MANUFACTURING & QUALITY AUDIT DIVISION
CENTRE FOR DRUG ADMINISTRATION

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GUIDANCE NOTES ON STERILITY TESTING

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GUIDANCE NOTES

Subject: STERILITY TESTING

1.0 INTRODUCTION

This guide on sterility testing provides guidance for local manufacturers and contract testing laboratories on sterility testing of sterile pharmaceutical products. It should be noted that these guidelines are not mandatory for the industry. The official requirements for sterility tests in Singapore are those specified in the British Pharmacopoeia 1998 (BP), the 1998 Supplement to the Third Edition of the European Pharmacopoeia (EP) or United States Pharmacopoeia (USP).

2.0 SCOPE

2.1 Inclusions

The sterility test that is performed either by the manufacturer or its contract testing laboratory on the finished product as a batch release quality control test is specified in this document. The recommendations in this document are based on the requirements of clause *2.6.1 Sterility* described in the *European Pharmacopoeia, Third Edition, Supplement 1998*[1] and revised by *Rapid Implementation, Resolution AP.CSP(98)6*, which was promulgated on 1 September 1998. At the time of issue this document reflected the current state of the art. It is not intended to be a barrier to technical innovation or the pursuit of excellence.

2.2 Exclusions

A product batch release test that relies on the sterility of a biological indicator, although often referred to as a "sterility test" by some manufacturers, is not considered to be a sterility test and is not described in this document.

3.0 DEFINITIONS

For the purposes of this document, the following definitions apply:

3.1 colony forming unit (CFU): Visible outcome of growth of micro-organisms arising from a single or multiple cells.

3.2 **growth promotion test:** Also referred to as fertility or nutritive properties test, which is performed on the media used during the sterility test to demonstrate that it is capable of supporting the growth of micro-organisms.

3.3 **negative controls:** Refers to the sterility test controls that may be used to identify a "false positive" test result. Growth in the media sterility test, or environmental monitoring, or negative product controls may contribute to the verification of a "false positive" test finding and an invalid test result.

3.4 **negative product controls:** Product or simulated product of known or undoubted sterility that is tested during the same test session as the product test samples. Negative product controls should be exposed to a terminal sterilisation process, such as exposure to steam sterilisation, gamma-irradiation etc, and be packaged in a similar manner to the test sample in terms of manipulations required of the test operator.

3.5 **positive controls:** Refers to the sterility test controls that may be used to define a "false negative" test result. An absence of growth of test challenge micro-organisms in the growth promotion, validation or "stasis" tests would result in a "false negative" test finding and an invalid test result.

3.6 **stasis test:** Also referred to as an inhibition test, which is performed to ensure that there are no inhibitory substances remaining in the product and that the media is still capable of supporting the growth of micro-organisms at the end of the sterility test incubation period. This test is not mandatory but it may be useful to confirm the inactivation of antimicrobial substances in products where a marginal test methodology is employed routinely, after an initial successful test validation.

3.7 **validation test:** Also referred to as a bacteriostasis and fungistasis test, which is performed in the presence of the product to determine whether inhibitory properties in the product have been neutralised at the beginning of the sterility test incubation period.

4.0 BACKGROUND

4.1 Although "sterility" is an absolute term, the assurance that any given item is sterile is a probability function, commonly expressed as a negative power to the base ten. The minimum acceptable Sterility Assurance Level (SAL) for terminally sterilised drugs is generally based on the probability of a non-sterile unit of 10^{-6}.

4.2 In practice, the sterility of a product is defined by the absence of viable and actively multiplying micro-organisms when tested in specified culture media. Turbidity in the broth media usually indicates contamination. This test is performed on the end-product and is one of the quality control tests specified for release of a batch of sterile product. The sterility test cannot be used to demonstrate the sterility of the entire batch but it may assist in identifying a non-sterile batch of product.

4.3 It is acknowledged that the sterility test suffers from significant statistical limitations and this contributes to the low probability of detecting anything less than gross contamination. However, these limitations can be reduced considerably by performance of the test under conditions that optimise the recovery of micro-organisms.

5. TRAINING

5.1 Sterility testing should only be performed by personnel who have been trained, qualified and certified to perform the various tasks and procedures related to sterility testing. The examination of test and control containers during and at the end of the incubation period should be included as part of the operator training program.

5.2 Supervisors should ensure that all personnel are monitored and follow Standard Operating Procedures (SOPs). Personnel should undergo periodic re-certification, particularly when problems are detected during the course of routine environmental and negative control monitoring, or when operators perform the test infrequently.

5.3 Personnel training should be documented and records maintained.

6.0 STERILITY TEST FACILITIES

The requirements for sterility testing facilities are available in reference documents eg. *PIC - PIC/S Annex to the Guide to Good Manufacturing Practice for Medicinal Products - Manufacture of Sterile Medicinal Products*. The recommendations in Section 8 of this document are applicable to the performance of the sterility test in a standard clean room environment.

6.1 Clean room design

Sterility testing should be performed under aseptic conditions, which are preferably consistent with the standard of clean room required for the aseptic manufacture of pharmaceutical products. Premises and equipment should be qualified according to the relevant principles of Installation Qualification and Operational Qualification (IQ/OQ).

6.1.1 Classification

6.1.1.1 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. Sterility testing should be carried out in a work zone that offers sufficient space and material should be placed in such a way that it does not disrupt the laminar airflow.

6.1.1.2 The test environment, which includes the laminar airflow cabinet or isolator, should be certified at least annually by a competent person for compliance with the specified standard conditions.

6.1.2 Air supply

6.1.2.1 Air supplied to the environment should be provided through terminal HEPA filters, which should be fitted with audible and/or visual alarms to indicate any sustained, out of specification pressure differentials across the HEPA filters.

6.1.2.2 There should be a pressure differential of not less than 10 to 15 Pascals (guidance value) between each of the areas, i.e. ambient/airlock and airlock/test room. Pressure readings should be taken and recorded from externally mounted gauges unless a validated continuous monitoring system is installed. As a minimum, readings should be taken prior to operator entry to the test suite. Pressure gauges should be labelled to indicate the area served, the acceptable specification, and whether or not the reading is absolute or differential.

6.2 Airlock and aseptic gowning

6.2.1 Airlock conditions

6.2.1.1 Entry to the clean room should be via an airlock in which operators are required to change into clean room garments.

6.2.1.2 The airlock should be designed to facilitate movement of the operator between the relatively unclean and clean areas of the room without compromising the aseptic gowning procedure. A step-over bench is a suitable division between these areas.

6.2.1.3 The airlock should contain: a full-length wall mirror; gowning instructions; hand washing, disinfection and drying facilities. If clean room garments are stored in the airlock, then adequate and appropriate storage facilities should be provided.

6.2.2 Aseptic gowning

6.2.2.1 The sterility test operator should change into sterile clean room garments consisting of a one-piece coverall suit, head cover, beard cover (if applicable), overshoes, gloves and mask. The use of sanitised garments may be acceptable if the process has been validated and their use is not routinely used to justify the performance of repeat sterility tests.

6.2.2.2 Protective garments should be changed for each work session or at least once a day if justified from the results of microbiological monitoring and validation studies.

6.2.2.3 Records should be kept of the sterilisation or sanitisation of the garments. This record may be in the form of a certification from an external supplier of sterile clean room garments provided that they have been approved by the sterility testing laboratory.

6.2.2.4 Each operator should be trained and certified in gowning procedures with training records maintained.

6.3 Clean room fittings and surfaces

6.3.1 All fittings, such as power outlets and light fittings should be flush with the wall or ceiling surfaces and sealed to prevent entrainment of unclean air. Surfaces should be smooth and impervious to the cleaning agents used.

6.3.2 The joints between ceiling/walls/floor should be coved to facilitate cleaning.

6.3.3 If supplied, intercom or communication systems should be designed to allow hands-free use, or their design should facilitate disinfection.

6.3.4 Chairs, storage cabinets and trolleys should be designed to facilitate cleaning and be suitable for use in a clean room environment.

6.3.5 There should be no extraneous equipment within the clean room environment.

6.3.6 Ultraviolet lamps may be fitted within pass-through cabinets only. Where there is more than one parallel tube, they should be shielded from each other and checked at least annually for performance, or whenever new lamps are fitted.

7.0 CLEANING, SANITISATION AND DISINFECTION

7.1 Outer surfaces of samples and equipment entering the testing suite should be disinfected, preferably with a sporicidal agent. Disinfection of surfaces and sample containers should be carried out in such a way as to avoid adventitious contamination of the samples by the chemical agent. Therefore, disinfectants should be free of microbiological contamination, which may be achieved through aseptic filtration or use of a product-compatible terminal sterilisation method.

7.2 Surfaces and operators' gloved hands should be disinfected regularly during the test session.

7.3 There should be protocols to cover all daily, weekly, and periodic cleaning, sanitisation, disinfection and fumigation procedures used in the testing environment.
If an isolator is used, the method of disinfection or sterilisation should be specified.

7.4 Prior to implementation, all cleaning, sanitising and disinfecting procedures should be validated from a microbiological perspective with respect to minimum disinfectant contact times and efficacy. Cleaning and disinfecting agents should be purchased to agreed and documented specifications.

7.5 Records should be retained in respect of routine preparation of cleaning and disinfecting agents, directions for their use, and validation of their efficacy.

8.0 ENVIRONMENTAL MONITORING

8.1 Environmental microbiological monitoring should include a combination of air and surface sampling methods, such as:
- active air sampling;
- settle (exposure) plates;
- surface contact (RODAC) plates, swabs or flexible films;
- operators' gloved hand plates.

8.2 Environmental monitoring should be performed under operational (dynamic) conditions either within the isolator or in the laminar airflow and associated background areas.

8.3 Location maps, exposure duration, and frequency of all types of microbiological environmental monitoring should be specified in written procedures.

8.4 The media used for each type of monitoring should be specified and the recovery of micro-organisms on the chosen media should be validated. Suitable inactivators of disinfectants and cleaning solutions may need to be incorporated into recovery media used for samples taken from surfaces.

8.5 There should be written specifications, including appropriate alert and action limits for microbial contamination. Guidance limits for microbiological environmental monitoring of clean rooms in operation may be found in reference documents eg. *PIC-PIC/S Sterile Annex*.

8.6 Records should be maintained of the numbers and type of organisms isolated and results presented in a format that facilitates early detection of trends. Routine identification of environmental micro-organisms to at least the genus level should assist in detecting trends. Sensitive techniques such as molecular typing techniques will be required for identification of micro-organisms if equivalence of identity of environmental and test isolates is the **sole** rationale used to invalidate the original sterility test (refer to clause 11.1).

9.0 STERILITY TEST DETAILS

9.1 Sampling

9.1.1 The number of containers tested per batch and quantity tested from each container should be, as a minimum, in accordance with the pharmacopoeial method followed.

9.1.2 Samples from aseptic fills should be selected from at least the beginning, middle and end of the batch fill. Additionally, SOPs should define criteria for inclusion and collection of samples immediately after interruptions and operator interventions during the filling process.

9.1.3 Samples from terminal sterilisation cycles should be selected from at least the potentially coolest part of the load if such a location was identified during validation studies, and from every load sterilised.

9.1.4 If an original test is declared invalid, then any samples used for the repeat sterility test should reflect the original samples in terms of sampling locations or aseptic processing times.

9.2 Test methodology

9.2.1 The test methodology should be in accordance with the pharmacopoeial method used. Membrane filtration of the product, with either an open or a closed system, is the preferred sterility test methodology. The filter should be pre-wetted, particularly when small volumes and antibiotics are tested. Filtration of the product should be followed by the minimum number of washes of the membrane with a suitable rinsing fluid established during validation studies. The membrane should not be permitted to dry out between filtration steps.

9.2.2 If the product cannot be filtered, then direct inoculation, immersion, in-situ incubation or combination methods as appropriate are acceptable.

9.3 Media types and manufacture

9.3.1 The media used should be in accordance with the pharmacopoeial method followed. Soya-bean casein digest (SCD) and fluid thioglycollate media (FTM) should normally be used. Alternative media are permitted and may be appropriate if the nature of the product or method of manufacture could result in the presence of fastidious organisms (e.g. vaccines, blood products, etc). Validation studies should demonstrate that alternative media are capable of supporting the growth of a wide range of micro-organisms. Inactivators of antimicrobials may be incorporated into growth media or rinse solutions as indicated by validation studies.

9.3.2 Media should be purchased from an approved supplier, or prepared in-house according to standard operating procedures that are based on validated sterilisation processes. pH checks of media should be included in these procedures to ensure that the pH is within specifications at the time of use.

9.3.3 A batch number and a shelf-life should be assigned to all media and batch-manufacturing documentation should be maintained.

9.4 Incubation period

9.4.1 All test containers should be incubated at temperatures specified by the pharmacopoeial method for each test media for at least 14 days, regardless of whether filtration or direct inoculation test methodology is used.

9.4.2 The temperature of incubators should be monitored and there should be records of calibration of the temperature monitoring devices.

9.4.3 Test containers should be inspected at intervals during the incubation period and these observations recorded.

9.4.4 If the product produces a suspension, flocculation or deposit in the media, suitable portions (e.g. 2-5 percent) of the contents of the containers should be transferred to fresh media under clean room conditions, after 14 days, and re-incubated for a further 7 days.

9.5 Negative test controls

9.5.1 Media sterility test

9.5.1.1 All media should be pre-incubated for 14 days at appropriate test temperatures to demonstrate sterility prior to use. Alternatively, this control test may be conducted concurrently with the product sterility test. Media sterility testing may involve either a representative portion or 100 percent of the batch.

9.5.1.2 Parametric release of sterile product may be approved by the competent authority according to clause *5.1.1 Methods of Preparation of Sterile Products* of the *European Pharmacopoeia*. The concept of parametric release could be extended to sterility test media that has been terminally sterilised to provide an equivalent level of sterility assurance to that expected for parametric release of sterile product. In this case, media sterility testing is not required.

9.5.2 Negative product controls

9.5.2.1 Negative product controls, which are similar in type and packaging to the actual product under test, should be included in each test session. These controls facilitate the interpretation of test results, particularly when used to declare a test invalid because of contamination in the negative product controls.

9.5.2.2 A minimum of ten negative product control containers may be adequate to simulate manipulations by the operator during a membrane filtration test. An equivalent number of samples to the test samples may be necessary to simulate the manipulations of the product by the operator/s during a direct inoculation test.

9.5.2.3 The negative control contamination rate should be calculated and recorded.

9.6 Positive test controls

All positive control tests in this section use viable challenge micro-organisms. These tests should be conducted in a laboratory environment separate from either the isolator or aseptic area where the product is tested.

9.6.1 Growth promotion test

9.6.1.1 Challenge organism strains that are used to verify the fertility of each batch of standard test media should be selected from those reference strains specified by the pharmacopoeial method. Environmental or fastidious organisms may be used if alternative non-selective enrichment media have been selected for the sterility test.

9.6.1.2 Media purchased from external vendors should be accompanied by certification of the growth promotion test performed on each batch of media. The test need not be repeated by the sterility testing laboratory provided there is documented control over the conditions used to transport media between the media manufacturer and the sterility testing laboratory.

9.6.1.3 The media should be inoculated with 10-100 CFU of challenge organisms.
The challenge inoculum should be verified by concurrent viable plate counts.

9.6.1.4 Growth promotion challenge organisms should show clearly visible growth in the test media within 3 days for bacteria and 5 days for fungi.

9.6.1.5 There should be written instructions and protocols covering all procedures for the preparation, maintenance and cultivation of test organism strains. The identity (morphological and physiological properties) of the strains should be checked periodically. At the time of use, cultures maintained by seed lot culture techniques should be no more than five passages from the original type culture strain which was obtained from a recognised reference culture supplier.

9.6.1.6 The growth promotion test may be performed concurrently with the product sterility test.

9.6.2 Validation (bacteriostasis and fungistasis) test

9.6.2.1 The test methodology should be validated by inoculation with 10-100 CFU of challenge organism strains to the media/product container at the beginning of the test incubation period. The challenge inoculum should be verified by concurrent viable plate counts.

9.6.2.2 The preferred validation method involves addition of challenge organisms directly to the product prior to direct inoculation or membrane filtration. However, where this is not practical due to inhibition or irreversible binding by the product, the challenge organisms should be added directly to the media containing the product in the case of direct test methodology, or to the last rinse solution if membrane filtration methodology is used.

9.6.2.3 The test is declared invalid if validation challenge organisms do not show clearly visible growth of bacteria within 3 days,

and fungi within 5 days in the test media containing product. In most cases, unless the sterile product causes turbidity in the media, visual recovery times should be comparable to those of the growth promotion test.

9.6.2.4 Validation should be performed on all new products, and repeated whenever there is a change in the experimental conditions. Although it is not a pharmacopoeial requirement, it is good laboratory practice to re-validate under the current experimental conditions every 12 months. Records of validation and/or re-validation tests should be maintained in the change control procedure protocol.

9.6.3 Stasis test

9.6.3.1 It is not mandatory, but it is recommended that a stasis test be performed when antibiotics, inherently antimicrobial or preserved products are tested. This test is sometimes referred to as an *inhibition test* by veterinary testing laboratories. The test is performed by inoculation of 10-100 CFU of challenge organisms directly to representative test containers of media containing product, which do not display any signs of contamination at the end of the test incubation period.

9.6.3.2 This test is particularly important for antibiotics, slow release sterile products and for direct inoculation methods where validity of the test method depends on the use of an exact amount of product (i.e. marginal methodology). The stasis test has also identified problems with dehydrated commercial media, which were not apparent when a validation test was conducted at the beginning of the incubation period.

9.6.3.3 Stasis test challenge organisms should show clearly visible growth in the test medium within 3 days for bacteria and 5 days for fungi, otherwise the test is invalid.

9.6.3.4 It is recommended that the stasis test is repeated at least every 12 months on the relevant categories of products or

when product is reformulated or media is changed. Records of stasis tests should be maintained.

10.0 RESULTS

10.1 Apparent or suspected growth in media should be verified by subculture to solid microbiological media and micro-organisms identified at least to genus but preferably to species level.

10.2 Automated or semi-automated biochemical organism identification systems should be subjected to periodic verification using reference strains of organisms that can be traced to a recognised reference culture collection, such as the American Type Culture Collection (ATCC), Maryland, USA or the National Collection of Type Cultures (NCTC), London, UK, etc.

10.3 Results of sterility testing for test samples and negative controls should be presented in a format that allows easy recognition of trends.

11.0 INTERPRETATION AND REPEAT TESTS

11.1 A test may be repeated only when it can be demonstrated that the test was invalid for causes unrelated to the product being examined. The *European Pharmacopoeia* restricts criteria to one or more of the following conditions only:

(a) the data of the microbiological monitoring of the sterility testing facility show a fault;

(b) a review of the testing procedure used during the test in question reveals a fault;

(c) microbial growth is found in the negative controls;

(d) after determination of the identity of the micro-organisms isolated from the test the growth of this species or these species may be ascribed unequivocally to faults with respect to the material and/or the technique used in conducting the sterility test procedure.

NOTE 1: When conditions (a), (b) or (c) apply then the test should be aborted prior to the completion of the incubation period.

NOTE 2: If a stasis test is performed at the end of the test incubation period, failure of challenge micro-organisms to grow in this test also invalidates the test.

NOTE 3: For condition (d) to apply as the **sole** criterion used to invalidate a test, it is necessary to demonstrate that a micro-organism isolated from the product is identical to an isolate from the materials and/or the environment. This determination entails the use of a sensitive typing technique such as a molecular typing technique or other techniques similar to those used for epidemiological studies. However, if tests are performed competently in a clean room environment the chance of simultaneous adventitious contamination occurring in the environment, test sample and negative controls is negligible. Provisions that allow repeat testing based on morphological or biochemical characterisation of environmental and/or product contaminants should not be permitted. It is possible for the environment to become contaminated by the samples under test, which may contain multiple micro-organisms that are difficult to differentiate without employing sensitive typing techniques.

11.2 If contamination, which is established to be unrelated to the product, occurs in the original test, the test may be repeated with the same number of test samples as used in the original test, with negative product controls tested concurrently.

11.3 If contamination is detected in the repeat test performed on the same number of test samples, the product does not comply with the test for sterility and the entire batch should be rejected. The *European Pharmacopoeia* does not permit further testing of the sample under any circumstances.

12.0 REFERENCES

PIC/S documents PE 001/1 : Recommendations on Sterility Testing

END OF DOCUMENT

GUIDANCE NOTES

Subject: STERILITY TESTING

REVISION HISTORY

Rev No.	DCC Ref No.	Author	Date of Revision	Remarks of Revision
001	-	-	01 Jan 2000	New.
002	-	-	03 Aug 2001	Change of organisation name
003	-	Terrance Ong	09 Dec 2002	Update document format.
004	208	Terrance Ong	01 Mar 2004	1) Change 3D HSA logo to 2D HSA logo. 2) Change Centre name to Centre for Drug Administration 3) Detached Revision History from Guidance Notes.
005	256	Terrance Ong	06 Sep 2004	1) Re-located Revision History to the main document.