GMP無菌作業技術論壇(二)

從法規要求與科學基礎探討滅菌蒸氣的品質

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大綱

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1. 溼熱滅菌製程與滅菌用蒸氣的種類

1. 滅菌方法
2. 溼熱滅菌法
3. 滅菌用蒸氣種類

1.1 滅菌方法
PIC/S GMP Guide Annex 1Draft, 2019

■ Sterilization by heat	條文	8.47-8.51 (5)
■ Moist heat sterilization		8.52 – 8.62 (11)
■ Dry heat sterilization		8.63 – 8.67 (5)
■ Sterilization by radiation		8.68–8.70 (3)
■ Sterilization with ethylene oxide		8.71–8.76 (6)
■ Filter sterilization of products which cannot be sterilized in their final container		8.77- 8.91(15)

1.1 滅菌方法

USP <1229 >STERILIZATION OF COMPENDIAL ARTICLES

- 1229.1 Steam Sterilization by Direct Contact
- 1229.2 Moist Heat Sterilization of Aqueous Liquids
- 1229.3 Monitoring of Bioburden
- 1229.4 Sterilizing Filtration of Liquids
- 1229.5 Biological Indicators for Sterilization
- *1229.6 Liquid Phase Sterilization (Formalin, H_2O_2 …)*
- *1229.7 Gaseous Sterilization (EO, ClO_2…)*
- 1229.8 Dry Heat Sterilization
- 1229.9 Physicochemical Integrators and Indicators for Sterilization
- 1229.10 Radiation Sterilization
- *1229.11 Vapor Phase Sterilization (H_2O_2 , Peracetic Acid.)*
- 1229.12 New Sterilization Method

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1.1 滅菌方法

Sterilization vs. Decontamination

- 問題：Isolator內使用VHP去汙染過程可降低BI 10^6以上，是否可稱為滅菌，用於膠塞缽？

- Agency is being asked, why Vapour Hydrogen Peroxide (VHP) cannot be used for “sterilisation” of these direct and indirect product contact parts. After all, pharmacopeias refer to VHP as a sterilising agent. However, our concern is that although under ideal conditions, VHP can achieve a reduction of biological Indicator spores of up to 6 logs, the process itself is incredibly fragile.
- VHP failure due to very minor occlusion, even to the degree that fatty acids from a fingerprint may “protect” contaminating organisms from the VHP demonstrate the true fragility of the process as a sterilant.

Ref. Andrew Hopkins, VHP (Vapour Hydrogen Peroxide) Fragility MHRA Blog, Apr.2018

1.1 滅菌方法

USP <1229>STERILIZATION OF COMPENDIAL ARTICLES

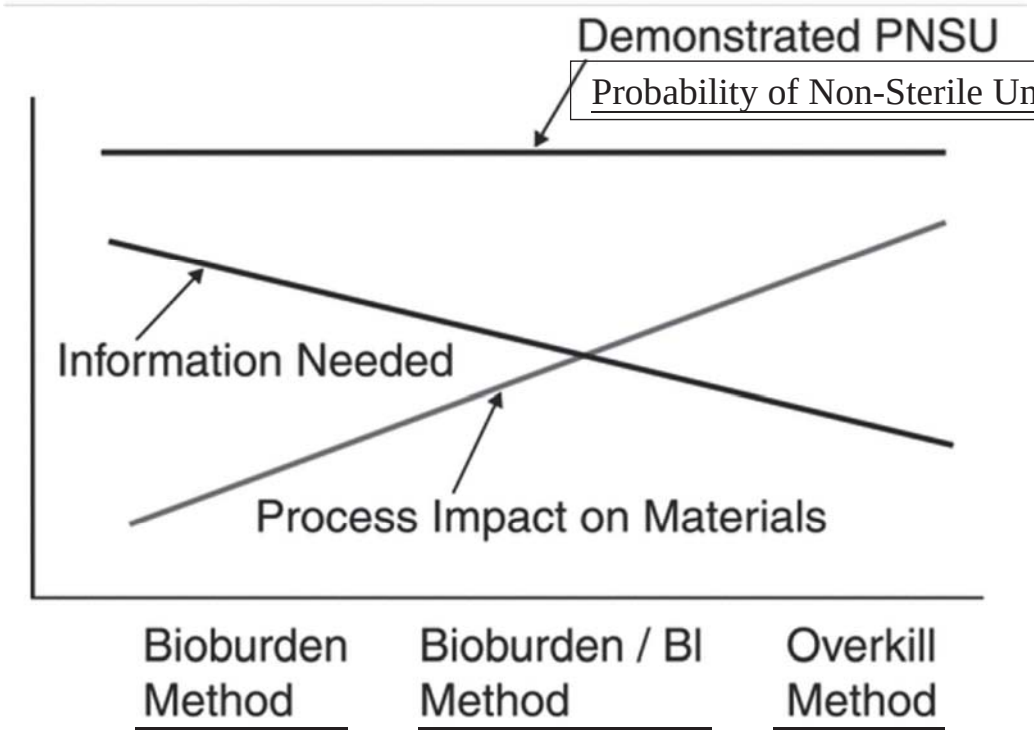

Figure 1. A basic comparison of various validation methods.

1.1 滅菌方法

EMA

EMA Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container, Mar. 2019

- Steam sterilisation performed with finished product temperature below 115 ˚C during the holding phase is an exceptional case and should be scientifically justified and supported by additional data as described in Table 1.

- If temperatures below 110 ˚C are included (during heat-up and cool-down) in the determination of F_0, this should be justified.

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Figure 1 Decision tree for sterilisation choices for aqueous products

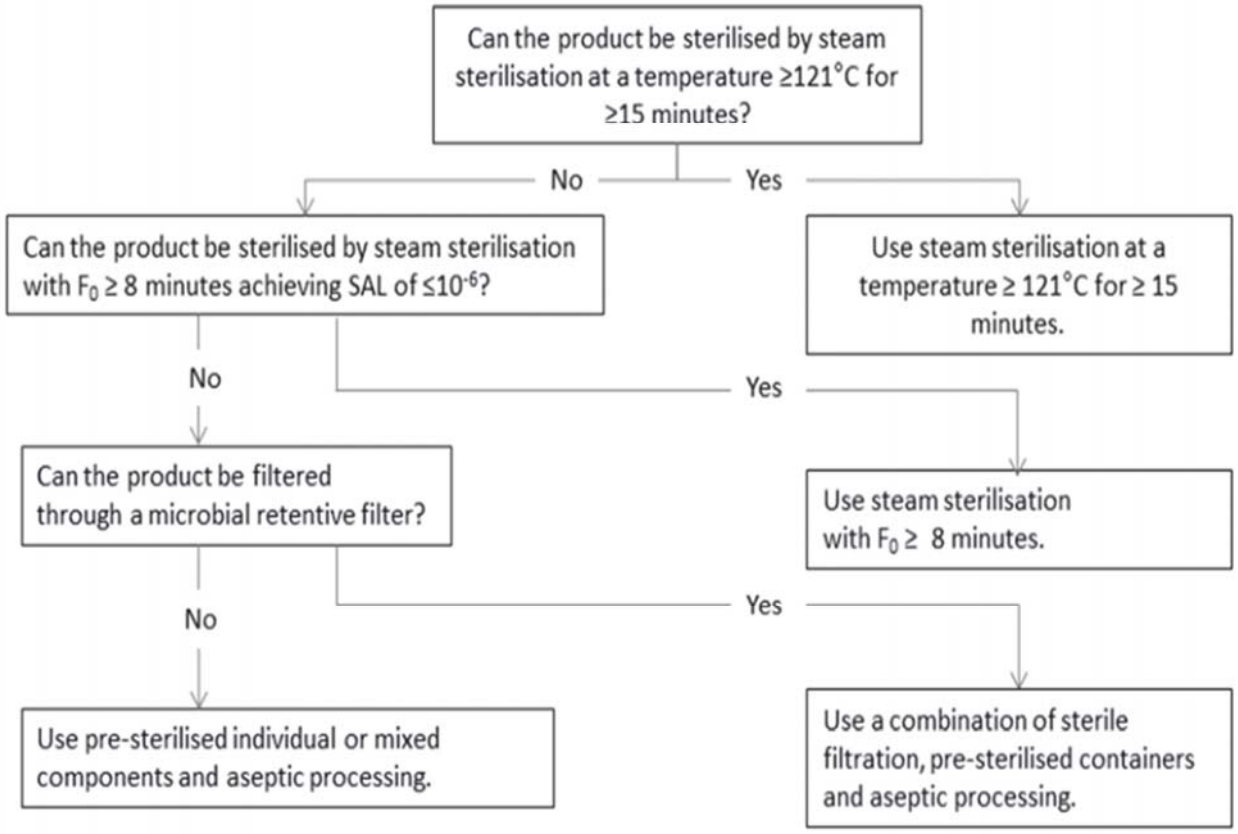

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Figure 2 Decision tree for sterilisation choices for dry powder products, non-aqueous liquid or semi-solid products

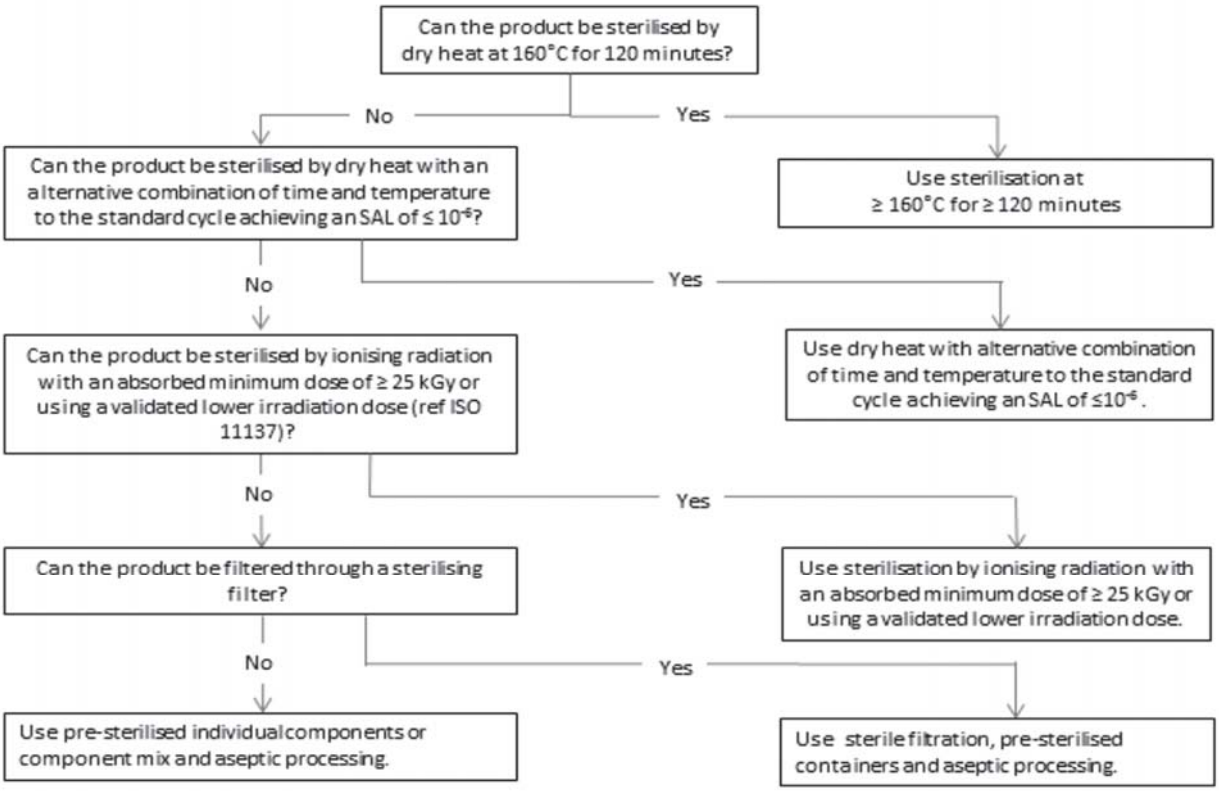

Table 1 Cycles for steam sterilisation and post-aseptic processing terminal heat treatment and corresponding data required in the quality dossier

Cycle	Type of process	Information in dossier*	Bioburden level before steam sterilisation or terminal heat treatment	Bioburden Characterised	Process hold temperature
Ph. Eur. 5.1.1 Reference Cycle	Sterilisation	1, 6	100 CFU/100ml (non-routine)	No	≥ 121 °C for ≥15 minutes
Overkill cycle F_o >12 min	Sterilisation	1, 2, 3, 4, 7	100 CFU/100ml (non-routine)	No	≥ 121 °C
F_o > 8 min	Sterilisation	1, 2, 3, 4, 7	100 CFU/100ml (routine)	No	> 115 °C
F_o > 8 min	Sterilisation	1, 2, 3, 5, 7, 8	100 CFU/100ml (routine)	Yes**	> 115 °C
F_o > 8 min	Sterilisation	1, 2, 3, 4, 7	100 CFU/100ml (routine)	Yes	> 110 °C
F_o > 8 min	Sterilisation	1, 2, 3, 5, 7, 8	100 CFU/100ml (routine)	Yes**	> 110 °C
F_o <8 min	Post-aseptic processing terminal heat treatment	1, 2, 3, 4, 7, 8	0 CFU/100ml, aseptic filtration and processing prior to terminal heat treatment (routine)	Yes***	> 110 °C****
F_o <8 min	Post-aseptic processing terminal heat treatment	1 2, 3, 5, 7, 8	0 CFU/100ml, aseptic filtration and processing prior to terminal heat treatment (routine)	Yes***	> 110 °C****

* For clarification of the code numbers, see below

** In-process control demonstrating acceptable heat resistance of bioburden

*** The bioburden prior to the sterilisation step (i.e. filtration) should be characterised for heat resistance

**** Temperatures below 110 °C may be used if justified. The requirement for additional documentation for such cycles is evaluated on a case by case basis

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1.1 滅菌方法
美國與歐盟的差異性討論

美國：

- 兼顧無菌保證與產品安定性
- 無菌製造環境汙染的微生物大多來自人體(G+細菌)
  - D(60) values **of Staphylococcus aureus** ranged from 4.8 to 6.5 min
  - Reported D- and z-values for Staphylococcus spp. in acid and pasteurized foods at 82.2°C are 0.4 s and 7°C, respectively
  - **Staphylococcus epidermis** at 0.87 water activity, a D-value at 70C of 500 s was recorded

歐盟：

- 無菌性對病人的風險不應妥協
- 可以例外，但必須提出充足的科學證據

1.2 溼熱滅菌法
PIC/S GMP Guide Annex 1 Draft, 2019

Section 8 Introduction

- Moist heat sterilization utilises steam or superheated water,
- Moist heat sterilization of hard goods or porous loads is primarily affected by latent heat of condensation of clean steam and the quality of steam is therefore important to provide consistent results.

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1.2 溼熱滅菌法
PIC/S GMP Guide Annex 1 Draft, 2019

- For aqueous liquid-filled containers, energy from moist heat is transferred through conduction and/or convection to the contents of the container without direct contact with the autoclave steam. In these cases, time and temperature are the key parameters and steam quality does not have the same impact to the process.
- Superheated systems are typically used for the terminal sterilization of product in flexible containers where the pressure differential associated with the steam would cause damage to the primary container.

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1.2 溼熱滅菌法
裝載型態 - 多孔性/硬質物件裝載

- 多孔性/硬質物件裝載多採用飽和蒸汽滅菌，當飽和蒸汽在被滅菌的物件表面上直接凝結時，熱就被傳遞。

- 多孔性/硬質物件裝載例
  - 過濾器
  - 橡膠塞與其他聚合物的瓶塞物料
  - 管子和軟管
  - 服裝
  - 清潔用的設備
  - 機器更換的零件

Ref. Technical Report No.1: Validation of Moist Heat Sterilization Processes: Cycle Design, Development. Qualification and Ongoing Control; PDA 2007

1.2 溼熱滅菌法
裝載型態 – 液體裝載

- 充填液體已密封的容器，由來自同一批之單一尺寸(size)、單一充填量的容器所構成，通常是均一的。

- 濕熱能量透過傳導及/或對流而傳遞到已裝填液體的容器內。

- 液體裝載例
  - 在最終產品容器(例如：管瓶、袋、注射器或安瓿)內的配方(溶液、懸浮液及/或乳劑)
  - 試驗或操作後，可能含有病原微生物的廢液

1.2 溼熱滅菌法 - 過程型態(Processes Types)

3.1 飽和蒸汽過程(Saturated Steam Process)

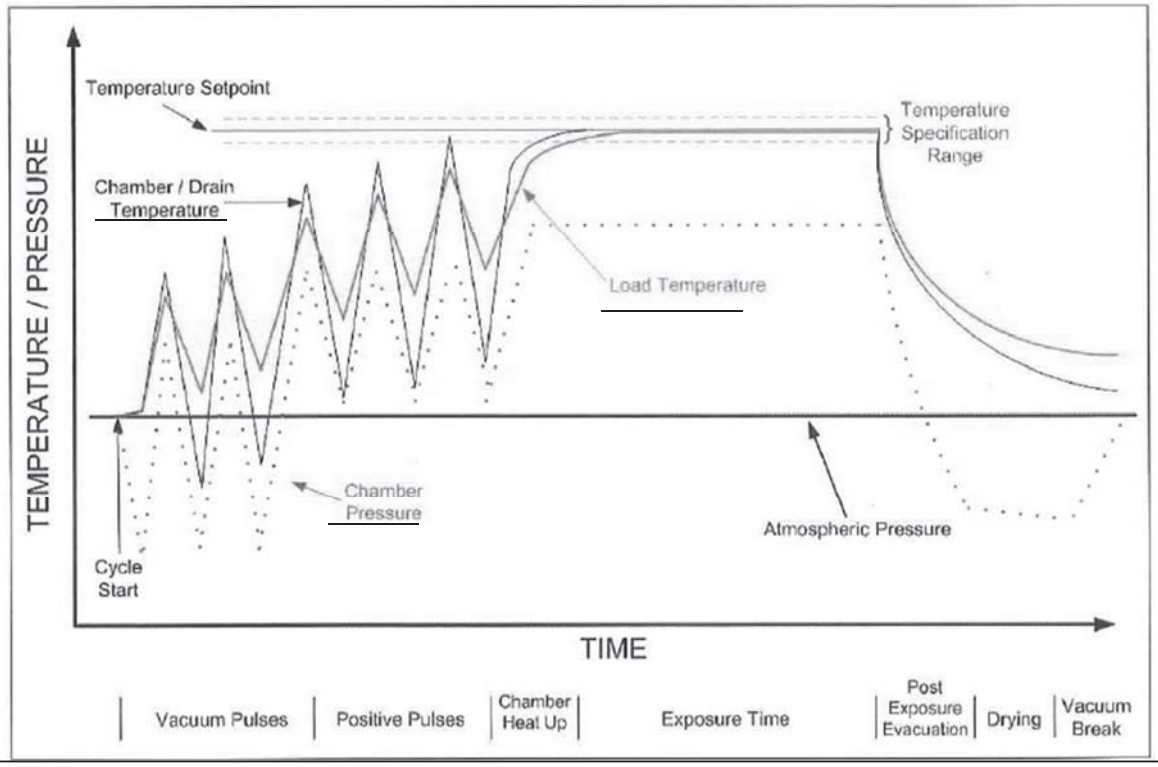
- 3.1.1 預真空過程 (Pre-vacuum Process)
- 3.1.2 重力置換過程 (Gravity Displacement Process)

3.2 超壓空氣過程(Air Overpressure Processes)

- 3.2.1 蒸汽/空氣混合物(SAM)過程 (Steam-Air Mixture Process)
- 3.2.1 超熱水過程 (Superheated Water Process)

1.2 溼熱滅菌法 - 過程型態(Processes Types)
預真空過程

Figure 4.3.1.1–1 ■ Prevacuum Process Cycle Example



1.2 溼熱滅菌法 - 過程型態(Processes Types)
重力置換過程

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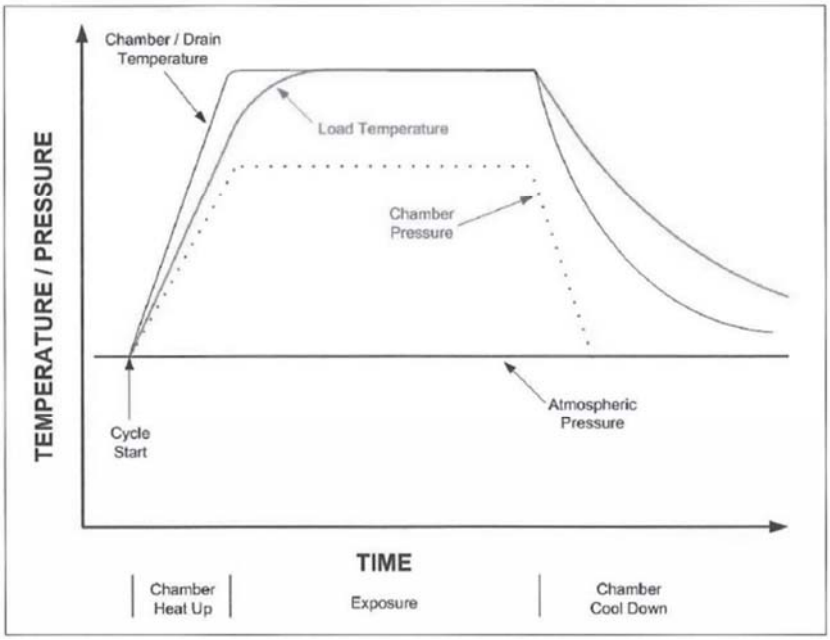
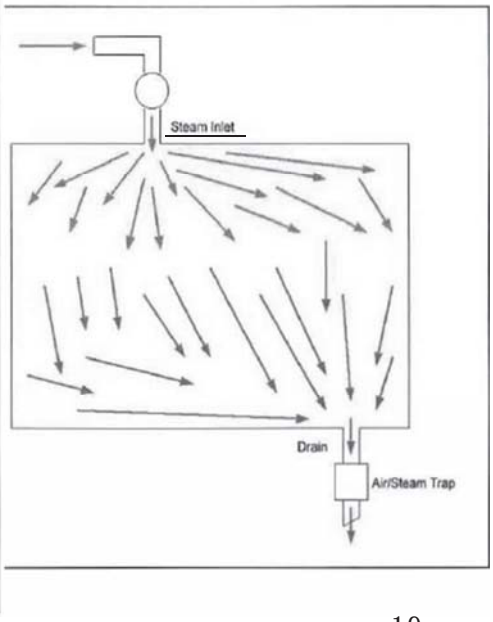
Figure 4.3.1.2–2 ■ Gravity Displacement Process Cycle Example



ıre 4.3.1.2–1 ■ Gravity Displacement Sterilizer



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1.2 溼熱滅菌法 - 過程型態(Processes Types)
蒸汽/空氣混合物(SAM)過程

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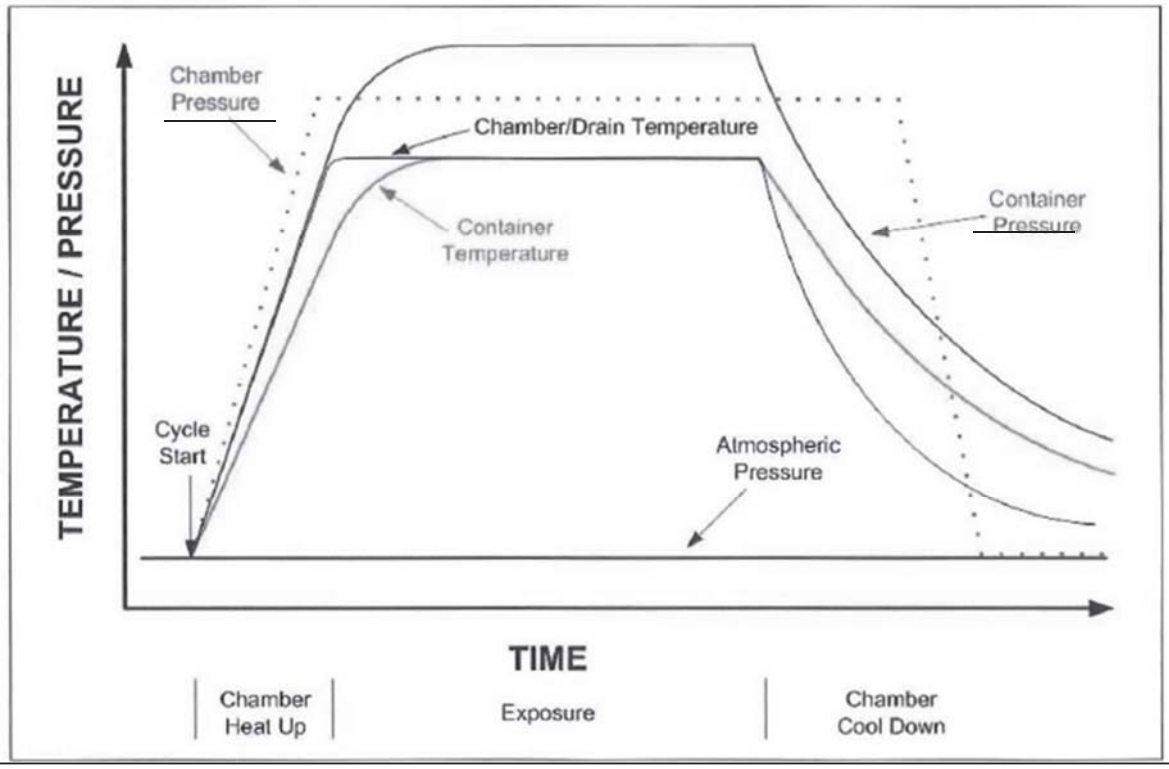
Figure 4.3.2.1–1 ■ Steam-Air Mixture Process Cycle Example



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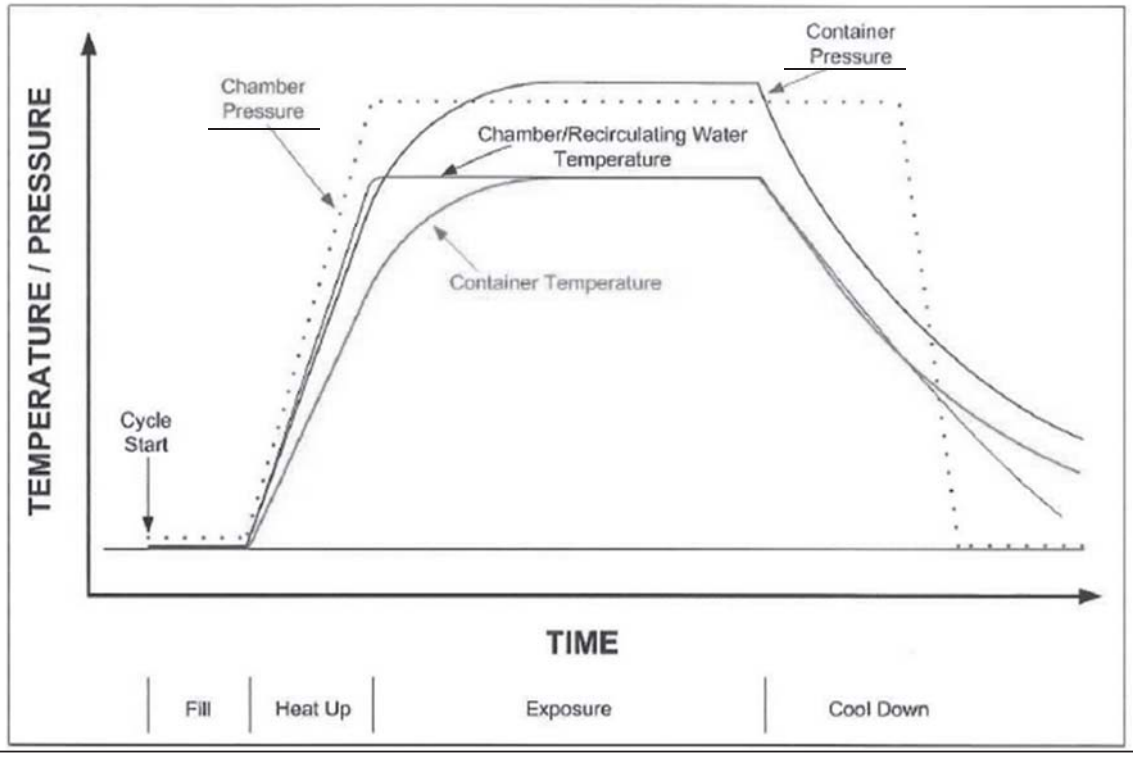
1.2 溼熱滅菌法 - 過程型態(Processes Types)
超熱水過程

Figure 4.3.2.2–1 ■ Superheated Water Process Cycle Example



1.3 滅菌用蒸氣種類

- 工廠蒸汽 (Plant Steam)
  - 適合於提供給滅菌機之夾層
  - 用於熱交換器之非衛生端的超熱水過程的加熱
- 過程蒸汽 (Process Steam)
  - 類似於工廠蒸汽但使用被管制的原水(不含揮發性添加物amines或hydrazines)
  - 在滅菌之前，容器已被充填並密封之情況下，過程蒸汽可適用於液體裝載之濕熱滅菌。
- 純蒸汽 (Pure Steam)
  - 純蒸汽是其冷凝水符合注射用水(WFI)規格的蒸汽。
  - 可使用軟化水、去離子水、與純淨水當原水。
  - 純蒸汽多使用於多孔性/硬質物件裝載之滅菌。

2. 直接滅菌用的蒸氣品質的法規要求

1. PIC/S GMP 附則一
2. EN 285

2.1法規要求 - PIC/S GMP Guide Annex 1 Draft, Dec 2017

Steam used as a direct sterilising agent

7.17 Purified water, with a low level of endotoxin, should be used as the minimum quality feed water for the pure steam generator.

7.18 Steam used for sterilization processes should be of suitable quality and should not contain additives at a level which could cause contamination of product or equipment.

The quality of steam used for sterilization of porous loads and for Steam-In-Place (SIP) should be assessed periodically against validated parameters. These parameters should include consideration of the following examples: non-condensable gases, dryness value (dryness fraction), superheat and steam condensate quality.

2.2 法規要求 - EN 285, 2015

13.3 Steam supply to the sterilizer chamber

13.3.1 Non-condensable gases

The sterilizer shall be designed to operate with saturated steam containing up to 3,5 ml noncondensable gases collected from 100 ml condensate when tested as described in 21.1.

NOTE

This method does not necessarily express the true content of NCG in steam. The limiting value was defined experimentally in the 1960s in relation to the sensitivity of air detectors commonly used in the UK at that time. Repeated measurements give an idea of the true picture of NCGs in the steam supply.

2.2 法規要求 - EN 285

13.3 Steam supply to the sterilizer chamber

13.3.2 Dryness value

The sterilizer shall be designed to operate with saturated steam with a dryness value not less than 0.95, where the dryness value denotes the mass of the gas fraction in the mass of saturated steam, when tested as described in 21.2.

註：**13.3.3 Dryness value** The sterilizer shall be designed to operate with saturated steam with a dryness value down to 0,95 for metal loads and 0,90 for other types of load when tested as described in 22.2. (EN 285 2006)

13.3.3 Superheat

When the supplied steam is expanded to atmospheric pressure the superheat shall not exceed 25 K when tested in accordance with 21.3.

2.2 法規要求 - EN 285

13.3 Steam supply to the sterilizer chamber

13.3.4 Contaminants

The sterilizer shall be designed to operate with steam that does not contain contaminants in quantities that can impair the sterilization process or harm or contaminate the sterilizer or sterilized load.

NOTE 1 Suggested maximum values of some contaminants are given in Table 4.

NOTE 2 A method for obtaining a condensate sample is given in 21.4.

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2.2 法規要求 - EN 285

Table 4 — Suggested maximum values of contaminants in condensate from steam supply to the sterilizer chamber

Determinant	Condensate
Silicate	≤ 0,1 mg/l
Iron	≤ 0,1 mg/l
Cadmium[c]	≤ 0,005 mg/l
Lead[c]	≤ 0,05 mg/l
Rest of heavy metals except iron, cadmium, lead[b]	≤ 0,1 mg/l
Chloride	≤ 0,1 mg/l
Phosphate	≤ 0,1 mg/l
Conductivity (at 20 °C)[a]	≤ 4,3 µS/cm
pH (20 °C) value	5 to 7
Appearance	Colourless clean without sediment
Hardness (Σ Ions of alkaline earth)	≤ 0,02 mmol/l

[a] See European Pharmacopeia.

[b] If the condensate meets the requirements on conductivity, it is not necessary to perform heavy metal tests.

[c] The limiting values meet the requirements for potable water.

2.2 法規要求 - EN 285

13.3 Steam supply to the sterilizer chamber

13.3.5 Pressure fluctuation

If steam is supplied from an external source, the sterilizer shall be designed to operate with a pressure fluctuation not exceeding ± 10 % of the pressure measured at the inlet to the final pressure reduction valve.

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2.2 法規要求 - EN 285

13.3 Steam supply to the sterilizer chamber

13.3.6 Feed water

If a dedicated steam generator is used (see 5.2.1), the sterilizer shall be designed to operate with steam produced from water free from contaminants in a concentration that can impair the sterilization process or harm or contaminate the sterilizer or sterilized load. If the quality of feed water can affect the quality of steam supplied to the sterilizer chamber (see Annex B) it shall be specified [see 25.2 m)].

NOTE Non-condensable gases dissolved in the feed water can cause an increase in non-condensable gases in the steam, see 13.3.1.

3. 滅菌蒸氣品質法規要求的科學探討
3.1 濕熱熱力學

Ref. Technical Report No.1: Validation of Moist Heat Sterilization Processes: Cycle Design, Development. Qualification and Ongoing Control; PDA 2007

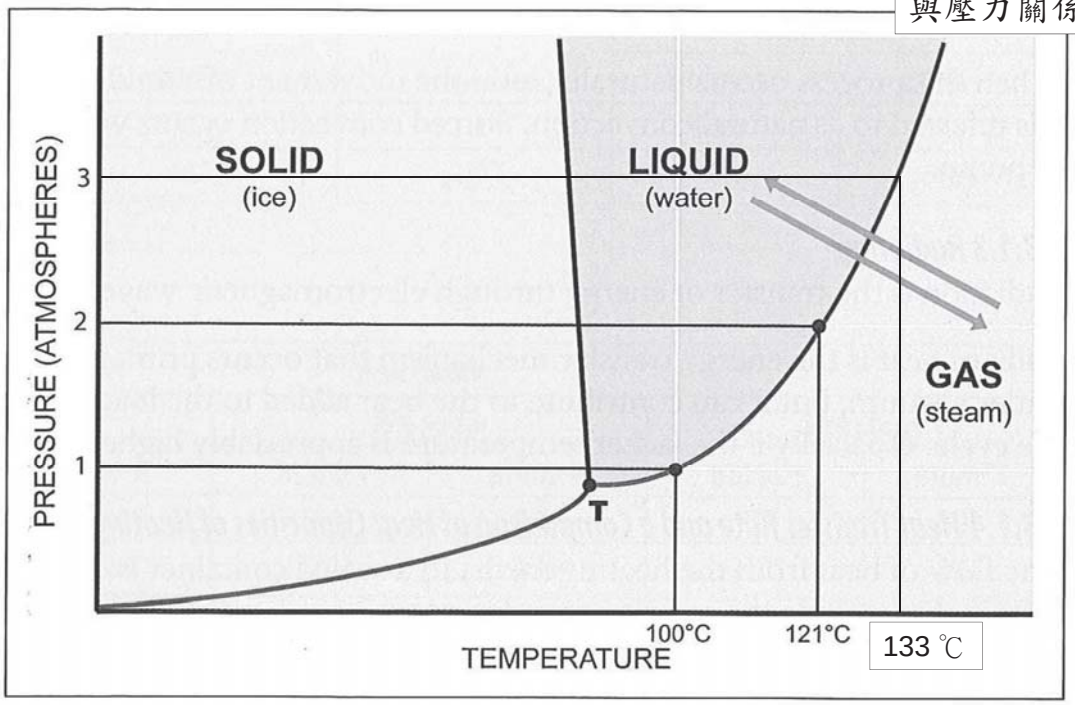
溫度、熱與濕熱

- 濕熱(Moist Heat)：
  - 飽和蒸汽(Saturated Steam)
  - 蒸汽/空氣混合物 (Steam-Air Mixture) (SAM)
  - 超熱水(Superheated Water)：溫度超過100^{o}C的液態水，需要超壓以保持這種狀態
- 溫度(Temperature)：溫度是熱能的量測值
  (溫度分佈) Temperature Distribution
- 熱(Heat)：一個物體和它的環境之間的溫差而被移轉的能量　(熱滲透) Heat Penetration

溫度、壓力與水之三相圖

Figure 3.3.1–3 ■ Pressure and Temperature Phase Diagram

飽和蒸汽之溫度與壓力關係恆定

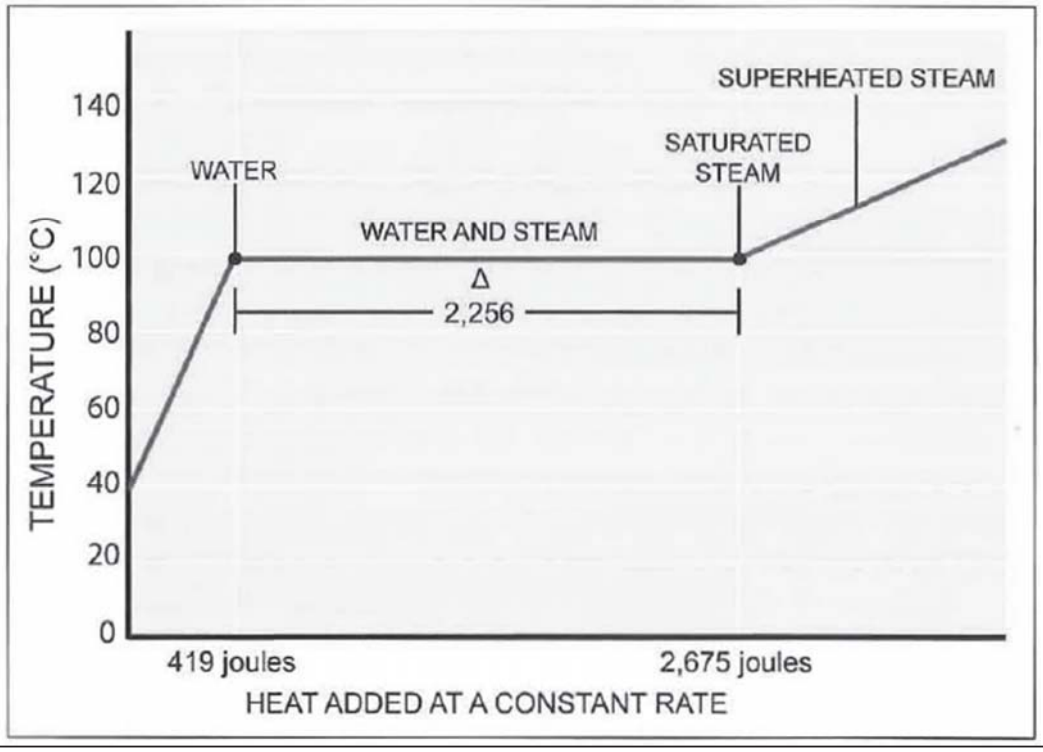


飽和蒸汽的熱能

- 在25°C和1.0大氣壓下，使一克液體水改變溫度1°C，需4.1焦耳的熱。
- 蒸發/凝結的熱是在飽和蒸汽過程中把能量傳給被滅菌物件的主要機制。
- 飽和蒸汽在100 °C含有2,675 J/g的能量，這是水本身的能量(419 J/g)加上變成蒸汽所需的能量(2,256 J/g)或在100 °C之下的蒸發/凝結熱的加總。
- 一克的蒸汽凝結時，會把2,256焦耳的能量傳送給在100°C的物體。

飽和蒸汽熱能

Figure 3.3.1–1 ■ Heating Curve of Water at 1.0 Atmosphere



飽和蒸汽熱能

表 3.3.1-2 飽和蒸汽表

飽和水與蒸汽之性質(公制單位)				
溫度	壓力	焓(內部的熱能) J/g		
℃	Bar**	Water h_l	Δh*	Steam h_v
100	1.013	419	2256	2675
115	1.692	483	2216	1699
120	1.987	504	2202	2706
121	2.026	508	2199	2707
125	2.322	525	2188	2713

* 冷凝或蒸發之潛熱($\Delta h^* = h_v - h_l$)

** 1.0 Atmosphere = 1.013 bar = 14.71 psia

熱能移轉的機制

- **傳導(Conduction)**：傳導是能量經由分子攪動(agitation)而移轉的機制。例如
  - 經過容器的瓶壁以傳送到被滅菌的液體
  - 滅菌媒介物(例如：蒸汽或熱水) 與物件直接接觸，而將能量移轉到物件的表面
- **對流(Convention)**：對流是經由與一個移動中的流體相接觸而將能量移轉的過程。例如
  - 自然對流
  - 強迫式對流。
- **輻射(Radiation)**：輻射是能量經由電磁波而移轉的機制。 例如
  - 真空下的乾燥階段
  - 當夾層溫度高於艙室溫度的時候。

濕熱滅菌過程之能力

表 3.3.1.4-1 滅菌過程之能力與需求概要

滅菌過程		熱移轉速率	需要循環	溫度分佈挑戰	裝載考量
飽和蒸汽		高	否	低	不需總壓力大於飽和蒸汽壓力之多孔性/硬質物件與液體之裝載
蒸汽/空氣混合物		蒸汽到水之速率與流速的函數	是	高	需要總壓力大於飽和蒸汽壓力之液體與可能某些多孔性/硬質物件之裝載
超熱水	超壓空氣/水噴	中高，流速的函數	是	中度	需要總壓力大於飽和蒸汽壓力之液體之裝載
	超壓空氣/水浸	高，但為流速的函數	是	中度	需要總壓力大於飽和蒸汽壓力之液體之裝載

濕熱滅菌過程之能力

表 3.3.1.4-2 飽和蒸汽

溫度	隱潛的熱容量(Δh)	
	BTU/lb.℉	BTU/ft³ ℉
212℉	970.3	36.1
250℉	945.3	68.3

Condensation to water will cause a volume decrease in excess of 99% for saturated steam cycles.

表 3.3.1.4-3 水

溫度	熱容量	
	BTU/lb.℉	BTU/ft³ ℉
212℉	1.001	59.87
250℉	1.003	58.98

表 3.3.1.4-4 蒸汽/空氣混合物

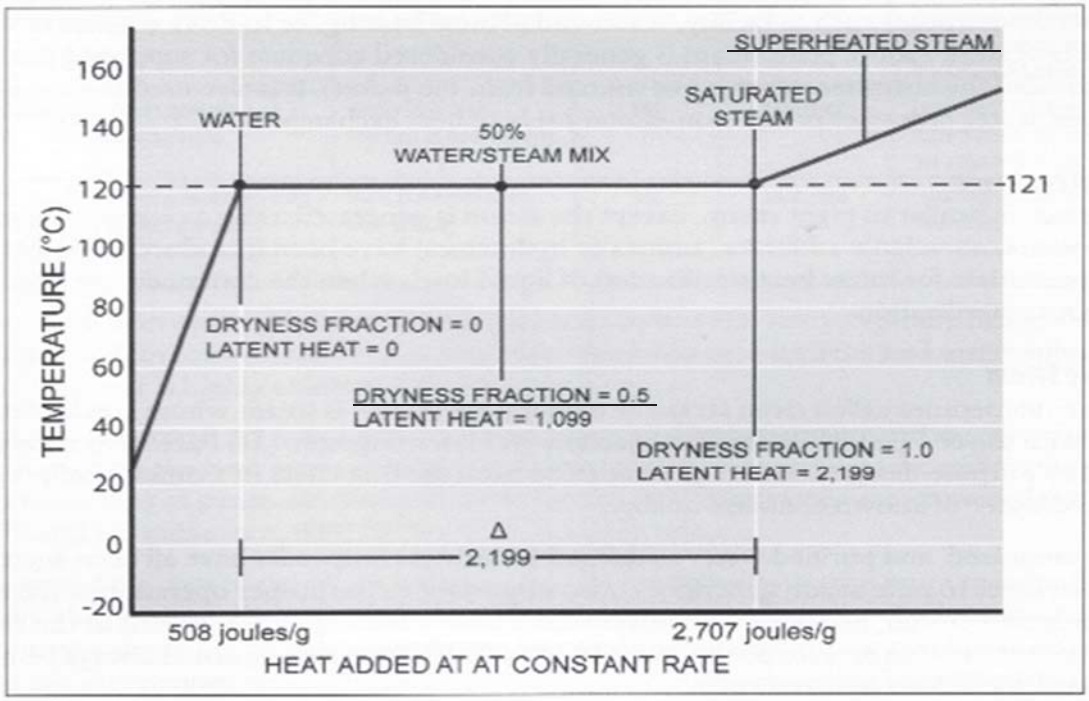
溫度	熱容量					
	60%蒸汽		75%蒸汽		90%蒸汽	
℉	BTU/lb.℉	BTU/ft³℉	BTU/lb.℉	BTU/ft³℉	BTU/lb.℉	BTU/ft³℉
212℉	13.06	0.61	19.94	0.86	27.6	1.10
250℉	11.56	0.72	17.21	1.12	24.29	1.65

純蒸汽的蒸汽品質試驗

- 非可冷凝氣體(Non-condensable Gases)
  - 可能被蒸汽從蒸汽發生器所帶過來的氣體(例如：空氣、氮氣與二氧化碳)　　規格：NMT3.5%V/V - EN285
- 乾燥部分與乾燥值(Dryness Fraction and Dryness Value)　　規格： NLT 0.95–EN285
  - 蒸汽的乾燥值(一個經試驗確定的乾燥部分)是適用於飽和蒸汽過程之蒸汽所攜帶的液態水之數量的量測值。
- 超熱(Superheat)　　規格： LT 25K–EN285
  - 超熱蒸汽是在特定的壓力下，溫度高於水蒸發的平衡曲線所指出的溫度的蒸汽。
  - 超熱的主要原因是：
    - 在使用點附近的過度降壓
    - 滅菌機之艙室的夾層的溫度比艙室的溫度高

乾燥值與超熱

Figure 3.3.3.2–1 ■ Dryness Fraction at 2.0 Atmospheres (Absolute)



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謝謝聆聽！

敬請指教！

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