a representative average, it should be noted that reported values range from 13 to 28 °C for dry heat with the majority of values ranging between 17 to 23 °C (1, 9, 13, 19, 25, 28, 40).

In some cases a dry heat process is intended only to provide sterilization, while in other cases, both sterilization and endotoxin inactivation (depyrogenation) are desired. The purpose of a cycle will dictate the validation approach. There are a number of approaches for validating dry heat processes. This Technical Report presents three of the many possible approaches.

The first approach, for dry heat processes designed for sterilization only, establishes cycle parameters based on the commodity bioburden and its thermal resistance characteristics. The cycle is designed to assure that the probability of survival of the native microflora is no greater than one cell in one million units of the commodity (10^{-6} probability of non-sterility). This approach is used with extremely heat labile materials. Highly heat resistant microorganisms (biological indicators) are used to demonstrate the adequacy of the process.

The second approach, which is also intended for sterilization purposes only, is the overkill method. This approach is used when materials can withstand greater thermal exposure. The overkill approach provides assurance of sterilization well in excess of the 10^{-6} probability of nonsterility. For example an F_H provided by an overkill cycle may produce a 12-log reduction of a biological indicator that exhibits a high resistance to dry heat.* The need for routine bioburden assessments is not necessary in this approach, and biological indicators may be deleted in some cases. However, individuals may elect to conduct appropriate studies occasionally in order to verify the adequacy of barriers to microbial contamination.

The third approach to process validation applies to cycles where the purpose is both sterilization and depyrogenation. Whenever depyrogenation is a desired end point, relatively high temperatures and/or extended heating times are necessary. Thus, the microbial lethality delivered by these cycles provides a margin of safety far in excess of a 10^{-6} probability of nonsterility.

* Although the Task Group has selected a 12-log reduction as one criterion for an overkill cycle, it should be recognized that other criteria may be chosen. The 12-log reduction concept is one which has been used for various types of sterilization processes for a number of years (2, 7, 10, 13, 21, 22, 29).

2.0 SCOPE

A dry heat sterilization process may be considered validated when the results of appropriate studies confirm that the process does what it is intended to do. This manual provides general information and guidelines for validating dry heat sterilizers that are used in the manufacture of pharmaceutical products. It is a compilation of various theories, sterilization variables, engineering, and microbiological studies that could be used alone or in combination to validate a dry heat sterilization/depyrogenation process.

The concepts and methods presented are not intended to be interpreted as the current practice in the industry nor do they represent standards that must be followed to validate a dry heat sterilization/depyrogenation process. Other technically equivalent methods for achieving this purpose exist and may be used. This technical report presents a representative program that may be followed to achieve validation.

This technical report does not encompass all the details of a validation program for dry heat sterilization/depyrogenation processes. Specifications for sterilizer performance, documentation, routine monitoring, and revalidation are not discussed.

3.0 CONVECTION HEATING PROCESSES

Convection heating is the method of transferring heat through a medium by motion of its parts. There are two types of convection heating: natural and forced convection heating. Natural convection heating is a result of the buoyancy forces generated by differences in density caused by temperature gradients in the fluid mass. Forced convection heating is effected by the action of a mechanical device.

The basic equation for convective heat transfer is:

$$q/c = \bar{h}_c A \Delta T$$

where:

q/c = rate of heat transfer by convection, BTU/hr
$\bar{h}_c$ = the average unit thermal convective conductance (this is often called the surface coefficient or heat transfer coefficient), BTU/hr ft^2 °F
A = area ft^2
ΔT = difference between the surface temperature and the temperature of the fluid at some specified location, °F

An example of a batch convection sterilizer is the typical batch forced hot air unit most commonly used to sterilize glassware (see Figures 1 and 4).

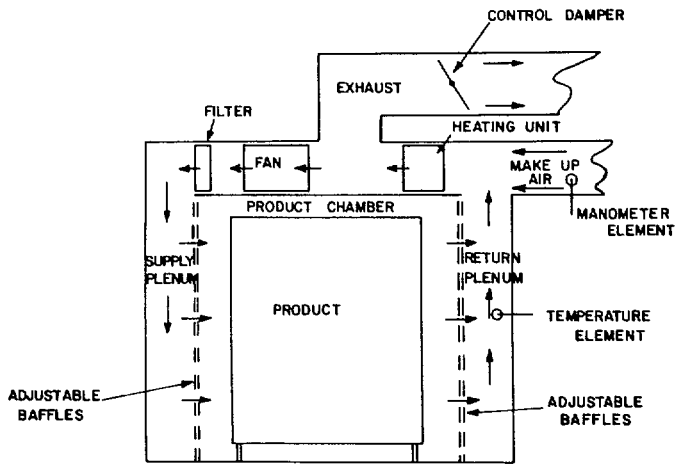

Figure 1—Example of a dry heat sterilizer for convection batch process

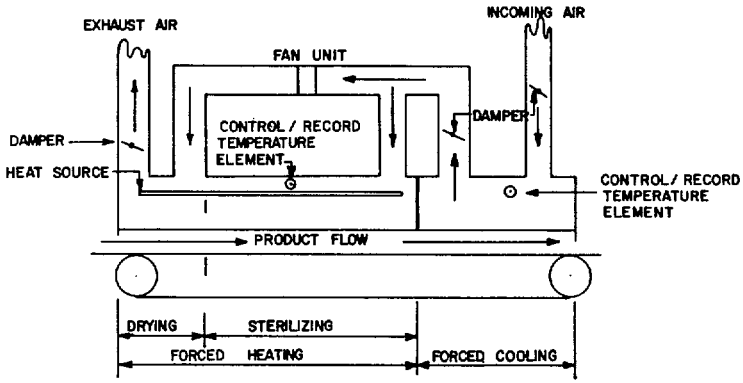

Figure 2—Example of a dry heat sterilizer for convection continuous process

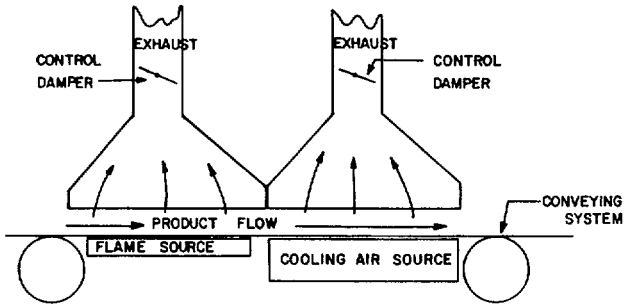

Figure 3—Example of a flame sterilizer for continuous conduction process

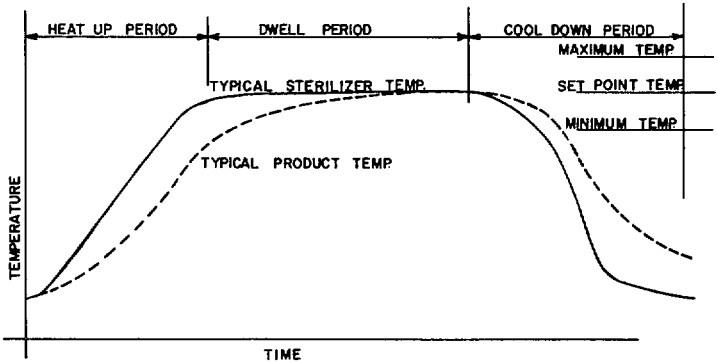

Figure 4—Example of a batch process

3.1 Convection Batch Process Certification

3.1.1 Definition of a Convection Batch Process

A convection batch process is one in which a predetermined quantity of commodity is simultaneously subjected to a convection cycle to effect sterilization and/or depyrogenation. Any sterilization process meeting these criteria shall be considered a convection batch process.

3.1.2 Acceptance of Equipment Installation

Verify and document that the equipment has met the following criteria:

- New equipment adheres to the original purchase specifications. Exceptions have appropriate documentation. Existing equipment has appropriate documentation to support each modification. A documentation package should be instituted in cases where no written information exists.
- The structural installation meets manufacturer's suggested guidelines, e.g., leveling, insulation, and sealing.
- All utility connections such as electrical, pneumatic, and HVAC meet the design limits and codes.

3.1.3 Verification of Basic Equipment Performance

This section involves the actual operation of the various systems that comprise the sterilizer. Each system is individually checked, where possible, for proper operation.

A series of tests is performed on the unit to determine if the electro/mechanical operations described in the equipment specification perform as stated.

The final check involves execution of a series of cycles to verify that each system component interacts correctly and repeatably in the programmed sequence of events.

Below is a suggested list of items that can be checked during this phase of the verification:

- *Electrical Logic*—Ensure that each step is in the correct sequence and that it is repeatable.

- *Cycle Set Point Adjustability*—Verify limit switch sequencing.
- *Door Interlocks*—Must work correctly, not allowing access during the cycle.
- *Gasket Integrity*—Check for positive/negative pressure seal of all door gaskets.
- *Vibration Analysis*—Check blowers for correct dynamic balancing to minimize vibration.
- *Louvre Balance Ability*—Check that louvre/linkage mechanisms can be actuated and adjusted for balance.
- *Blower Rotation*—Check that blowers rotate in the specified direction.
- *Blower RPM*—Verify that the correct blower RPM is achieved.
- *Heater Elements*—Check that all heater elements operate.
- *Room balance*—Check that the ΔP balance is positive from the sterile core to the preparation area when one door is opened.
- Verify integrity of HEPA filters if they are used.

3.1.4 Calibration of Equipment

There are two basic modes of equipment calibration; when the piece of equipment to be calibrated is removed from the sterilizer and calibrated, and when the piece of equipment to be calibrated is calibrated *in situ*. A combination of both is recommended to achieve an accurate calibration.

It is recommended that the following pieces of equipment be calibrated prior to validation in accordance with the individual company's metrology policy.

- Temperature sensing devices and recording systems
- Temperature controllers (preferably *in situ*)
- Pressure gauges
- Cycle set point switches (preferably *in situ*)
- Timing devices

- Velometers

3.1.5 Verification of Thermodynamic Characteristics of the Unit

In the convective mode of dry heat sterilization, an important parameter is the uniformity of the heating medium. This uniformity may be measured by obtaining the flow rates of the heating medium (i.e., air) at the source discharge of the actual sterilization chamber.

The flow rate is equal to the product of the velocity and the cross sectional area at the discharge source.

$$Q = Av$$

where Q is the volumetric flow in ft^3/min (CFM), A is the cross-sectional area of the discharge orifice in ft^2, and v is the velocity of the air in feet per minute.

Adjust source discharge louvres so that a controlled flow is obtained across the face of the discharge section. Monitor the particulate quality of the discharge air and ensure that it falls within pre-established acceptable limits.

Once the air flow pattern is established, the temperature profile of the sterilization chamber can be obtained, e.g., tests are performed to determine differential temperatures, hot and cold spots, and zones which are slow to heat.

Data obtained from the empty sterilizer testing will be used as a basis for all future flow pattern modifications. The number and type of tests necessary to demonstrate repeatability may be determined from an evaluation of the results obtained.

3.1.6 Engineering Qualification of the Process

Due to the sensitivity of a hot air sterilization process to significant variations in load configuration, load initial temperature, and the specific heat of the load components, the following studies are suggested:

- Perform heat distribution and penetration studies with the probes placed in patterns which give the greatest amount of temperature information per run. These studies will determine minimum cycle times and the placement

of microbial challenges for the microbial validation of the process.

- Test load variations (e.g., minimum, maximum, etc.) for differences in temperature and slowest to heat zone. This data base may be used to identify the load types which demonstrate similar heating characteristics.

Once the major load classification is established, a worst case representation pilot load from this group may be used to establish a time-temperature relationship for the entire group.

Using the pilot load, exposure times, set points, and other variables necessary for the cycle can be firmly fixed. Verify that these parameters are mechanically repeatable. A suggested list of variables that may be checked for mechanical repeatability is provided below:

- Temperature set point
- Exposure time
- Controller sensitivity

3.1.7 Microbial Validation of the Process

Replicate heat distribution and heat penetration runs are conducted in the unit. No equipment or process adjustments are made during these runs. During the heat penetration studies microbiological challenge units (e.g., biological indicators and/or endotoxin challenges) are placed adjacent to probed units. The microbiological challenge verifies the thermodynamic parameters of the established cycle (see Section 6.0).

Cycle parameters are set at the minimum cycle specifications to assure process efficiency under minimum cycle conditions.

3.1.8 Review and Collation of All Test Data

Data from runs are collated and analyzed. The results are judged as acceptable or unacceptable. If unacceptable, either a new test is performed or the test parameters changed.

If the data are acceptable, per the specifications of the test procedure, then the results are placed in a report which can be available for review by interested individuals.

3.1.9 Final Certification of the Document Package

When reviewing the final package, the following considerations can be addressed:

- Do all ΔT values and heat history summations for the sterilization runs correspond to the company specifications?
- Do the results of microbiological challenges conform to the biological intent of the process? (see Section 6.0)
- All the data in a finalized format are reviewed and, if acceptable, are approved by the various disciplines involved.

The tested unit is considered validated and is released for use if the established cycle specifications are met.

3.2 Convection Continuous Process Certification

3.2.1 Definition of a Convection Continuous Process

A continuous convection process is one in which a predetermined quantity of commodity is conveyed at a predetermined rate through a convection cycle to effect sterilization and/or depyrogenation. Any sterilization process meeting these criteria shall be considered a continuous convection process (see Figures 2 and 5).

3.2.2 Acceptance of Equipment Installation

Verify and document that the equipment meets the following criteria:

- New equipment adheres to the original purchase specifications. Exceptions have appropriate documentation. Existing equipment has appropriate documentation to support each modification. A documentation package should be instituted in cases where no written information exists.
- The structural installation meets manufacturer's suggested

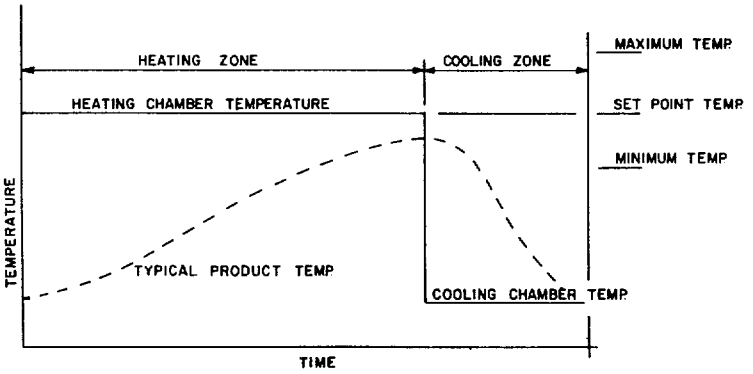

Figure 5—Example of a continuous cycle

guidelines, e.g., leveling, insulation, and air flow requirements.

- All utility connections such as electrical and HVAC meet the design specifications and codes.
- Materials of construction of both the unit and the facility meet all design limits and codes.

3.2.3 *Verification of Basic Equipment Performance*

This section involves the actual operation of the various systems that comprise the continuous sterilizer. Each system is individually checked, where possible, for proper operation.

A series of tests is performed on the unit to determine if the electro/mechanical operations described in the equipment specification perform as stated.

The final check involves operation of the unit to verify that each system component interacts correctly.

Below is a suggested list of items that may be checked during this phase of the verification:

- *Electrical Logic*—Ensure that each step is in the correct sequence and that it is repeatable.
- *Cycle Set Point Adjustability*—Verify limit switch sequencing.
- *Commodity Interlocks Overload*—Must work correctly,

not allowing excess commodity buildup during processing.

- *Gasket Integrity*—Check that the leakage rate does not exceed a predetermined value at all panel gaskets from zone to zone.
- *Air Balance Ability*—Check that baffle/linkage mechanisms can be actuated and adjusted for balance.
- *Blower Rotation*—Check that blowers rotate in the specified direction.
- *Vibration Analysis*—Check blowers for correct dynamic balancing to minimize vibration in each phase of the tunnel.
- *Air Balance*—Check that the ΔP is positive with respect to the preparation section through the tunnel.
- *Blower RPM*—Verify that the correct blower RPM is achieved.
- *Heater Elements*—Check that all heater elements operate.
- *Belt Speed*—Check that the belt and belt speed recorder are operable.
- Verify integrity of HEPA filters if they are used.

32.4 Calibration of Equipment

There are two basic modes of equipment calibration; when the piece of equipment to be calibrated is removed from the sterilizer and calibrated, and when the piece of equipment to be calibrated is calibrated *in situ*. A combination of both can be used to achieve an accurate calibration. The following pieces of equipment are calibrated prior to validation in accordance with individual company's metrology policy.

- Temperature sensing devices and recording systems
- Temperature controllers (preferably *in situ*)
- Pressure gauges
- Belt speed recorder
- Belt speed controller
- Cycle set point switches (preferably *in situ*)
- Velometers

3.2.5 *Verification of Thermodynamic Characteristics of the Unit*

In the convective mode of dry heat sterilization, a critical parameter is the uniformity of the heating medium. This uniformity may be measured by obtaining the flow rates of the heating medium (i.e., air) at the source discharge of the sterilization and heat up zones of the tunnel.

The flow rate is equal to the product of the velocity and the cross sectional area at the discharge source.

$$Q = Av$$

where Q is the volumetric flow in ft^3/min (CFM), A is the cross-sectional area of the discharge orifice in ft^2, and v is the velocity of the air in feet per minute.

Adjust the source discharge so that a controlled flow is obtained across the face of the discharge section. Monitor the particulate quality of the discharge air and ensure that it falls within preestablished acceptable limits.

Once the air flow pattern is established, the temperature profile of the heat up and sterilization can be obtained (e.g., tests are performed to determine differential temperatures, hot and cold spots, and those sections which are slow to heat).

Data obtained from the empty sterilizer testing will be used as a basis for all future flow pattern modifications. The number and type of tests necessary to demonstrate repeatability may be determined from an evaluation of the results obtained.

3.2.6 *Engineering Qualification of the Process*

Due to the sensitivity of a hot air sterilization process to significant variations in load density, initial load temperature, and the specific heat of the load components, the following procedures can be checked.

- Load variations should be tested for variations in temperature and slowest to heat portion of the belt by placing sensors across the width of the belt. The data base may be

used to classify the load types into those loads which demonstrate similar heating characteristics.

Once the major load classifications are established, a worse case representative pilot run from this group can be used to establish a line speed-temperature relationship for the entire group.

From the pilot run, exposure times, set points, and other variables necessary for the cycle can be fixed. Verify that these parameters are mechanically repeatable. A suggested list of variables that may be checked for mechanical repeatability is provided below.

- Temperature set point per zone
- Belt speed
- Controller sensitivity

3.2.7 Microbial Validation of the Process

Replicate heat distribution and heat penetration runs are conducted in the unit. No equipment or process adjustments should be made during these runs. During the heat penetration studies microbiological challenge units (e.g., biological indicators and/or endotoxin challenges) are placed adjacent to probed units. The microbiological challenge verifies the thermodynamic parameters of the established cycle (see Section 6.0).

The cycle parameters are set at the minimum cycle specifications to assure process efficiency under minimum cycle conditions.

3.2.8 Review and Collation of All Test Data

Refer to Section 3.1.8.

3.2.9 Final Certification of the Document Package

Refer to Section 3.1.9.

4.0 CONDUCTION HEATING PROCESSES

Conduction heating is accomplished via two mechanisms. The first is that of molecular interaction whereby molecules at higher energy levels impart energy to adjacent molecules at lower energy levels.

The second mechanism is via "free" electrons. Pure metallic solids contain the highest concentration of free electrons and non-metals contain the lowest. Thus, the ability of solids to conduct heat varies directly with the free electron concentration; pure metals are the best conductors and non-metals are the poorest conductors of heat.

4.1 Conduction Batch Process Certification

No commercially available conduction batch sterilizers are known to the Task Group at the time of this writing.

4.2 Conduction Continuous Process Certification

4.2.1 Definition of a Continuous Conduction Process

A continuous conduction process is one in which a predetermined quantity of commodity is subject to a continuous conduction cycle at a predetermined rate to effect sterilization and/or depyrogenation. Any sterilization process meeting these criteria should be considered a continuous conduction process (see Figures 3 and 5).

4.2.2 Verification of Equipment Installation

Verify and document that the equipment has met the following criteria:

- New equipment adheres to the original purchase specifications. Exceptions have adequate documentation. Existing equipment has appropriate documentation to support each modification. A documentation package should be instituted in cases where no written information exists.
- The structural installation meets manufacturer's suggested

guidelines such as leveling, insulation, and utility requirements.

- All utility tie-ins such as electrical, pneumatic, gas and HVAC meet the design specifications and codes.
- Materials of construction of both the unit and the facility meet all design limits and codes.

4.2.3 Verification of Basic Equipment Performance

This section involves the actual operation of the various systems that comprise the sterilizer. Each system is individually checked, where possible, for proper operation.

A series of tests is performed on the unit to determine if the mechanical operations described in the equipment specification are performed as stated.

The final check involves operation of the unit to verify that each system component interacts correctly.

Below is a suggested list of items that may be checked, if applicable, during this phase of the verification:

- *Electrical Logic*—Ensure that each step is in the correct sequence and that it is repeatable.
- *Cycle Set Point Adjustability*—Verify limit switch sequencing.
- *Vibration Analysis*—Check blowers for correct dynamic balancing to minimize vibration.
- *Air Balance Ability*—Check that baffle/linkage mechanisms can be actuated and adjusted for balance.
- *Blower Rotation*—Check that the blowers rotate in specified direction.
- *Heat Shields*—Verify that all heat shields are correctly installed.
- *Blower RPM*—Verify that the correct blower RPM is achieved.
- *Burner Element*—Check that all burner elements perform correctly.
- Verify integrity of HEPA filters if they are used.

4.2.4 *Calibration of Equipment*

There are two basic modes of equipment calibration; when the piece of equipment to be calibrated is removed from the sterilizer and calibrated, and when the piece of equipment to be calibrated is calibrated *in situ*. A combination of both can be used to achieve an accurate calibration.

It is recommended that the following pieces of equipment be calibrated prior to validation in accordance with the individual company's metrology policy.

- Temperature sensing devices and recording systems
- Temperature controllers (recommend *in situ*)
- Pressure gauges
- Cycle set point switches (recommend *in situ*)
- Gas flowmeters
- Velometers

4.2.5 *Verification of Thermodynamic Characteristics of the Unit*

In the conductive mode of dry heat sterilization, a critical parameter is the uniformity of the heating medium. This uniformity may be measured by obtaining product temperatures at the discharge source of the commodity from the sterilization chamber.

Louvres, if applicable, may be adjusted such that a controlled flow is obtained across the face of the product discharge section. Monitor the particulate quality of the discharge air and ensure that it falls within pre-established acceptable limits.

Data obtained from the empty sterilizer testing will be used as a basis for all future flow pattern modifications. The number and type of tests necessary to demonstrate repeatability may be determined from an evaluation of the results obtained.

4.2.6 *Engineering Qualification of the Process*

Due to the sensitivity of a conductive sterilization process

to significant variations in load configuration, initial load temperature, and the specific heat of the load components, the load variations should be tested for delta temperature and slowest to heat zone. The data base may be used to classify the product types into those products which demonstrate similar heating characteristics.

Once the major product classification is established, a worst case representative pilot run from this group may be used to establish a time-temperature relationship for the entire group.

Using the pilot load, exposure times, set points, and other variables necessary for the minimum cycle can be firmly fixed. Verify that these parameters are mechanically repeatable. A suggested list of variables that may be checked for mechanical repeatability is provided below.

- Temperature set point
- Process rate
- Controller sensitivity

4.2.7 Microbial Validation of the Process

Replicate heat distribution and heat penetration runs are conducted in the unit. No equipment or process adjustments should be made during these runs. During the heat penetration studies microbiological challenge units (e.g., biological indicators and/or endotoxin challenges) are placed adjacent to probed units. The microbiological challenge verifies the thermodynamic parameters of the established cycle (see Section 6.0).

The cycle parameters are set at the minimum cycle specifications to assure process efficiency under minimum cycle conditions.

4.2.8 Review and Collation of All Test Data

Refer to Section 3.1.8.

4.2.9 Final Certification of the Document Package

Refer to Section 3.1.9.

5.0 RADIANT HEATING PROCESSES

Radiant Heating is a process by which energy flows from a high temperature body to a lower temperature body when the bodies are separated in space even when a vacuum exists between them. Heat is emitted in the form of finite batches or quanta of energy without the aid of an intervening medium (16).

Basic Transfer Equation

$q_r = \sigma.A.T^4{}_1$

q_r = Rate of heat flow in BTU/hr

A = Surface area of the emitting object in ft^2

T_1 = Surface temperature of the emitting object in degrees Rankine

σ = Stefan-Boltzmann constant 0.1714×10^{-8} BTU/hr $ft^2.°R$

Because of the nature of the mechanism for radiant heat transfer, several additional factors should be incorporated into the formula when it is concerned with dry heat sterilization. The amount each factor contributes to the process will be inherent to the process equipment and will be different for various equipment designs. The important point is that these factors be considered and weighed during a process validation.

5.1 Radiant Heat Process Factors

5.1.1 Radiant Heat Source

The nature of the source of radiant energy in a dry heat unit should be considered when verifying the process. The wavelength range—hence, the amount of energy—will be determined by the temperature the source can attain. Resistance wire and quartz tube are two common sources. The wavelength operation ranges and power levels for these systems will vary and thus influence the initial design parameters.

5.1.2 Exposure Section and Source Geometry

The geometry of both the source and the exposure section

of the unit will affect the uniformity of the radiation density within a unit. The geometry of a source will directly affect the emissive power and initial distribution of radiation. The placement of the source and the geometry of the exposure enclosure will indirectly affect the reflecting radiation within the unit.

5.1.3 Target Properties

There are three target properties which should be considered. They are: the geometry of the object being irradiated, the color of the object, the surface and composition of the object being irradiated.

The geometry of the object will affect what percent of the object will receive a majority of the radiant energy. The color of the object will determine what wavelength is most absorbed, and the surface characteristics and composition of the object will determine the reflectance to transmittance ratio. These properties will determine heat up and exposure time in a unit.

5.2 Radiant Heat Batch Process

No commercially available radiant heat batch sterilizers are known to the Task Group at the time of this writing.

5.3 Radiant Heat Continuous Process Certification

5.3.1 Definition of a Continuous Radiant Heat Process

A continuous radiant heat process is one in which a predetermined quantity of commodity is subjected to a continuous radiant heat cycle at a predetermined rate to effect sterilization and/or depyrogenation. Any sterilization process meeting these criteria shall be considered a continuous radiation process.

5.3.2 Verification of Equipment Installation

Verify and document that the equipment has met the following criteria:

- New equipment adheres to the original purchase specifications. Exceptions have adequate documentation. Existing equipment has appropriate documentation to support each modification. A documentation package should

be instituted in cases where no written information exists.

- The structural installation meets manufacturer's suggested guidelines such as leveling, insulation, and utility requirements.
- All utility tie-ins such as electrical, pneumatic, and HVAC meet the design specifications and codes.
- Materials of construction of both the unit and the facility meet all design limits and codes.

5.3.3 Verification of Basic Equipment Performance

This section involves the actual operation of the various systems that comprise the sterilizer. Each system is individually checked where possible for proper operation.

A series of tests is performed on the unit to determine if the mechanical operations described in the equipment specification perform as stated.

The final check involves operation of the unit to verify that each system component interacts correctly.

Below is a suggested list of items that may be checked during this phase of the verification:

- *Electrical Logic*—Ensure that each step is in the correct sequence and that it is repeatable.
- *Cycle Set Point Adjustability*—Verify limit switch sequencing.
- *Vibration Analysis*—Check blowers for correct dynamic balancing to minimize vibration.
- *Air Balance Ability*—Check that baffle/linkage mechanisms can be actuated and adjusted for balance.
- *Blower Rotation*—Check that blowers rotate in specified direction.
- *Heat Shields*—Verify that all heat shields are correctly installed.
- *Blower RPM*—Verify that the correct blower RPM is achieved.

- *Heater Elements*—Check that all heater elements perform correctly.
- *Belt Speed*—Check that belt speed and belt speed recorder are operable.
- *Air Balance*—Check that the ΔP is positive with respect to the preparation section through the tunnel.
- Verify integrity of HEPA filters if they are used.

5.3.4 Calibration of Equipment

There are two basic modes of equipment calibration; when the piece of equipment to be calibrated is removed from the sterilizer and calibrated, and when the piece of equipment to be calibrated is calibrated *in situ*. A combination of both is recommended to achieve an accurate calibration.

It is recommended that the following pieces of equipment be calibrated prior to validation in accordance with the individual company's metrology policy.

- Temperature sensing devices and recording systems
- Temperature/electrical controllers (preferably *in situ*)
- Cycle set point switches (preferably *in situ*)
- Belt speed controller
- Belt speed (preferably *in situ*)
- Air balance
- Velometers

5.3.5 Verification of Thermodynamic Characteristics of the Unit

In the radiant heat mode of dry heat sterilization, the critical parameters are covered in Sections 5.1.1 through 5.1.3.

Since there is no heating medium present as is the case in conductive and convective modes of dry heat sterilization, it is not appropriate to determine thermodynamic characteristics of the radiation sterilizer. The Engineering Qualification of the process can be followed logically.

5.3.6 Engineering Qualification of the Process

Due to the sensitivity of a radiant heat sterilization process

to significant variations in load configuration, initial load temperature, and the specific heat of the load components, the load variations should be tested for delta temperature and slowest to heat zone. The data base may be used to classify the product types into those products which demonstrate similar heating characteristics.

Once the major product classification is established, a worst case representation pilot run from this group may be used to establish a time/energy relationship for the entire group.

5.3.7 Microbial Validation of the Process

Once minimum process settings have been established, replicate runs are conducted in which microbiological challenges (e.g., biological indicators and/or endotoxin challenges) are placed in a predetermined number of containers. No equipment or process adjustments should be made during these runs. The microbiological challenge verifies the minimum thermodynamic parameters of the established cycle (see Section 6.0).

Cycle parameters are set at the minimum cycle specifications to assure process efficiency under minimum cycle conditions.

5.3.8 Review and Collation of All Test Data

Refer to Section 3.1.8.

5.3.9 Final Certification of the Document Package

Refer to Section 3.1.9.

6.0 Biological Validation

6.1 Biological Intent

Some dry heat processes are intended only to provide sterilization. Others are intended for both sterilization and depyrogenation. The biological intent of the cycle will dictate the validation approach.

6.1.1 Sterilization Only—Probability of Survival Approach

Materials affected by heat require a sterilization process that provides adequate assurance of sterility without resulting in thermal degradation. Since overheating or underprocessing may result in an unacceptable product, validation of sterilization cycles for these materials is critical. The objective of this approach is to determine the minimum amount of dry heat required to assure that the probability of survival of the bioburden is not greater than 10^{-6}. This process lethality may be expressed as F_H in dry heat sterilization. F_H is defined as the equivalent time, in minutes at 170 °C, which has been delivered to the product by the process and assumes a z value of 20 °C. In order to establish this parameter, the following studies may be performed:

6.1.1.1 Laboratory studies to determine the number and heat resistance of microorganisms associated with the product. This information will determine the minimum F_H required to obtain the specified probability of survival, e.g., 10^{-6}.

6.1.1.2 Calibration of biological indicators that are used to monitor process lethality.

6.1.1.3 Plant studies (in production sterilizers) to determine:

6.1.1.3.1 Acceptability of sterilizer for providing a uniform heating medium (temperature distribution).

6.1.1.3.2 Minimum F_H provided by sterilization cycle.

6.1.1.3.3 Reproducibility of cycle to ensure that the minimum F_H is consistently delivered to the location in the loading pattern that is the slowest to heat.

6.1.2 Sterilization Only—Overkill Approach

The overkill approach is used when the material(s) can withstand excessive heat treatment without adverse effects. This approach implies that sufficient lethality is imparted by the cycle to provide at least a 10^{-6} probability of survival regardless of the number and resistance of naturally occurring microorganisms. In fact, the F_H value used will generally insure a probability of survival of considerably less than 10^{-6} for the bioburden.

When employing the overkill approach, it is suggested that the minimum F_H provide, for example, a 12-log reduction of a biological indicator that exhibits a high resistance to dry heat. For example, a biological indicator with a $D_{170\ °C}$ of 2.5 min would require a minimum F_H of 30 min. in order to attain a 12-log reduction. Since the majority of mesophilic sporeformers have $D_{170\ °C}$ values less than 0.5 min (4, 5, 6, 39), an F_H that assures a 12-log reduction of a biological indicator with a $D_{170\ °C}$ of 2.5 min would provide a considerable margin of safety.

The overkill approach offers the advantage of eliminating routine bioburden and resistance studies in developing and validating dry heat sterilization processes. Instead cycle parameters are adjusted to ensure that the coolest area in the loading pattern receives an F_H that will provide, for example, a 12-log reduction of a biological indicator that exhibits a high resistance to dry heat.

The overkill approach does not imply that less precaution is necessary in preventing microbial contamination. Controlled manufacturing environments and good manufacturing practices which provide barriers to microbial contamination still are important in order to minimize the bioburden on the equipment and prevent pyrogen formation. However, individuals may elect to conduct appropriate studies on occasion in order to verify the adequacy of barriers to microbial contamination.

When employing the overkill approach, the following studies can be performed to establish that materials are exposed to at least the minimum lethality.

6.1.2.1 Calibration of biological indicators that are used to monitor process lethality.

6.1.2.2 Plant studies (in production sterilizers) to determine:

6.1.2.2.1 Acceptability of sterilizer for providing uniform heating medium (temperature distribution).

6.1.2.2.2 Slowest to heat location in the loading pattern (heat penetration).

6.1.2.2.3 Minimum F_H delivered by the cycle provides a 12-log reduction of a biological indicator that exhibits a high resistance to dry heat.

6.1.2.2.4 Reproducibility of the cycle to ensure that the slowest to heat location in the loading pattern consistently receives the specified F_H.

6.1.3 Sterilization and Depyrogenation

It is well-known that pyrogens can be destroyed by dry heat. Consequently, the pharmaceutical industry has traditionally employed dry heat as a means of depyrogenating instruments, metal equipment, heat stable materials, and glassware. Such processes usually employ very high temperatures. For example, continuous processes often use temperatures greater than or equal to 300 °C, and batch processes usually employ temperatures greater than or equal to 200 °C.

In this approach cycle parameters are adjusted to ensure that the slowest to heat location is the loading pattern receives adequate endotoxin inactivation. The heat lethality delivered by these cycles will provide a large margin of safety with regard to sterility since dry heat resistance organisms such as *Bacillus subtilis* spores have D values of only a few seconds at temperatures used for depyrogenation (34). One can, therefore, anticipate microbial reductions well in excess of 10^{100}, and cycle lethality can actually be defined on the basis of endotoxin inactivation.

Estimates of the depyrogenating ability of a dry heat process can come from a knowledge of the level of the ambient endotoxin load. Cycles designed for depyrogenation will take into account the endotoxin level present prior to heat treatment. If quantitative measurement of the endotoxin level is not possible, cycles can be designed to inactivate levels of

endotoxin which are not expected to be encountered in practice. Demonstration of the depyrogenating ability of a dry heat process can, therefore, be achieved by inactivating known challenges of purified endotoxin.

To establish that materials are consistently exposed to sufficient heat when employing this approach, the following studies may be performed:

6.1.3.1 Laboratory studies to determine the endotoxin content. These studies will determine the endotoxin challenge for establishing cycle parameters.

6.1.3.2 Plant studies (in production sterilizers) to determine:

6.1.3.2.1 Acceptability of vessel for providing uniform heating medium (temperature distribution).

6.1.3.2.2 Slowest to heat location in loading pattern (heat penetration).

6.1.3.2.3 Minimum endotoxin inactivation provided by cycle (endotoxin challenge).

6.1.3.2.4 Reproducibility of cycle to ensure that the minimum endotoxin inactivation is consistently achieved at the slowest to heat location in the loading pattern.

6.2 Laboratory Studies

6.2.1 Cycles Designed for Sterilization only—Probability of Survival Approach

6.2.1.1 Bioburden Studies

Determine the presterilization microbial load on the commodity being sterilized. As mentioned previously, these studies are necessary to determine the minimum dry heat sterilization process (e.g., minimum F_H) that will provide an acceptable assurance of sterility. Techniques and methods for recovering microorganisms from powders, oils, and solid surfaces are available from several sources (8, 12, 17, 20).

6.2.1.2 Laboratory *D* Value Studies

A *D* value is defined as the time required to reduce the microbial population by 90% or one logarithm at a particular set of exposure conditions. *D* value studies of product isolates are necessary to determine the minimum dry heat sterilization process (e.g., F_H) that will provide an acceptable assurance of sterilization. *D* values are also necessary for calibrating biological indicators.

6.2.1.2.1 Laboratory *D* value determinations:

- Biological indicators whether prepared in-house or purchased commercially.
- Resistant bioburden isolates (e.g., screening test survivors).

6.2.1.2.2 Heat Resistance Screening Test for Bioburden

Screening tests are conducted to select the more heat resistant bioburden isolates. Survivors can be characterized with respect to their *D* values.

6.2.1.2.3 Type of Carrier

In all heat resistance studies it is preferable to use the product or item in question as the carrier since the heat resistance (*D* and *z* values) may be influenced by the type of carrier. Therefore, when validating dry heat sterilization cycles for a given type of material, *D* values are generally obtained by inoculating samples of the item in question. In addition, to allow comparison between different lots of biological indicators, *D* values should be determined on a standard carrier under a standard set of conditions.

6.2.1.3 Laboratory *D* Value Methodology

Obtain a commercially prepared biological indicator or use a suspension containing a known number of spores to inoculate replicate samples of carrier materials (e.g., glass, metal, powder, paper strips, etc.).

6.2.1.3.1 Equilibrate the biological indicators at standard environmental conditions (e.g., conditions simulate production conditions).

6.2.1.3.2 Positive controls to determine the number of spores on the biological indicator to be tested. Although the number of spores required will be based on the individual experimental design, 1×10^5 to 1×10^7 spores per sample are generally used.

6.2.1.3.3 Expose at least 5 samples at specified temperature for at least three different time intervals.

6.2.1.3.4 The number of survivors can be determined by colony count or by the fraction negative method (see Sections 6.2.1.4 and 6.2.1.5).

6.2.1.4 Estimation of *D* Value by Survival Curve Method

6.2.1.4.1 In this method the logarithm of plate count data are plotted against time at a given temperature (see Figure 6). The *D* value is determined from a linear regression analysis of the data points by the following equation:

$$y = a + bx$$

where:

y = log number of survivors at time x
x = time at test temperature
a = y intercept at time 0
b = slope of the line

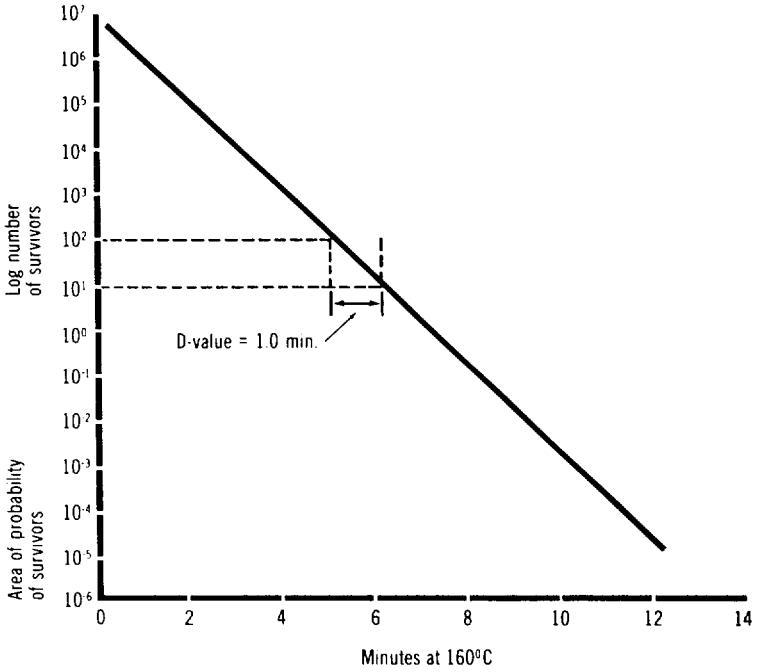

Figure 6—Microbial death rate curve

The D value is the negative reciprocal of the slope.

6.2.1.4.2 For a more detailed analysis of the D value calculation by the survival curve method, including the intercept ratio, consult reference 26.

6.2.1.5 Estimation of D Value by Fraction Negative Method

6.2.1.5.1 At least five replicate samples having the same number of microorganisms are heated at each time interval.

6.2.1.5.2 After heating, the samples are transferred to a suitable broth medium such as Soybean Casein Digest and incubated under appropriate conditions.

6.2.1.5.3 Record the fraction of samples negative for growth at each time interval.

6.2.1.5.4 The data may then be evaluated by the MPN method of Stumbo et al. (30). When employing this method, data can only be analyzed when a fraction of the replicates is negative. The D value is estimated according to the formula:

$$D = \frac{U}{\text{Log } a - \text{Log } b}$$

where:

U = heating time at specified temperature

a = initial number of microorganisms per replicate

b = number of microorganisms after treatment U; b is found using the formula $2.303 \log_{10} (p/q)$, where p is the number of replicates and q is the number of negative replicates

A D value may be determined at each time interval according to the above formula. The average D value is then determined by calculating the arithmetic mean of the individual D values at the different time intervals.

Additional methods for analyzing MPN data can be found in references (27, 31).

6.2.1.6 Correction for Heating and Cooling Lags When Determining Laboratory D Values

If heat-up and cool-down times are significant when heating or cooling, corrections should be made.

6.2.1.6.1 Measure temperature by attaching a thermocouple to the item being heated. Record

the time and temperature at frequent intervals until the item reaches the set temperature. Repeat this operation for the cooling phase by recording time and temperature immediately after the item has been removed from the oven.

6.2.1.6.2 Calculate the lethality for each time interval as described in Section 6.3.1.1.

6.2.1.6.3 The lethality accrued during heat-up and cool-down is added to the time at temperature to determine the value of U.

$$U = L_h + L_c + T$$

where:

L_h = lethality during heat-up

L_c = lethality during cool-down

T = Time at set temperature

6.2.1.6.4 Calculate D values with the adjusted heating time.

6.2.1.7 Determination of z Values

The z value of a microorganism is a measure of how heat resistance changes with changes in temperature. The z value is defined as the number of degrees (Fahrenheit or Celsius) that are required to change the D value by a factor of ten. It allows integration of the lethal effect of heat as the temperature changes during the heating and cooling phases of a sterilization cycle. The z value is necessary to make calculations that allow comparison of microbial lethality at different temperatures. A z value can be determined as described below:

6.2.1.7.1 Determine the D value of an organism at a minimum of three different temperatures.

6.2.1.7.2 Construct a Thermal Death Curve by plotting the logarithm of the D value on the

ordinate of the graph versus the temperature on the abscissa as illustrated in Figure 7.

6.2.1.7.3 Although it is appropriate to assume a z value of 20 °C (36 °F) for dry heat sterilization, the z value should be verified for biological indicators when they are used to measure the integrated lethality of a dry heat sterilization cycle. Discrepancies may occur between F_H values determined from thermocouple data and from biological indicator data if the z value of the biological indicator varies significantly from 20 °C.

6.2.1.8 Apparatus for Obtaining Heat Resistance Data

There are several types of apparatus that may be used for determining dry heat resistance in an accurate and reproducible manner. For a detailed de-

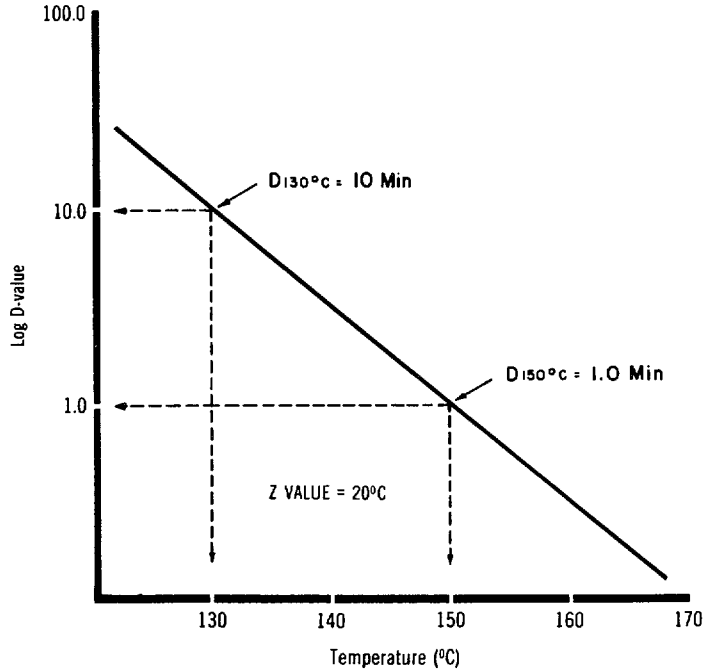

Figure 7—Assessment of z Value

scription of these apparatus consult reference 27.

Two types of apparatus commonly used are listed below. Other types can be used.

6.2.1.8.1 Planchet System

Depositing spores in metal planchets and then heating them in a laboratory oven or on a hot plate is a common way of studying the dry heat destruction of microorganisms on surfaces.

6.2.1.8.2 Glass Tube System

Placing the spores in a screw-cap test tube or in sealed glass tubes and heating them in an oil bath or heating block is a common way of studying the dry heat destruction of entrapped spores.

6.2.1.9 Use of Bioburden Data, z and D Values to Calculate Probability of Survival and Minimum F_H Value Required for Sterilization

When making process calculations, the microbial population used to represent the bioburden is generally the maximum number of microorganisms found per unit. This is a conservative approach since the entire population may not be sporeformers. When concerned with the adverse effects of excessive heat, it would be acceptable to consider bioburden as the maximum number of sporeforming bacteria per unit. In addition, the D values used in process calculations are generally those obtained from the most resistant isolates. This is also a conservative approach since it assumes that the entire population consists of the most heat resistant microorganisms.

6.2.1.9.1 Determination of Probability of Survival

The number and resistance of indigenous microorganisms are required to determine the level of sterility assurance provided by a previously established dry heat sterilization cycle or to determine the F_H value

when developing a new sterilization cycle. When an F_H value is known for a sterilization cycle, the probability of microbial survival for that cycle is calculated according to:

$$\text{Log } b = \text{Log } a - F_H/D$$

where:

b = probability of survival
a = bioburden per item
D = $D_{170\ °C}$ = time at 170°C to reduce population of most resistant microorganism found in product or environment by 90%
F_H = equivalent minutes at 170 °C (assume z = 20 °C)

Example:

Minimum F_H provided by a production cycle is 4.0 min (previously determined by thermocouple).

a = 1000 spores/item (previously determined by bioburden studies)
$D_{170°C}$ = 0.4 min (most resistant indigenous flora)
Log b = 3-[4/0.4] = − 7
b = 10^{-7} probability of survival

6.2.1.9.2 Determination of Minimum Required F_H Value

The bioburden of the product, the resistance of the indigenous microorganisms, and the maximum acceptable level for microbial survival must be known when determining the lethality required for a dry heat sterilization cycle. The following formula may be used to establish the minimum F_H of the process.

$$F_H = D_{170\,°C} (\text{Log } a - \text{Log } b)$$

where:

F_H = minimum lethality required (assume z = 20 °C), expressed as the number of minutes equivalent to time at 170 °C that the slowest to heat item in the load should be heated

b = maximum acceptable level for probability of survival

a = bioburden per item

$D_{170\,°C}$ = time to reduce a population of the most resistant microorganism found in or on the product by 90%.

Example:

b = 10^{-6}

a = 10,000 spores per item (established from bioburden studies).

$D_{170\,°C}$ = 1.2 min (established from laboratory resistance studies

F_H = 1.2 [4-(−6)] = 12 min

The above process calculation assumes a simple logarithmic model for microbial destruction by dry heat. It is recognized that all microbial dry heat destruction data may not fit the logarithmic thermal destruction model exactly. However, this model is the most convenient and usable model available today. Besides, a review of the research performed by investigators studying the dry heat destruction of homogeneous cultures of a single species of microorganisms indicates that almost all have obtained the predicted "straight-line" survivor curve. Where there have been exceptions, the deviation that occurred was that the D value was smaller in the first

heating period than the D value for all later heating periods (18).

6.2.1.9.3 Determination of Process Time

The process time of a dry heat sterilization process required to deliver the minimum F_H value can be determined as follows:

- Establish the slowest to heat location by thermocouple analysis and adjust sterilization time such that the coolest zone is at process temperature for the specified time (e.g., 12.0 minutes in the example mentioned above). This method does not consider the additional lethality received by the item during the heating and cooling phase of the sterilization cycle.
- Establish the load cool point by thermocouple analysis and adjust the sterilization time such that the integrated lethality at the slowest to heat location is equal to or greater than the F_H required.

6.2.2.0 Equivalent Times

When process temperatures other than 170 °C are used, equivalent times can be determined through use of the following formula:

$$F_t^z = F_H/L \qquad \text{(a)}$$

F_t^z = the equivalent time at temperature t delivered to an item for the purpose of sterilization with a specific value of z (e.g., 20 °C).

F_H = the equivalent time of 170 °C delivered to an item for the purpose of sterilization; a z value of 20 °C is employed

$$L = \text{lethal time} = \log^{-1} \frac{(To - Tb)}{z}$$

$$L = 10^{(To-Tb)/z}$$

To = temperature within item other than 170 °C
Tb = base temperature (170 °C)

Equation (1) demonstrates that the following time temperature relationships provide equivalent lethality (assume z = 20 °C)

$F_{140°C}^{z=20°C}$ = 12.0/.032 = 375.0 min at 140°C

$F_{150°C}^{z=20°C}$ = 12.0/0.1 = 120.0 min at 150°C

$F_{160°C}^{z=20°C}$ = 12.0/.316 = 38.0 min at 160°C

$F_{170°C}^{z=20°C}$ = 12.0/1 = 12.0 min at 170°C

$F_{180°C}^{z=20°C}$ = 12.0/3.16 = 3.8 min at 180 °C

6.2.2.1 Biological Indicators

Biological indicators are microorganisms that exhibit a high resistance to a particular type of sterilization (e.g., dry heat). Calibrated biological indicators can be used to monitor the lethality of a dry heat sterilization process since they respond to the effects of time and temperature. In most instances, however, biological indicator data are used to supplement the information obtained with thermocouples in heat penetration studies. Nevertheless, the F_H can be calculated by using calibrated biological indicators as described in Section 6.2.2.1.2 below. In addition, biological indicators can be used as an additional tool to ensure that the probability of nonsterility is less than 1×10^{-6}.

6.2.2.1.1 Type and Number of Challenge Microorganisms Used as Biological Indicator

Spores of *B. subtilis* have been widely used as the challenge organism for dry heat. These spores have been selected because they exhibit a relatively high resistance to dry heat sterilization. However, spores of other bacterial species may be used if they are properly calibrated. Indeed, the resistance and spore population are important criteria when selecting biological indicators for challenging dry heat sterilization.

6.2.2.1.2 The number of spores used to monitor a sterilization cycle is dependent on the expected process lethality (i.e., F_H) and the resistance of the microorganism. This relationship is described by:

$$F_H/D_{170\,^\circ C} = (\text{Log } a - \text{Log } b)$$

where:

Log a − Log b = spore log reduction (SLR)

6.2.2.1.3 Biological indicators may be designed to indicate whether the sterilization cycle is providing sufficient lethality to assure a 10^{-6} probability of microbial survival with respect to the bioburden. In this mode the criteria for the biological indicator may be derived as follows:

$$\text{cycle time} = (\text{Log } N_s - \text{Log } 1 \times 10^{-6})\, D_s$$

where N_s is the bioburden on the product to be sterilized, 10^{-6} is the probability that the bioburden will survive the sterilization cycle, and D_s is the D value for the natural contaminants.

The initial number of microorganisms on the biological indicator, N_i, can then be derived from the following equation that was suggested by Bruch (7):

$$(\text{Log } N_i + 1)\, D_i = (\text{Log } N_s - \text{Log } 1 \times 10^{-6})\, D_s$$

where D_i is the D value of the biological indicator microorganism.

6.2.2 Cycles Designed for Sterilization Only—Overkill Approach

This approach is employed when sterilizing heat stable materials. With this approach F_H values are generally used that ensure a probability of survival of considerably less than 10^{-6} for the heat resistant challenge microorganism. In order

to ensure such microbial overkill, F_H values that will provide a 12-log reduction of a biological indicator that exhibits a high resistance to dry heat are employed.

6.2.2.1 Type and Number of Challenge Microorganism Used as Biological Indicators

It should be recognized that most biological indicators will not have sufficient resistance and number of spores to monitor F_H values delivered by overkill cycles. Cycles having F_H values greater than 20 min will inactivate the majority of biological indicators currently in use by the pharmaceutical industry.

When employing overkill sterilization cycles for heat stable commodities it is suggested that *B. subtilis* spores be used as the biological indicator because of their relatively high resistance to dry heat. The number of spores used to monitor a sterilization cycle is dependent upon the expected process lethality and the resistance of the microorganism. This relationship is described by:

$$F_H/D_{170\ °C} = \text{Log } a - \text{Log } b$$

where:

Log a − Log b is the spore log reduction (SLR).

As a general approximation, the number of spores required for each carrier can be determined by:

$$N_0 = \text{Log}^{-1} [F_H/D_{170\ °C}]$$

when employing fraction negative methods. (N_0 = the number of spores on each carrier).

6.2.2.2 Determination of Survivors

The process time required to deliver the minimum required F_H value can be determined as described in Section 6.2.1.9.3.

6.2.2.3 Equivalent Times

If process temperatures other than 170 °C are used,

equivalent times can be determined as described in Section 6.2.2.0.

6.2.3 Cycles Designed for Sterilization and Endotoxin Inactivation

When endotoxin inactivation is the desired endpoint in a dry heat sterilization process, relatively high temperatures and extended heating times are employed. If the dry heat cycle is run long enough to destroy endotoxin, the chance of microorganisms surviving is remote. Consequently, in this approach cycle parameters are adjusted to ensure that the slowest to heat location in the loading pattern receives adequate endotoxin inactivation.

6.2.3.1 Bioburden and Heat Resistance Studies on Product Isolates

Refer to Sections 6.2.1.1 and 6.2.1.2.

6.2.3.2 Endotoxin Studies

The use of endotoxin challenges for monitoring a depyrogenation process requires a knowledge of the endotoxin levels in the commodity prior to processing, especially if the commodity supports growth of gram negative microorganisms.

Techniques and methods for recovering endotoxin from various commodities are available from several sources (3, 14, 17, 20, 34).

6.2.3.3 Endotoxin Testing Methodology

Two methods commonly used for detecting endotoxin are listed below:

6.2.3.3.1 USP Pyrogen Test

The current USP rabbit test consists of injecting the product into an ear vein of a rabbit under specified conditions and recording the body temperature for 3 hr after injection to see if there is a significant rise

in temperature. For a detailed description of the test consult USP XX (36). This test is subject to the inherent variability characteristic of all animal tests (38).

6.2.3.3.2. Limulus Amebocyte Lysate (LAL Test)

This method has been shown to be the most sensitive method presently available for detecting endotoxins. It is an *in vitro* procedure which utilizes lysate prepared from the amebocytes of the horseshoe crab *Limulus polyphemus*. The test is simple, specific, rapid, and inexpensive compared to the USP pyrogen test.

The LAL test consists of combining a specified amount of limulus amebocyte lysate with the solution sample, incubating at 37 °C for a specified period of time, and checking the sample for evidence of clotting. A positive endpoint is indicated by the formation of a solid gel clot endpoint, a turbidity endpoint, or a colorimetric endpoint (35). The test is read by visual examination of the clot or by spectrophotometric techniques. Quantitation can be achieved by comparing the test endpoint with that of a known endotoxin positive control. Details of the procedure can be found in the Guideline for the Use of Limulus Amebocyte Lysate Test (LAL) for Pyrogen Testing of Medical Devices (11) or directions provided by LAL manufacturers.

6.2.3.4 Biological Indicators

If it is desired to employ biological indicators in production cycles during reproducibility studies, the necessary details for determining the type and number of challenge microorganisms used as biological indicators are described in Section 6.2.2.1.

6.2.3.4.1 Calibration of Biological Indicators

Since heat resistant organisms such as *B. subtilis* have D values of only a few seconds at temperatures generally used for endotoxin inactivation, the microbial lethality delivered by cycles designed for endotoxin inactivation will provide a large margin of safety with regard to sterility. It should be recognized that the resistance and number of spores of dry heat biological indicators currently in use are not sufficient to accurately monitor the F_t^z values delivered by cycles designed for endotoxin inactivation. Such cycles can be monitored by physical means (e.g., thermocouples) at a level of lethality far in excess of that which can be monitored by biological indicators. Nevertheless, biological indicator challenges may be performed to verify that a large margin of safety is being achieved. It should be noted, however, that the F_t^z value obtained with biological indicators in such cases will be a very conservative estimate (refer to Section 6.3.2.2.2).

6.2.3.4.2 Correlation of Laboratory Resistance Values with F_t^z Values

An alternative method would be to correlate the laboratory obtained dry heat resistance values (e.g., D and z values) for the biological indicators or resistant bioburden organisms with the F_t^z values obtained with thermocouples in production challenges. Such an approach would allow the calculation of predicted microbial kills and the establishment of cycle safety factors. This method would, therefore, eliminate the need for biological indicators in the production cycles. An example of this method is described below.

Example:

The minimum $F_{t=220\,^{\circ}C}^{z=20\,^{\circ}C}$ value detected by thermocouples for a particular commodity in a production run was 120 min. Laboratory dry heat resistance values for the biological indicator with the commodity in question gave, z = 20 °C, $D_{220\,^{\circ}C}$ = 0.004 min. Therefore, the spore log reduction (SLR) provided by the cycle can be determined by:

$$\text{SLR} = \frac{F_{t=220\,^{\circ}C}^{z=20\,^{\circ}C}}{D_{220\,^{\circ}C}}$$

$$= 120 \text{ min}/.004 \text{ min}$$
$$= 30{,}000$$

Or, a cycle with an $F_{t=220\,^{\circ}C}^{z=20\,^{\circ}C}$ of 120 min would provide a $10^{30,000}$ reduction of the biological indicator.

6.2.3.5 Calculation of Delivered Process Lethality

The process lethality can be estimated by integrating the lethal rates obtained for the slowest to heat location within the load or by determining the time that the coldest point in the load is at process temperature as described in Section 6.3.1.

6.3 Plant Studies

6.3.1 Calculation of Delivered Process Lethality (Alternative Approaches)

6.3.1.1 Integration of Lethal Rates

6.3.1.1.1 A temperature of 170 °C is recognized as the sterilization standard for dry heat sterilization by the U.S. Pharmacopeia and other official compendia (37). Therefore, to demonstrate the magnitude of sterilization assurance associated with a dry heat process the temperature data collected during heat penetration studies is con-

verted to lethal rates at specified time intervals by the following formula:

$$L = \text{Log}^{-1} \frac{(To - Tb)}{z} = 10^{(To-Tb)/z}$$

where:

To = temperature within the commodity
Tb = reference temperature (i.e., 170 °C)
z = temperature increment required to change the D value by a factor of 10. A z value of 20 °C is an appropriate general dry heat z value.

A table of lethal rates can be compiled for routine use. An example of such a table is provided in Appendix A.

6.3.1.1.2 The F_H is then determined by integrating the lethal rates throughout the heating process. Analytically, the calculation of the lethality of the process (F_H) is described by the equation:

$$F_H = \int L dt$$

Several methods have been developed by which the lethal rates of a steam sterilization process can be determined (26, 27, 31). These same principles can be applied for dry heat sterilization processes. One of the least difficult methods which provides a reliable estimate of the F values is the Patashnik Method (23). An example of this method is displayed in Appendix B.

6.3.1.2 Time at Temperature

The time that the slowest to heat location in the loading pattern is at process temperature can be used to express the lethality. This is a conservative approach since it does not consider the additional

lethality that accumulated during the heating or cooling phases.

6.3.1.3 Sterilization at Temperature Other Than 170 °C

When process temperatures other than 170 °C are used for sterilization, the amount of time providing equivalent lethality to that at 170 °C can be calculated as described in Section 6.2.2.0.

6.3.2 Biological Indicator Challenges

6.3.2.1 Procedure for Use of Biological Indicators

6.3.2.1.1 Inoculate the commodity in question or a carrier material of choice with a known volume of spore suspension. Equilibrate at environmental conditions prior to sterilization.

6.3.2.1.2 Determine the viable spore count on representative biological indicator units.

6.3.2.1.3 Perform the microbial challenge studies during the heat penetration reproducibility studies (refer to Sections 3.1.7, 3.2.7, 4.2.7, and 5.3.7). A minimum of ten challenge units should be employed per challenge. Position the majority of the challenge units in the slowest to heat location of the loading pattern. Place challenge units adjacent to commodities that contain thermocouples.

6.3.2.1.4 The loading pattern should be the same as that specified for normal production use. If variable size loading patterns are used, conduct the challenge studies with the production conditions that yield the minimum F_H values.

6.3.2.1.5 Initiate the sterilization cycle.

6.3.2.1.6 After the specified sterilization cycle, recover the test units and at least one unchallenged unit as a negative control. In addition, at least one unprocessed biologi-

cal indicator should be tested as a viability control.

6.3.2.1.7 Enumerate survivors by appropriate plate count or fraction negative methods. Treat the negative control and the viability control in the same manner as the test samples.

6.3.2.2 Evaluation of Biological Indicator Results

The process lethality of the dry heat sterilization cycle as measured by biological indicators is determined in the following manner:

$$F_H = D_{170\ ^\circ C}\ (\text{Log}\ a - \text{Log}\ b) \qquad \text{(a)}$$

The $D_{170\ ^\circ C}$ of the indicator would have previously been determined by laboratory studies (Section 6.2.1.3).

6.3.2.2.1 Example Employing Survivor Count Results

Assume:

$D_{170\ ^\circ C} = 1.0$ min
$a = 1.0 \times 10^8$ spores/replicate
$b = 5.0 \times 10^1$ spores/replicate

Therefore:

$$F_H - 1.0(8 - 1.7) = 6.3 \text{ min}$$

6.3.2.2.2 Example Employing Fraction Negative Results

Assume:

$D_{170\ ^\circ C} = 1.0$ min
$a = 1 \times 10^7$ spores/replicate
b = 20 replicates/5 negative replicates

Therefore, according to equation (a)

$b = 2.303 \log_{10}(20/5) = 1.39$ spores/replicate
$F_H = 1.0(7 - .143) = 6.9$ min

With overkill sterilization cycles all biological indicators may be inactivated due to the high F_H values employed. In these situations, a minimum F_H value can be estimated by assuming one positive unit (the minimum F_H value obtained in this manner will be a conservative estimate). For example, if 20 challenge units were used and all were negative, a minimum F_H value could be calculated as follows:

Assume:

$D_{170\,°C}$ = 1.5 min (z = 20 °C)

a = 1.0×10^8 spores/unit

b = 20 units/19 units negative (one positive unit assumed)
Thus,

b = 2.303 log (20.19) = .05

Therefore,

$$F_H = 1.5(8 - [-1.3]) \geqq 14.0 \text{ min}$$

6.3.3 Endotoxin Challenges

6.3.3.1 Minimum Endotoxin Inactivation

A level of endotoxin in excess of the normal expected level in the commodity can be used to challenge the depyrogenation cycle. A reasonable margin of safety should be included in the challenge endotoxin level.

6.3.3.2 Procedure for Use of Endotoxin Challenge

6.3.3.2.1 Inoculate appropriate samples of commodities (or suitable carriers of the same composition if it is not possible to directly inoculate the commodity) with an aliquot containing an appropriate amount of *E. coli* lipopolysaccharide [Braude strain, 0 group 113:H10:K negative (reference endotoxin EC-2) or suitable equivalent

hereafter referred to as *E. coli* LPS]. As a general rule, commodities should be washed in the same manner as regular production commodities and should receive a volume of endotoxin equal to the mean volume of water present in the container after the wash cycle.

6.3.3.2.2 Appropriate positive and negative controls should be run to rule out the possibility of false positive tests due to reagent and/or equipment contamination and to determine that a sufficient amount of endotoxin is present at the time of use (11).

6.3.3.2.3 Perform the endotoxin challenge studies during the reproducibility studies described in Sections 3.1.7, 3.2.7, 4.2.7, and 5.3.7. Position the majority of the challenge units in the slowest to heat location of the load. Place challenge units adjacent to commodities that contain thermocouples.

6.3.3.2.4 The loading pattern should be the same as that specified for normal production use. If variable size loading patterns are used, conduct the challenge studies with the production conditions that yield the lowest F_H values.

6.3.3.2.5 Initiate the specified cycle.

6.3.3.2.6 After processing, recover the challenge units and determine the residual endotoxin in the challenge units as described in Section 6.2.3.3. Since a more accurate estimate of endotoxin inactivation can be provided if some endotoxin survives the challenge, the challenge level should be such that it would yield a measurable amount of endotoxin after "depyrogenation." However, this does not guarantee that some endoxin will survive. The cycle may be capable of destroying much greater amounts of endotoxin than can be conveniently provided during challenge studies.

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APPENDIX A

Dry Heat Lethal Rates (z = 20°C)*

Temp. (°C)	Lethal Rate (Min. at 170°C) (Min. at T)	Temp. (°C)	Lethal Rate (Min. at 170°C) (Min. at T)	Temp. (°C)	Lethal Rate (Min. at 170°C) (Min. at T)
110	0.001	144	0.050	164	0.501
111	0.001	145	0.056	164.5	0.531
112	0.001	146	0.063	165	0.562
113	0.001	147	0.071	165.25	0.579
114	0.001	148	0.079	165.5	0.596
115	0.002	149	0.089	165.75	0.613
116	0.002	150	0.100	166	0.631
117	0.002	150.5	0.106	166.25	0.649
118	0.002	151	0.112	166.5	0.668
119	0.003	151.5	0.119	166.75	0.688
120	0.003	152	0.126	167	0.708
121	0.003	152.5	0.133	167.25	0.729
122	0.004	153	0.141	167.5	0.750
123	0.004	153.5	0.150	167.75	0.771
124	0.005	154	0.158	168	0.794
125	0.006	154.5	0.168	168.25	0.818
126	0.006	155	0.178	168.5	0.841
127	0.007	155.5	0.188	168.75	0 866
128	0.008	156	0.200	169	0 891
129	0.009	156.5	0.211	169.25	0 917
130	0.010	157	0.224	169.5	0 944
131	0.011	157.5	0.237	169.75	0.972
132	0.012	158	0.251	170	1.000
133	0.014	158.5	0.266	170.25	1.029
134	0.016	159	0.282	170.5	1.059
135	0.018	159.5	0.299	170.75	1.090
136	0.020	160	0.316	171	1.122
137	0.022	160.5	0.335	171.25	1.155
138	0.025	161	0.355	171.5	1.189
139	0.028	161.5	0.376	171.75	1.223
140	0.032	162	0.398	172	1.258
141	0.035	162.5	0.422	172.75	1.296
142	0.040	163	0.447	172.5	1.334
143	0.045	163.5	0.473	172.75	1.372

APPENDIX A - (con't)

Dry Heat Lethal Rates (z = 20°C)*

Temp. (°C)	Lethal Rate (Min. at 170°C) (Min. at T)	Temp. (°C)	Lethal Rate (Min. at 170°C) (Min. at T)	Temp. (°C)	Lethal Rate (Min. at 170°C) (Min. at T)
173	1.412	187.5	7.500	218	251.189
173.25	1.454	188	7.943	219	281.838
173.5	1.496	188.5	8.414	220	316.228
173.75	1.540	189	8.913	221	354.813
174	1.585	189.5	9.441	222	398.107
174.25	1.631	190	10.000	223	446.683
174.5	1.679	191	11.220	224	501.187
174.75	1.728	192	12.589	225	562.341
175	1.778	193	14.125		
175.5	1.884	194	15.849		
176	1.995	195	17.783		
176.5	2.113	196	19.953		
177	2.239	197	22.387		
177.5	2.371	198	25.119		
178	2.512	199	28.184		
178.5	2.661	200	31.623		
179	2.818	201	35.481		
179.5	2.985	202	39.810		
180	3.162	203	44.668		
180.5	3.350	204	50.119		
181	3.548	205	56.234		
181.5	3.758	206	63.096		
182	3.981	207	70.795		
182.5	4.217	208	79.433		
183	4.467	209	89.125		
183.5	4.732	210	100.000		
184	5.012	211	112.202		
184.5	5.309	212	125.893		
185	5.623	213	141.254		
185.5	5.957	214	158.489		
186	6.311	215	177.828		
186.5	6.683	216	199.526		
187	7.079	217	223.872		

* $L = 10^{\frac{T - 170°C}{20°C}}$ = minutes at the 170°C reference temperature per minute at "T" °C.

APPENDIX B: Calculation of the Lethality of a Dry Heat Sterilization Process Using the Method of Patashnik

The time/temperature curves generated in the heat penetration studies provide us with the necessary data for calculating the F_H value of a dry heat sterilization process. For example, the temperature data collected are converted to lethal rates at specified time intervals by the formula:

$$L = 10^{\frac{T_t - 170\ °C}{z}}$$

where:

T = Temperature at time t
170 °C = Base temperature
z = Temperature increment required to change the D value by a factor of 10. A z value of 20 °C is assumed when calculating F_H.

Both Stumbo (31) and Pflug (26) have summarized the various methods of integrating the lethal rates during a sterilization cycle. Of the various methods available, the trapezoidal method of Patashnik (23) is the least difficult and provides a reliable estimate of the F_H value. In this method the area under the time/temperature curve is divided into equally spaced parallel cords. The distance between these cords, Δt, will be the constant time interval between successive temperature printouts from the recording potentiometer. The area under the curve is found by using the formula:

$$F_H = \Delta t(Y_1 + Y_2 + Y_3 + \ldots Y_{n-1})$$

where values of y are the lethal rates.

An evaluation of heat penetration data by method of Patashnik is seen in the accompanying table.

APPENDIX B (con't)

Calculation of the Lethality of a Dry Heat Sterilization Process by Patashnik Method

Time (min)	Temperature (C°)	Lethal Rate min at 170°C Min at T
5	105	0.0006
10	110	0.0010
15	120	0.0032
20	135	0.0178
25	150	0.1000
30	165	0.5623
35	170	1.0000
40	172	1.2589
45	174	1.5849
50	174	1.5849
55	174	1.5849
60	175	1.7782
65	165	0.5623
70	150	0.1000
75	140	0.0316
80	130	0.0100
85	110	0.0010
90	105	0.0006

Σ of lethal rates = 10.1922

F_H = Δt (Σ of lethal rates) = 10.1922

5 x 10.1922 = 50.961 min at 170°C