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Laboratory Aspects of Gamma and Ethylene Oxide Sterilisation Validation

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Sterile: Absence of all living or potentially living organisms. In practice bacteria, yeast and moulds due to technical limitation of the detection of

- Viruses
- Protozoa
- Rickettsiae
- Chlamydia
- Mycoplasma

Bioburden: The microorganisms that are present on a medical device.

Gamma Dose Setting: The procedure used to determine the sterilisation dose for a medical device.

References

- **ISO 14937:** Sterilization of health care products-General requirements.
- **ISO 11135-1:** Sterilisation of Healthcare Products-Ethylene Oxide.
- **ISO 11737-1:** Sterilisation of Medical Devices-Determination of a population of microorganisms on products.
- **ISO 11737-2:** Sterilisation of Medical Devices-Tests of Sterility.
- **ISO 11137-1:** Sterilisation of Healthcare Products-Radiation.
- **ISO 20857:** Sterilisation of Healthcare Products-Dry Heat.
- **ISO 17665-1:** Sterilisation of Healthcare Products-Moist Heat.

References-Continued

- **ISO 11138-1:** Sterilization of health care products-Biological Indicators-General Requirements.
- **ISO 11138-2:** Sterilization of health care products-Biological indicators for ethylene oxide.
- **ISO 10993-1:** Biological Evaluation and Testing.
- **ISO 10993-3:** Tests for genotoxicity, carcinogenicity and reproductive toxicity.
- **ISO 10993-5:** Test for Residual Cytotoxicity.
- **ISO 10993-7:** Tests for Residual Ethylene Oxide.
- **ISO 10993-12:** Preparation of sample and reference materials.

Sterility Testing or Test for Sterility

- **Tests used to inspect the sterility of pharmaceuticals and veterinary products.**
- **Uses TSB and Thioglycollate, 14 days at two temperatures:**
  - TSB at 22.5ºC (20-25ºC).
  - Thioglycollate at 32.5ºC (30-35ºC).
- **Test items can be pooled.**
- **Test Methodology in Australia is mandated by TGA.**
- **Mandated Test is BP/EP, appendix XVI A. Test for Sterility – no variations are allowed except for those proposed by the TGA in "TGA Guidelines for Sterility Testing of Therapeutic Goods".**
- **Sampling plans are contained in BP/EP.**
- **Test method must be validated.**

Test of Sterility

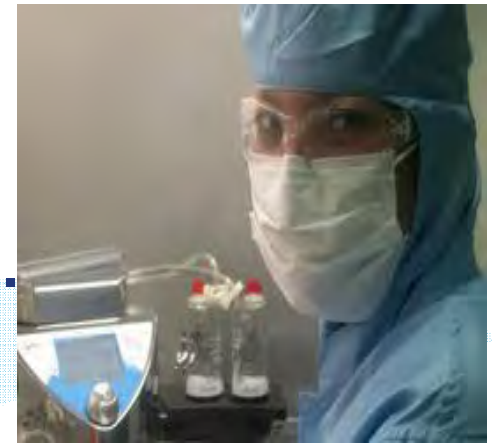
- Test used in the validation of a sterilisation process for medical devices.
- Uses only one media, usually TSB – 14 days at 30°C (28-30°C). Other media can be used.
- Test items can not be pooled-we need to know how many positive and negative results occur.
- Tests for contamination of a product sample with aerobic and aero tolerant mesophilic bacteria and fungi that can grow in TSB.
- Test must be validated-mostly by Stasis at the end of incubation period.

Sterility Testing Control

- **Cleanroom facility:**
  - **Positive pressure.**
  - **Staged entry into room for**
    - Personnel
    - Equipment and Materials.
  - **Disinfection of incoming samples and equipment.**
    - Sterilised utensils.
    - Garments.
    - Disinfection program on facility.
- **Operator training.**
- **Operator monitoring:**
  - Negative controls.
  - Glove impression plates.

Cleanroom Monitoring

- Settle plates.
- Surface samples.
- Room pressure maintained.
- **Contamination rate records maintained.**
- **Alert levels and actions.**
- **Trending Microbiological Data:**
- For critical areas statistical analysis is difficult or impossible because the results are too low.
- Mostly such facilities only yield 1 or 2 CFU from hundreds of sessions.



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Environment Monitoring Limits

PIC'S Guidelines Annex 1: Manufacture of Sterile Medicinal Products.

Location	Class	Action Limit		
		Settle Plate (CFU / 4hr.)	Swab (CFU / 100cm²) Contact plates (CFU / 25cm²)	Glove print (CFU / glove)
Test Area (LFC)	A	≥1	≥1	≥1
Clean Room	B	5	5	NA
Ante Room	C	50	25	NA

Test for Sterility

- **Bioburden Recovery Validation.**
  - ISO 11737 provides guidance.
  - Agar selection.
  - Incubation conditions.
  - Diluent selection.
- **The optimal conditions are then chosen.**
- **Routinely TSA (30°C) and SDA (25°C) with added antibiotics are used.**
- **Antimicrobial substance inhibition-required by international companies. Clients are asked to inform if antimicrobials are present.**

Bioburden Recovery Validation

- **ISO 11137 Method 1 and AAMI TIR27 are bioburden based methods, validation of bioburden enumeration is important.**
- **Bioburden Enumeration is Fundamental to the gamma dose chosen in the verification experiment.**
- **Validation must investigate growth and removal.**
- **Selection of Extraction Method:**
  - Stomaching
  - Shaking
  - Blending
  - Flushing
  - Sonication

Bioburden Recovery Validation

- **Two approaches:**
  - Use Natural bioburden and apply a repetitive or exhaustive extraction. Repeat Extraction process until little additional bioburden is recovered.
  - Use spore inoculation if natural bioburden is <100.
- **These studies lead to a correction factor that is then used to correct the data obtained from the routine method which usually is a single extraction of the medical device.**
- **Bioburden correction factor BCF = Extraction 1 as percentage of total bioburden.**

Spore Inoculation Method

- Usually a bacterial spore eg *Bacillus atrophaeus.*
- Inoculate the item and then recover the spores.
- Calculate the recovery factor.
- Triplicate devices from the same batch or different batches are used.
- The data must be used with caution as it may not reflect the natural bioburden.

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Sterilisation Process Validation

- **Need to Demonstrate a SAL of 10^{-6}.**
- **Sterility Assurance Level (SAL):** Probability of finding one viable microorganism in 10^n units processed.
- **Generally SAL =** 10^{-6} for healthcare products labeled sterile, i.e., not more than one microorganism in 1,000,000 product items.
- **Two Approaches:**
  - **Overkill Approach:** Demonstrate a log 12 kill of a resistant organism and it is assumed that the SAL is at least 10^{-6} .
  - **Bioburden Approach:** Demonstrate that the process achieves SAL of 10^{-6} using the bioburden of the item to be sterilised.

Overkill Process Validation – Rationale

- Therapeutic products (solid and liquid) rarely have microbial population of 10^6.
- Assume that there is a population of 10^6 and that they are resistant bacteria.
- Design sterilisation process to destroy 10^{12}.
- The sterilisation process therefore is assumed to have SAL of at least 10^{-6}.
- Less work than bioburden approach.
- The sterilisation process is harsh as it has been over estimated.
- Some products can not withstand an overkill sterilisation process.

Bioburden Based Process Validation

- Sterilisation process is targeted to the product and there is minimal over processing.
- Usually requires a significant amount of work.
- Requires an ongoing bioburden monitoring program and the data from this program is highly significant for verification of the continuing suitability of the sterilisation process.
- Microbiological work is a very important component and its importance must not be under estimated.

Bioburden Based Process Sterilisation Validation Methods

- Place a requirement on the manufacturer to control bioburden within limits.
- Bioburden data must demonstrate a consistency of bioburden.
- Bioburden must be monitored.
- Bioburden data must be analysed for trend.
- Control of bioburden is not always easy.
- A change in bioburden may alter the sterilisation dose needed and therefore initiate a re-validation.

ISO 11137

- Provide guidance and methods for the demonstration of a SAL of 10^{-6}.
- Two methods are given in ISO 11137, Method 1 and 2.
- Both methods are bioburden based methods, there are no overkill methods provided.

Product Definition

- **Products can be grouped into families.**
- **Purpose is to reduce resources required to establish and maintain Sterilization dose. Criteria to define and maintain a Product Family are detailed in ISO 11137-2.**
- **The type and number of Microorganisms present on or in the product shall be used as basis of selecting a product to represent a product family.**
- **Can be products of different forms.**
- **For all members of the family, factors that affect bioburden must be similar:**
  - Type and source of raw material.
  - Components.
  - Product design and size.
  - Manufacturing location, environment , process, equipment.

Product Family

- **Product to represent the Product Family for Verification Dose Experiment or Sterilization Dose Audit.**
- **Family can be represented by:**

A. **Product Master:** Product that presents the greatest challenge to sterilization

B. **Product Equivalent:** Group of products that were assessed as requiring the same sterilization dose. Selection can be random or scheduled and depend on manufacturing volume and availability.

C. **Simulated Product:** *(For example for expensive products under development):* A fabricated product with similar size, components and manufacturing environment, which presents an equivalent or greater challenge for Sterilisation.

ISO 11137 Method 1 Summary

- Determine if bioburden is Less or as Resistant to a Theoretical Population.
- Enumerate bioburden – 10 items from 3 batches.
- Items can be pooled to determine batch average, if bioburden is low (<10). SIP can not be pooled.
- Select verification gamma dose and the batch.
- Expose 100 product items to verification dose equivalent to SAL 10^{-2} – the verification experiment.
- Test product items. If 0, 1 or 2 positive tests verification is successful.
- Select sterilisation dose.
- Does not always work due to failure of the verification experiment.

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Verification Experiment Dose-Example

Average Bioburden	Sterility Assurance Level				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
1000	11.0	14.2	17.6	21.2	24.9
1100	11.1	14.4	17.8	21.3	25.0
1200	11.2	14.5	17.9	21.5	25.2

Verification Experiment Dose↑

Method 1 Failure

- Does happen.
- There is a small safety factor represented in the bioburden figures and doses in Method 1.
- Even if performed correctly there is a 8% probability of failure.
- Failure may occur if the bioburden is under estimated.
- Failure occur most often with textile devices.
- Means method 2 must be used unless method 1 can be invalidated.

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ISO 11137 Method 2 Summary

- **Perform incremental dose experiment Two methods**
- **Method 2A -** 540 product items: 20 items from each of three batches at 9 different doses.
- **Test product items:** 540 individual tests of sterility.
- **Method 2B -** 480 product items: 20 items from each of three batches at 8 different doses.
- **Test product items:** 480 individual tests of sterility.
- **Select verification batch, calculate verification dose and irradiate 100 items at that dose.**
- **Perform tests of sterility.**
- **Calculate sterilisation dose.**
- **Usually gives a result.**

VD_{max} Method

- Method for substantiation of 15kGy or 25kGy as a sterilisation dose.
- Designed to give less probability of failure of verification experiment than method 1 of ISO 11137.
- Use only 10 devices in verification experiment. Instead of the 100 used in Method 1 of ISO 11137.
- Can only be used if bioburden for whole device is ≤1,000 CFU for 25kGy and ≤15 CFU for 15kGy.
- The inclusion of Method VD_{max} for 15kGy provides an alternative to Method 1 for dose establishment for product of low average bioburden.
- To distinguish the two applications of Method VD_{max}, a superscript of "25" or "15" has been added to the term VD_{max} where appropriate, VD_{max}^{25} and VD_{max}^{15}.

Gamma Irradiation Dose Audit

- After dose is set the medical device sponsor must ensure that the dose is audited regularly.
- This provides evidence that dose remains valid and that the resistance of the bioburden has not changed so that the dose is no longer valid.
- The sponsor must make a logical and defendable decision on audit frequency.
- ISO 11137 recommends quarterly audits but allows a lesser frequency to be argued.
- Guidelines provided in ISO 11137-2 for each method.

Ethylene Oxide Sterilisation

- **Fractional Cycle:** Process in which the exposure time is reduced compared to that specified in the sterilization process.
- **Half Cycle:** Sterilization cycle in which the exposure time is reduced by 50 % compared with the sterilization process.
- **Parametric Release:** Declaration that product is sterile, based on records demonstrating that the process parameters were delivered within specified tolerances. This method of process release does not include the use of biological indicators.
- **Process Challenge Device (PCD):** Item designed to constitute a defined resistance to the sterilization process and used to assess performance of the process.

Annex A

- **Determination of lethal rate of the sterilization process-Biological indicator / bioburden approach. Two Methods;**
- **Direct Enumeration Method:** Consists of an enumeration or physical count of the survivors, exposing BIs or PCDs to the fractional cycle, removing the challenge and performing counts on the samples or biological indicators.
- **The Fraction Negative Method:** Uses growth/no growth during fractional cycles, involves running sterilization cycles in which some, but not all, of the biological indicators are inactivated.
- **Either of these methods may be used for Annexes A or B.**

Annex B

- **Conservative determination of lethal rate of the sterilization process-overkill approach.**
- **Two Methods;**
  - **Half Cycle Approach:** Demonstrate total inactivation of a 10^6 BI at a half-cycle exposure time. When exposure time is doubled, a minimum 12 Spore Log Reduction (SLR) is delivered.
  - **The Cycle Calculation Approach:** Aimed to achieve a 12 SLR process by Methods given in Annex A.

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Thank You !