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 22.5°C (20-25°C).
  - Thioglycollate at 32.5°C (30-35°C).
- 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 30°C (28-32°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 - 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 Products**

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<th>Location</th>
<th>Class</th>
<th>Action Limits</th>
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<td>Settle Plate (CFU/4hr.)</td>
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<td>Swab/Contact Plates* CFU per 100 cm²/25 cm²</td>
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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.
  - \( \geq 10\mu m \) has a cumulative mean of \( \leq 25/mL \)
  - \( \geq 25\mu m \) has a cumulative mean of \( \leq 3/mL \)
- 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.
  - \( \geq 10\mu m \) has a cumulative mean of \( \leq 6000/container \)
  - \( \geq 25\mu m \) has a cumulative mean of \( \leq 600/container \)
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 (10^5-10^6 cfu/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*
      - *Serratia marcesans*
      - *Brevundimonas diminuta*
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