5.1.1 & 5.1.2

V'Iain Fenton-May
Chairman Group 1

Methods of Preparation of Sterile Products

Changes started in 2008 and were mainly driven under the Chairmanship of Prof Hans van Doorne

Methods of Preparation of Sterile Products

5.1.1 is intrinsically linked to 5.1.2

- The texts have been revised and rewritten
- Methods of obtaining sterile product have been revised and elaborated on.
- The main focus was to ensure that the processes
  - – were valid and
  - –could be shown to be valid.

Methods of Preparation of Sterile Products

- 5.1.2 has been extensively changed in order to ensure the validity of the test.
- There is now a requirement to show that the BIs are suited to the process (a reduced cycle is used to avoid surviving organisms at full cycle)

5.1.2 (Biological Indicators)

Traditionally Bio Indicators have been treated as a ‘comfort’ test on the sterilising process. The test was considered to be outdated and required change

The changes are to introduce scientific rigour to the test.

The User is responsible for ensuring the validity of the BIs for the process (either by testing or audit of the manufacturer)

Proof that the indicator will test the lethality of the product/cycle, requires some survivors at the end of the cycle.

Revision of 5.1.2 (history)

1) Position paper: *Biological Indicators, Tools to Verify the Effect of Sterilisation Processes* K. Haberer, H. van Doorne - March 2011

2) Pharmeuropa 24.1 in December 2011, >80 comments many critical

Following consultation it was deemed unacceptable to have a test that allowed surviving organisms at the end of a sterilising process. The objective could be met if a reduced cycle was used for the validation

3) Pharmeuropa 27.3 in March 2015: new version, well received

4) Publication in **Supplement 9.2** in January 2017

5.1.2 Biological indicators and related microbial preparations used in the manufacture of sterile products

The main changes

1) New title so as to cover BI and microbial preparation for sterilisation grade filtration

2) Description of different types of BI and quality requirements

3) Guidance on how BIs are selected and how they are used to characterise sterilisation processes

4) Quantitative approach to be used for cycle development

A new Structure
1. General introduction
2. Biological indicators for sterilisation processes
3. Biological indicators for heat sterilisation
3.2 Biological indicators for moist heat sterilisation
3.3 Biological indicators for dry heat sterilisation
4. Biological indicators for gas sterilisation
5. Biological indicators for ionising sterilisation
6. Microbial preparations for sterilisation grade filtration

5.1.2 Biological indicators and related microbial preparations used in the manufacture of sterile products

Scope of the Chapter

The use of BIs is intended to cover the sterilisation of finished products and relevant related sterilisation processes (i.e. sterilisation processes for items coming into direct contact with the final sterilised product).

outside the scope: to validate the sterilisation of other non-terminal units

BIs are test systems containing viable micro-organisms (usually spores of bacteria) that provide a defined challenge to verify the required effectiveness of a specified sterilisation process.

BIs are intended for the *development and validation* of the sterilisation processes and not for routine monitoring unless otherwise stated in this general chapter.

5.1.1 The processes covered

- Steam
- Dry Heat
- Ionising radiation
- Gas
- Filtration

These are all now described in the same format

Covering: Principal, Equipment, Cycle, Effectiveness & Control

- Aseptic assembly is included as a means of retaining a sterile product

5.1.1 General comments

Viral safety is covered by 5.1.7

Efficacy of a process is dependant on conditions; the inactivation of micro-organisims follows an exponential curve.

Hence '*steps designed to reduce microbial contamination will contribute significantly to sterility assurance.'*

There is always a non-zero possibility of survival!

Steam Sterilisation

Highlights

- The standard cycle is defined

  (*15min at 121 ℃*)

  with the *minimum* temperature permitted

  (*110 ℃*)

- Cycle effectiveness correlated with biological effectiveness. Cycle validation is verified by exposure to Biological indicators (see 5.1.2).
- Routine control is by physical means not BIs.

Dry Heat Sterilisation

Highlights

- The standard cycle is defined

  (*160 ℃ for at least 2hrs*)

- Cycle validation is verified by exposure to Biological indicators (see 5.1.2).
- Routine control is by physical means not BIs.

Sterilisation by Ionising Radiation

Highlights

- The standard cycle requires an adsorbed dose of 25kGy (Kilograys)
- Cycle validation is by physical means
  - BUT may be verified by exposure to Biological indicators (see 5.1.2) for
    - tissues,
    - cell products and
    - products with a potential to protect spores.
- Routine control is by physical means not BIs.

Gas Sterilisation

Highlights

- No standard cycle is specified
  - Two classes of compounds are described
    - Alkylating Agents
    - Oxidising Agents
- Cycle validation of the complex mix of parameters is by physical and biological methods
  - Giving consideration to:
    - Conditioning – humidity, temperature & load configuration
    - Sterilisation – time & concentration with penetration
    - Aeration
- Routine control is by physical means **and** biological.

General comments

- Reference to other Official Guidance has been removed

  (GMP & EN on Ionising radiation)

- The efficacy of the process is dependent on
  - The processing conditions
    - Including Bioburden & physical parameters
- The inactivation is exponential and therefore there is a non-zero possibility of survival.

The revised chapter 5.1.2: Biological Indicators (BI's) in validation of sterilisation processes - a brief overview of the essential elements of the update of the chapter

Peter Annel, DVM, Danish representative in EDQM Group 1

Changes at a glance

- 1. General introduction
- 2. Biological indicators for sterilisation processes
- 3. Biological indicators for heat sterilisation
  - 3.2 Biological indicators for moist heat sterilisation
  - 3.3 Biological indicators for dry heat sterilisation
- 4. Biological indicators for gas sterilisation
- 5. Biological indicators for ionising sterilisation
- 6. Microbial preparations for sterilisation grade filtration
- 3. Biological indicators for heat sterilisation
  - 3.2 Biological indicators for moist heat sterilisation
  - 3.3 Biological indicators for dry heat sterilisation

Changes at a glance

- **2. Biological indicators for sterilisation processes**
  - 2-1 Description of biological indicators for sterilisation processes
  - 2-2 Quality requirements for biological indicators
    - Data required to be known by the user per delivery
    - 2-2-1 URS
    - 2-2-2 QC
    - 2-2-3 Suitability for purpose

Changes at a glance

- **2. Biological indicators for sterilisation processes**
  - Requirements for
    - No. of viable spores
    - D-value

    → Replaced by requirement of a SAL of 10^{-6} (5.1.1)
  - 2-1 Description of biological indicators for sterilisation processes
    - High level of confidence in the manufacturer's compliance in quality standards
    - Alternatively, the BI characteristics shall be verified

Changes at a glance

- **2-1 Description of biological indicators for sterilisation processes**
  - 2-1-1 Inoculated carriers
  - 2-1-2 Self-contained biological indicators

  Product or surface interaction - different reaction to sterilising conditions as compared to BI's
  Commercially available BI may not be suitable to test sterilisation effective-ness in the most difficult to sterilise locations
  An inoculum from a well-characterised spore suspension may be a better model:

  - 2-1-3 Characterised spore suspensions
  - 2-1-4 Custom-made biological indicators

Changes at a glance

- **2-2 Quality requirements for biological indicators**
- 2-2-1 URS
- Choice of BI based on the particular sterilisation process:
  - Microorganism
  - Type of BI
  - D-value
  - Initial spore count
  - Resistance suitable and worst case

Changes at a glance

- **Data to be known by the user per delivery of each batch**
  - Genus and species of the organism (CC no. as appropriate)
  - Unique reference (e.g. batch no.)
  - $Log_{10}(N_0)$
  - D-value (incl. confidence interval, variation range, if feasible)
  - Z-value (where relevant)
  - Type of sterilisation process incl. conditions
  - Method (FN, survivor curve, etc.)
  - Type of carrier
  - Type of packaging
  - Recovery method
  - Composition of the recovery medium
  - Type of indicator, if relevant
  - Storage conditions, expiry date

Changes at a glance

- **2-2 Quality requirements for biological indicators**
- 2-2-2 QC
- Users employing biological indicators outside of the manufacturer's recommendations should thoroughly characterise the biological indicators for the particular sterilisation process
- 2-2-3 Suitability for purpose
- The user shall ensure the that the biological indicator is inactivated to the expected survival rate by the particular range of sterilisation conditions used

Changes at a glance

- **4. Biological indicators for gas sterilisation**
  - Requirements adjusted (D-value relevant to the process etc.)
  - *Geobacillus stearothermophilus* suitable for VHP processes

Changes at a glance

- **5. Biological indicators for ionising radiation sterilisation**
  - May be required for certain types of processes (e.g. sterilisation of tissues, cell preparations or other specific cases…)
  - Reference dose transferred to 5.1.1

Changes at a glance

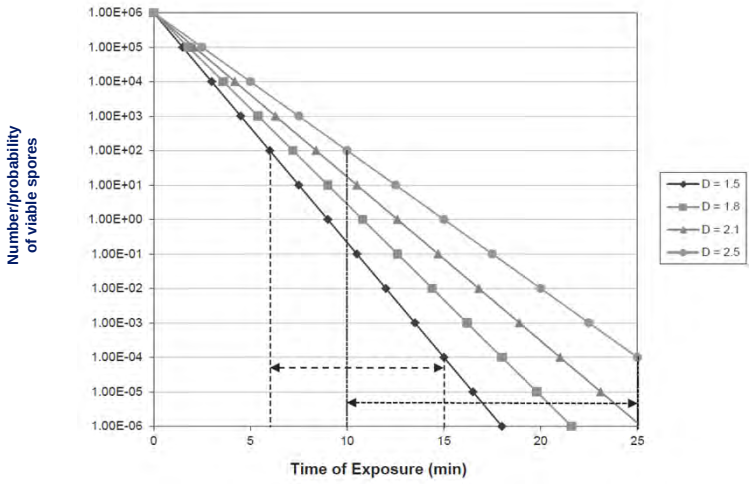
- **6. Microbial preparations for sterilisation grade filtration**
  - New section
  - Microbial challenge of 10^7 CFU/cm^2 filter area
    - ≤ 0.22 µm:
      - *Brevundimonas diminuta* recommended
      - Alternatively other microorganisms
    - ≤ 0.1 µm
      - *Acholeplasma laidlawii*

Changes at a glance

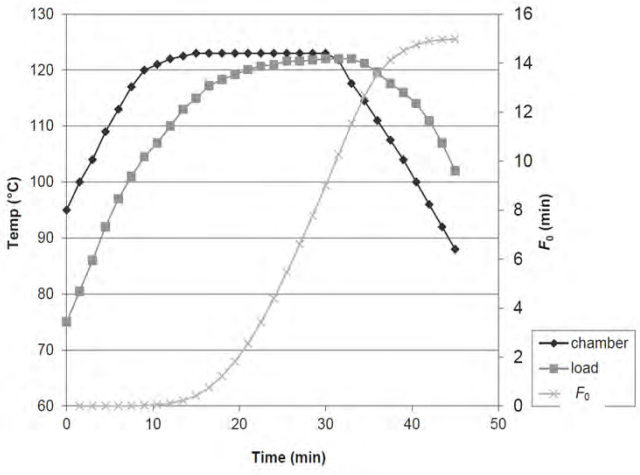
- **3. Biological indicators for heat sterilisation**
  - 3.2 Biological indicators for moist heat sterilisation
  - 3.3 Biological indicators for dry heat sterilisation

Sterilisation kinetics for bacterial spores

Sterilisation at 121 °C



Lethality effect of a steam sterilisation process



3-1-2 Establishment of validation cycle

Thus, at standard conditions (121 °C), the number (probability), N, of surviving spores at the time point t for a spore population with a certain D-value (D) and an initial spore population N_0 follows

$$log_{10}N = -t/D + log_{10}N_0$$

Survival time t_s is defined as

$$t_s = D \times (log_{10}N_0 - 2)$$

Kill time t_k is defined as

$$t_k = D \times (log_{10}N_0 + 4)$$

3-1-2 Establishment of validation cycle

<u>Demonstration of equivalence between physical and biological sterilisation effectiveness:</u>

Too high exposure time (t_{vl}) -> too low sensitivity to suboptimal sterilisation conditions (e.g. $t_{vl} \geq t_k$ (kill time))

Too short exposure time (t_{vl}) -> too high sensitivity to the variation in conditions (sterilisation process, D-value, initial spore count)

A theoretical surviving rate between 10^{-1} and 10^{-3} is considered an optimal compromise and is chosen (a reduction of the cycle might be needed):

Exposure time t_{vl} for development of the validation cycle:

$$D \times (log_{10}N_0 + 1) \leq t_{vl} \leq D \times (log_{10}N_0 + 3)$$

3-1-2 Establishment of validation cycle

The process can be accepted if

- the frequency of biological indicators with surviving microorganisms is
  - as expected
  - not due to inappropriate conditions
- complete inactivation of all BI's is obtained after a full cycle

3-1-2 Establishment of validation cycle

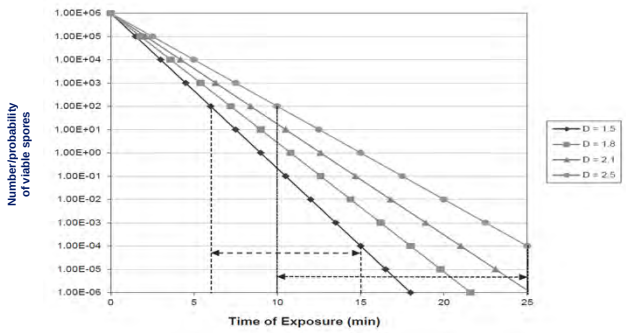
Example:

BI characteristics:

$D_{121°C}$ = 2.1 min.

$N_o = 10^6$

Exposure time t_{vl} for development of the validation cycle:



$$D \times (\log_{10}N_0 + 1) \leq t_{vl} \leq D \times (\log_{10}N_0 + 3)$$

$$14.7 \text{ min} \leq t_{vl} \leq 18.9 \text{ min}$$