



MANUFACTURING & QUALITY AUDIT DIVISION
CENTRE FOR DRUG ADMINISTRATION

Ref. No.: GUIDE-MQA-011-004

Effective Date : 06 SEP 2004

**GUIDANCE NOTES ON
VALIDATION OF TERMINAL MOIST HEAT
STERILISATION**

© HSA September 2004

Reproduction prohibited for commercial
purposes.

Reproduction for internal use is authorised,
provided the source is acknowledged.

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

INTRODUCTION

This document provides guidance for the submission of information and data in support of the efficacy of moist heat sterilisation processes.

The efficacy of a given sterilisation process for a specific drug product is evaluated on the basis of a series of protocols and scientific experiments designed to demonstrate that the sterilisation 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 manufacturer can conclude that the efficacy of the sterilisation process is validated. Sterilisation process validation data should be generated using procedures and conditions that are fully representative and descriptive of the procedures and conditions proposed for manufacture of the product.

INFORMATION FOR TERMINAL MOIST HEAT STERILIZATION PROCESSES

1. Description of the Process and Product

1.1 The Drug Product and Container-Closure System

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

1.2 The Sterilisation Process

A description of the sterilisation process used to sterilise the drug product in its final container-closure system, as well as a description of any other sterilisation process(es) used to sterilise 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.

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

1.3 The Autoclave Process and Performance Specifications

A description and of the autoclave process, including pertinent information such as cycle type (e.g., saturated steam, water immersion, and water spray), and statement of cycle parameters and performance specifications including minimum and maximum F_0 , temperature, the pressure and time including the time requirement for venting the sterilizer of air, the product come-up time to the desired temperature and cooling time. Identify the autoclave(s) to be used for production sterilisation, including manufacturer, model and serial number.

1.4 Autoclave Loading Patterns

A description of representative autoclave loading patterns and loading-carrying devices (e.g. metal basket, trolley or perforated tray), should be provided.

1.5 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.

When an automated autoclave with computer controlled program is used, routine monitoring of the sterilising temperature and pressure should not be relied solely on the information from the print-out data. Additional routine checks for each production cycle are necessary such as the use of biological indicators or calibrated maximum thermometer placed inside the autoclave.

1.6 Requalification of Production Autoclaves

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

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

1.7 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.

1.8 Maintenance of Autoclaves

A schedule for maintenance of autoclaves should be documented and followed strictly.

2. Thermal Qualification of the Cycle

2.1 All equipment used in studying the steam steriliser, such as temperature and pressure instrumentation must be calibrated with calibration instruments traceable to the national reference instruments. Information on calibration program for the equipment used should also be provided.

2.2 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 sterilisation cycle should be provided for each autoclave. Results from a minimum of three consecutive, successful cycles should be provided to ensure that the results are consistent and meaningful.

Heat distribution studies should include two phases: 1) heat distribution in an empty autoclave chamber and 2) heat distribution in a loaded autoclave chamber. Identify the location of the cool spot and the effect of the load size and/or configuration on the cool spot location on a reproducible basis. The difference in temperature between the coolest spot and the mean chamber temperature should be not greater than $\pm 2.5^{\circ}\text{C}$.

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

Heat penetration studies should include the determination of the F_0 value of the cold spot inside the commodity located at the cool spot previously determined from heat-distribution studies container mapping studies. The container cold spot for containers $\geq 100\text{ml}$ is determined using container-mapping studies. The studies should include minimum and maximum loading configurations. Statistical analysis of the F_0 values achieved at each repeated cycle may be conducted to verify the consistency of the process and the confidence limits for achieving the desired F_0 value.

Any changes in the load size, load configuration, or container characteristics (volume, geometry, etc) must be accompanied by repeat validation studies.

Repeat validation studies maybe required, if the autoclave has undergone repair or moved to a new location that has different facility supplies (e.g. steam supply).

2.3 Thermal Monitors

The number of thermal monitors used (≥ 10) and their location in the chamber should be described. A diagram is helpful.

Accuracy of thermocouples should be $\pm 0.5^\circ\text{C}$. Thermocouples should be calibrated before and after a validation experiment at two temperatures: 0°C and 125°C . Any thermocouple that senses temperature more than 0.5°C away from the calibration temperature bath should be discarded. Stricter limits i.e., $<0.5^\circ\text{C}$, may be imposed according to the user's experience and expectations. Temperature recorders should be capable of printing temperature data in 0.1°C increments.

2.4 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 (including the time requirement for venting the sterilizer of air, the product come-up time to

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

the desired temperature and the cooling 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 sterilisation of the product that is the subject of the application.

3. 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 sterilisation 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.

3.1 Identification and Characterisation of Bioburden Organisms

Describe the methods and results from studies used to identify and characterise 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.

Where overkill cycles are not employed, the indigenous bioburden must be characterised in un-sterilised products for at least 3 lots initially and at predefined intervals for an extended period thereafter.

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

3.2 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.

3.3 Identification, Resistance, and Stability of Biological Indicators

Correlation between physical and microbiological validation should be performed using suitable biological indicators, e.g., *Bacillus stearothermophilus*. Information and data concerning the identification, supplier, spore population on the carrier, resistance (D and Z values), survival and kill time, storage conditions 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, purity and resistance, and provide performance specifications.

3.4 The Resistance of the Biological Indicator Relative to That of Bioburden

Studies characterising 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.

3.5 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 sterilise conditions (e.g., the most difficult to sterilise 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

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

solutions in which it is most difficult to kill the biological indicator under sterilisation cycles that simulate production conditions.

Maximum allowable manufacturing time limits between initial product compounding and sterilisation of the last filled container must be established to ensure pre-sterilisation bioburden does not increase beyond established levels.

4. Computerised Systems used in Sterilisers

- 4.1 Computerised systems e.g. programmable logic controllers (PLC) in sterilisers shall be validated to meet the requirements of international standards such as Good Automated Manufacturing Practice (GAMP) with relevant validation life-cycle documents maintained.
- 4.2 Print outs from sterilisers and electronic data generated are to be treated as records with retention periods defined as per site procedures.
- 4.3 Levels of access and system security shall be defined for softwares that permit changes to critical parameter setting for sterilisation.

5. Microbiological Monitoring of the Environment

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.

6. Steam Quality

Steam used for sterilisation should be of suitable quality. It should not contain impurities at a level that would cause contamination of product or equipment and the steam condensate should meet the analytical specification for Water for Injection.

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

The steam quality must be tested periodically to ensure that

- 6.1 moist heat (rather than dry-heat) sterilising conditions are achieved;
- 6.2 superheating does not occur;
- 6.3 wet loads are avoided;
- 6.4 non-condensable gases is below 3.5%; and
- 6.5 mineral and organic impurities (including bacteria and pyrogens) are below specified maximum levels.

The three basic steam quality tests are the superheat test, dryness value and non-condensable gas tests.

Even if the quality of the steam is satisfactory, another aspect of the steam supply, i.e. variations in steam pressure, may adversely affect the process. The pressure of the steam supply line to the autoclave should not fluctuate by more than $\pm 10\%$ under all conditions.

7. 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:

7.1 Simulation of the Stresses from Processing

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

7.2 Demonstrate Integrity Following the Maximum Exposure

Container-closure integrity should be demonstrated on product units that have been exposed to the maximum sterilisation 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.

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

7.3 Multiple Barriers

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

7.4 The Sensitivity of the Test

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

7.5 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 A of this guidance.)

8. Bacterial Endotoxins Test and Method

The bacterial endotoxins test should be validated and carried out in accordance with the requirements of the compendial methods. The validation of gelation method should include initial qualification of the laboratory such as equipment qualification and technician qualification, test for confirmation of labelled sensitivity of the LAL reagent, inhibition and enhancement testing and determination of noninhibitory concentration and maximum valid dilution.

At present, the gelation test for bacterial endotoxins is the official method of analysis. Four types of quantitative assays may be recognised, namely turbidimetric end point method, kinetic turbidimetric method, chromogenic peptide end-point method and kinetic chromogenic peptide method. The method used should be shown to meet the requirement for linear regression, i.e, a significant slope and non-significant deviations from linear regression. For end point methods, an additional requirement of having an intercept not significantly different from zero should be fulfilled

Test for confirmation of labelled sensitivity of the LAL reagent must be repeated for every new lot of reagent used. Qualification of the laboratory technician is required for new technician before carrying out the test.

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

Revalidation of bacterial endotoxins test is required when conditions that are likely to influence the test result change (e.g., change in manufacturer of the LAL reagent or the formula of the product).

Routine bacterial endotoxin test should include verification of labelled LAL reagent sensitivity and positive and negative controls in duplicates as per requirement of the compendial method.

9. Sterility Test Methods and Release Criteria

Sterility test methods used for the product should be validated and carried out in accordance with the requirements of the compendial methods, including the release criteria. Protocols should be provided to include samples size and selection of representative units during production should be provided. Test should be performed within barrier systems and information concerning validation of the barrier system should be provided. Revalidation of sterility test methods is required when conditions that are likely to influence the test result change (e.g., change in formula of the product).

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

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. 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 tests should be performed. Standard methods for microbial challenge tests for effectiveness of antimicrobial preservatives are prescribed in the British Pharmacopoeia, the European Pharmacopoeia and the United States Pharmacopoeia. For purposes of the stability protocol, the first three production lots should be tested with microbial challenge tests 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. Bacterial Endotoxin Test

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.

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

REFERENCES

1. Guidance for industry for the submission of documentation for sterilisation process validation in applications for human and veterinary drug products. FDA.
2. PDA Technical Report No 30: Parametric release of pharmaceuticals terminally sterilised by moist heat. PDA Journal of Pharmaceutical Science and Technology. Jul-Aug 1999. Vol 53(4); 219-222
3. Moist heat sterilisation in the UK Pharmaceutical Industry. PDA Journal of Pharmaceutical Science and Technology. Sep-Oct 2000. Vol 54(5); 413-420
4. British Pharmacopoeia
5. United States Pharmacopeia
6. European Pharmacopoeia
7. Pharmaceutical Process Validation, Second Edition (1993), edited by Ira R. Berry and Robert A. Nash, Marcel Dekker, Inc., New York

END OF DOCUMENT

GUIDANCE NOTES

Subject: VALIDATION OF TERMINAL MOIST HEAT STERILISATION

REVISION HISTORY

Rev No.	DCC Ref No.	Author	Date of Revision	Remarks of Revision
001	-	-	01 Nov 2001	New document.
002	-	-	09 Dec 2002	Update document format.
003	215	Terrance Ong	01 Mar 2004	1) Change 3D HSA logo to 2D HSA logo. 2) Change Centre name to Centre for Drug Administration 3) Detached Revision History from Guidance Notes.
004	263	Terrance Ong	06 Sep 2004	1) Re-located Revision History to the main document.