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Tests to Support “Sterility” Claim

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Sterile Product

- As per TGO 77, a sterile product must comply with the
- requirements of the following tests:
  - Sterility Test
  - Bacterial Endotoxins Test
- Appendix XIII Particulate - Contamination – requires injections and infusions to pass sub visible particle test
- Particulate contamination of injections and infusions consists of extraneous, mobile undissolved particles, other than gas bubbles, unintentionally present in the solution.

Methods of Sterilisation

- Healthcare products intended to be sterile should be sterilized in their final sealed container (terminal sterilization).
- ISO/TC 198 has prepared standards for terminal sterilization of health care products
  - By irradiation (series ISO 11137)
  - By moist heat (ISO 17665-1)
  - By dry heat (ISO 20857, in preparation)
  - By ethylene oxide (ISO 11135-1)

Sterility Testing / 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.
- Guiding document is ISO 11737-2.
- Uses only one media, usually TSB – 14 days at 30ºC (28-32ºC). 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 - Method Development

- The method for performing the Sterility Test must be confirmed before for Method Suitability (Validation)
  - Filterability
  - Chemical Compatibility
  - Rinsing Fluids & Volumes
  - Potential Inhibition issues
  - Membrane Compatibility
  - Quantity of Samples to be tested
- Products containing bacteriostatic or fungistatic agents need to be neutralized to not inhibit the growth of viable microorganisms present in the product.
- Neutralization may be achieved via: dilution, pre-wetting, filtration and rinsing , chemical neutralization, enzyme activity, or a combination of these methods

Method Suitability TEST

- Method suitability test is performed:
  - When the test for sterility has to be carried out on a new product;
  - Whenever there is a change in the experimental conditions of the test.
- After transferring the contents of the container/containers to be tested, add a small number of viable micro-organisms (not more than 100 CFU) to the final portion of sterile diluent used to rinse the filter for MF and to the medium for DI.
- Same micro-organisms as those described under GPT with a positive control. Incubate all the containers containing medium for not more than 5 days.
- The method suitability test may be performed simultaneously with the test for sterility of the product to be examined.

QC test for culture media

- **Validation program:** per supplier, per lot number, per preparation
- **Suitability Tests:** have to be carried out before, or in parallel, with the test on the product to be examined
- **Sterility**
  - Pharmacopoeias require that testing should be performed to confirm the sterility of the microbiological medium. The medium is incubated under aerobic conditions.
  - Concurrent negative control with every sterility test of product
- **Growth promotion Test**
  - To confirm the ability of the test medium to support the growth and reproduction of selected microorganisms.
  - Incubate each culture media at the *temperature specified in the actual pharmacopoeia.*
  - Incubate each microorganism for the *time specified in the actual pharmacopoeia*
  - Examine for growth

Culture Media: Growth Promotion Test

- **FTM incubated at 30-35°C**
  - *Clostridium sporogenes* — anaerobic
  - *Pseudomonas aeruginosa* — aerobic
  - *Staphylococcus aureus* — aerobic
- **TSB incubated at 20-25°C**
  - *Aspergillus brasiliensis* — fungi (mould)
  - *Bacillus subtilis* — aerobic
  - *Candida albicans* — fungi (yeast)
- **Incubate for not more than *3 days in the case of bacteria and not more than 5 days in the case of fungi.***

Sterility Testing Methods-Membrane Filtration

- Advantages of membrane filtration
  - The antimicrobial activity of the sample can be eliminated by rinsing
  - No interaction between product and culture media
  - Testing of big volumes (from 100 ml - several liters)
  - Method more sensitive than Direct Inoculation
  - Use of less culture media than with Direct Inoculation
  - Oily products can be treated with emulsifying agents
- Limitations
  - Not usable with unfilterable products

Sterility Testing Methods-Direct Inoculation

- **Advantages**
  - Direct immersion of medical devices
  - Non-filterable products may be tested
- **Limitations**
  - Antimicrobial product activity may inhibit growth
  - Intrinsic product turbidity-Sub-culturing necessary
  - Aseptic technique training and validation required
  - Sample to media ratio can not be greater than 10%.
  - High risk of false positives

Sterility Test-Limitations

- Not for viruses or mycoplasma
- Destructive test
- Probability-based
  - Statistically poor at detecting contamination outside of gross contamination, not representative
  - Batch not entirely tested, only samples
  - Based upon presence of growth
- Long incubation period: 14 days
- Only 2 media to enable growth of: bacteria, fungi and yeasts

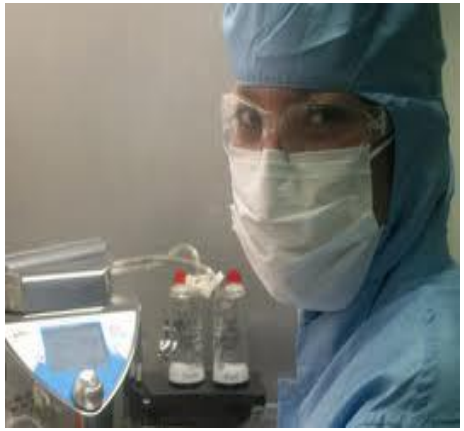
Where to conduct the sterility test?

- The sterility test should be conducted within a class A laminar airflow cabinet located within a class B clean room.
- Or in an isolator that need not be located within a controlled environment.
- The test may also be performed within a class A clean room, if available. (PIC/S, 2007)

Sterility Testing Control

- **Clean room 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.

Environment Monitoring Limits

PIC'S Guidelines Annex 1: Manufacture of Sterile Medicinal

Location	Class	Action Limits		
		Settle Plate (CFU/4hr.)	Swab/Contact Plates* CFU per 100 cm^2/25 cm^2 *	Glove Print CFU/Glove
Test Area (LFC)	A	≥ 1	≥ 1	≥ 1
Clean room	B	5	5	NA
Ante Room	C	50	25	NA

Aseptic Processing (ISO 13408)

- When a health care product is intended to be sterile and cannot be terminally sterilized, aseptic processing provides an alternative. Presterilization of product, product parts and/or components and all equipment coming into direct contact with the aseptically-processed product is required.
- Standard also dictates the procedures for process simulations by media fills (Incubation and inspection of media filled units).
- Incubation temperatures shall be within the range of 20 °C to 35 °C for 14 days
- If two temperatures are used for incubation, the units are typically incubated for at least 7 days at each temperature (starting with the lower temperature).

Test for Endotoxins

- Endotoxin is lipopolysaccharide component of the cell wall of Gram-negative bacteria which is heat stable and elicits a variety of inflammatory responses in animals and humans.
- Materials used to manufacture parenteral and other products required or claimed to be free from endotoxins shall comply with a limit test.
- This applies to raw materials (including water), intermediate products (such as bulk solutions or suspensions) and other components (such as container components) used as part of the product.
- The levels of endotoxin shall be determined by pharmacopoeial procedures.
- Method A, B, C, D, E and F are described in pharmacopoeia.

Sub Visible Particle Testing

- Injections or infusions often are contaminated with mobile and undissolved extraneous particles.
- There are two procedures specified under British Pharmacopoeia Appendix XIII;
  - Method 1 (Light Obscuration Particle Count Test)
  - Method 2 (Microscopic Particle Count Test) for detection of these particles.
- Solutions for infusion or injections with a nominal content of more than 100mL Passes the Sub-Visible Particle Count Test if the particle sizes detected are as follows.
  - ≥ 10µm has a cumulative mean of ≤ 25/mL
  - ≥ 25µm has a cumulative mean of ≤ 3/mL
- Solutions for infusion or injections with a nominal content of less than or equal to 100mL Passes the Sub-Visible Particle Count Test if the particle sizes detected are as follows.
  - ≥ 10µm has a cumulative mean of ≤ 6000/container
  - ≥ 25µm has a cumulative mean of ≤ 600/container

Seal Integrity Testing

- Two methods
  - Dye intrusion method
    - Immersion in a suitable solution of dye, under vacuum pressure
    - Visually inspected for dye intrusion
  - Microbial ingress method
    - Microbial challenge by liquid immersion test determines the ingress of microorganisms into a package that has been challenged with a liquid microbial suspension
    - Any containers with a compromised seal due to either breakage or damage can be detected by demonstration of microbial growth.
    - The test involves immersing media filled package units into a liquid suspension of microorganisms (10^5-10^6cfu/mL) for a specified period of time and then removing, rinsing, incubating and examining the units for microbial growth.
    - Selection of challenge organisms is based on the size and motility of the organism. Recommended organisms include
      - *Escherichia coli*
      - *Clostridium sporogenes*
      - *Staphylococcus epidermidis*
      - *Pseudomonas aeruginosa*
      - *Searratia marcesans*
      - *Brevundimonas diminuta*

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