Q4B Evaluation and Recommendation of Pharmacopoeial Texts for Use in the ICH Regions

Annex 8: Sterility Test General Chapter

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INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

DRAFT CONSENUS GUIDELINE

EVALUATION AND RECOMMENDATION OF PHARMACOPOEIAL TEXTS FOR USE IN THE ICH REGIONS

ON

STERILITY TEST GENERAL CHAPTER

Q4B ANNEX 8

Current *Step 2* Version 1
dated 13 November 2008

At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Steering Committee to the regulatory authorities of the three ICH regions (the European Union, Japan and the USA) for internal and external consultation, according to national or regional procedures.

Q4B Annex 8
Document History

Current *Step 2* version

Code	History	Date
Q4B Annex 8	Approval by the Steering Committee under *Step 2* and release for public consultation.	13 November 2008

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Draft ICH Consensus Guideline

Released for Consultation on 13 November 2008, at *Step 2* of the ICH Process

TABLE OF CONTENTS

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ON

STERILITY TEST GENERAL CHAPTER

Q4B Annex 8

1. INTRODUCTION

This annex is the result of the Q4B process for the Sterility Test General Chapter.

The proposed texts were submitted by the Pharmacopoeial Discussion Group (PDG).

2. Q4B OUTCOME

2.1 Analytical Procedures

The ICH Steering Committee, based on the evaluation by the Q4B Expert Working Group (EWG), recommends that the official pharmacopoeial texts, Ph.Eur. 2.6.1. Sterility, JP 4.06 Sterility Test, and USP <71> Sterility Tests, can be used as interchangeable in the ICH regions subject to the conditions detailed below. Testing conditions for medical devices, such as sutures, are outside the scope of the ICH recommendation.

2.1.1 Local texts identified by the black diamond symbol are not considered interchangeable in all regions.

2.1.2 Diluting and rinsing fluids should not have antibacterial or antifungal properties if they are to be considered suitable for dissolving, diluting, or rinsing an article under test for sterility.

2.2 Acceptance Criteria

The acceptance criteria are harmonized between the three pharmacopoeias.

3. TIMING OF ANNEX IMPLEMENTATION

When this annex is implemented (incorporated into the regulatory process at ICH Step 5) in a region, it can be used in that region. Timing might differ for each region.

4. CONSIDERATIONS FOR IMPLEMENTATION

4.1 General Consideration

When sponsors or manufacturers change their existing methods to the implemented Q4B-evaluated pharmacopoeial texts that are referenced in Section 2.1 of this annex, any change notification, variation, and/or prior approval procedures should be handled in accordance with established regional regulatory mechanisms pertaining to compendial changes.

4.2 FDA Consideration

Based on the recommendation above, and with reference to the conditions set forth in this annex, the pharmacopoeial texts referenced in Section 2.1 of this annex can be considered interchangeable. However, FDA might request that a company demonstrate that the chosen method is acceptable and suitable for a specific material or product, irrespective of the origin of the method.

4.3 EU Consideration

For the European Union, the monographs of the Ph. Eur. have mandatory applicability. Regulatory authorities can accept the reference in a marketing authorisation application, renewal or variation application citing the use of the corresponding text from another pharmacopoeia as referenced in Section 2.1, in accordance with the conditions set out in this annex, as fulfilling the requirements for compliance with the Ph. Eur. Chapter, Sterility: 2.6.1., on the basis of the declaration of interchangeability made above.

4.4 MHLW Consideration

The pharmacopoeial texts referenced in Section 2.1 of this annex can be used as interchangeable in accordance with the conditions set out in this annex. Details of implementation requirements will be provided in the notification by MHLW when this annex is implemented.

5. REFERENCES USED FOR THE Q4B EVALUATION

5.1 The PDG Stage 5B sign-off document: *Japanese Pharmacopoeial Forum*, Volume 16, number 4 (December 2007).

5.2 The pharmacopoeial references for Sterility Test for this annex are:

5.2.1 *European Pharmacopoeia* (Ph. Eur.):
Supplement 6.3 (official in January 2009), Sterility (reference 01/2009:20601).

5.2.2 *Japanese Pharmacopoeia* (JP):
The 4.06 Sterility Test will be made official via Ministerial Notification (March 2009). The draft English version of the JP text is appended.

5.2.3 *United States Pharmacopeia* (USP):
<71> Sterility Tests as presented in *Pharmacopeial Forum Volume 34(6), Interim Revision Announcement No. 6,* official December 1, 2008.

English text as provided to the Q4B EWG

This text below has been provided by the Ministry of Health, Labour and Welfare (MHLW) and represents an English translation of a Ministerial Notification to be published in March 2009.

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia.

The test is applied to substances, preparations or articles which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating micro-organism has been found in the sample examined in the conditions of the test.

1. Precautions against microbial contamination

The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any micro-organisms which are to be revealed in the test. The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.

2. Culture media and incubation temperatures

2.1. Introduction

Media for the test may be prepared as described below, or equivalent commercial media may be used provided that they comply with the growth promotion test.

The following culture media have been found to be suitable for the test for sterility. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. Soya-bean casein digest medium is suitable for the culture of both fungi and aerobic bacteria.

2.2. Fluid thioglycollate medium

Fluid thioglycollate medium

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose monohydrate/anhydrous	5.5 / 5.0 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or	0.5 g
Thioglycollic acid	0.3 mL
Resazurin sodium solution (1 in 1000), freshly prepared	1.0 mL
Water	1 000 mL
(pH after sterilization 7.1 ± 0.2)	

Mix the L-cystine, agar, sodium chloride, glucose, water-soluble yeast extract and pancreatic digest of casein with water, and heat until solution is effected. Dissolve the sodium thioglycollate or thioglycollic acid in the solution and, if necessary, add sodium hydroxide TS so that, after sterilization, the solution will have a pH of 7.1 ± 0.2. If filtration is necessary, heat the solution again without boiling and filter while hot through moistened filter paper. Add the resazurin sodium solution (1 in 1000), mix and place the medium in suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a colour change indicative of oxygen uptake at the end of the incubation period. Sterilize using a validated process. If the medium is stored, store at a temperature between 2 °C and 25 °C in a sterile, airtight container. If more than the upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink colour disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container. Do not use the medium for a longer storage period than has been validated.

Fluid thioglycollate medium is to be incubated at 30-35 °C.

For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycollate medium incubated at 20-25 °C may be used instead of soya-bean casein digest medium provided that it has been validated as described in growth promotion test.

Where prescribed or justified and authorized, the following alternative thioglycollate medium might be used. Prepare a mixture having the same composition as that of the fluid thioglycollate medium, but omitting the agar and the resazurin sodium solution (1 in 1000), sterilize as directed above. The pH after sterilization is 7.1 ± 0.2. Heat in a water bath prior to use and incubate at 30-35 °C under anaerobic conditions.

2.3. Soya-bean casein digest medium

Soya-bean casein digest medium	
Pancreatic digest of casein	17.0 g
Papaic digest of soya-bean meal	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate/anhydrous	2.5 / 2.3 g
Water	1 000 mL
(pH after sterilization 7.3 ± 0.2)	

Dissolve the solids in water, warming slightly to effect solution. Cool the solution to room temperature. Add sodium hydroxide TS, if necessary, so that after sterilization the solution will have a pH of 7.3 ± 0.2. Filter, if necessary, to clarify, distribute into suitable vessels and sterilize using a validated process. Store at a temperature between 2 °C and 25 °C in a sterile well-closed container, unless it is intended for immediate use. Do not use the medium for a longer storage period than has been validated.

Soya-bean casein digest medium is to be incubated at 20-25 °C.

3. Suitability of the culture medium

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined.

Sterility

Incubate portions of the media for 14 days. No growth of micro-organisms occurs.

Growth promotion test of aerobes, anaerobes and fungi

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Suitable strains of micro-organisms are indicated in Table 4.06 -1.

Inoculate portions of fluid thioglycollate medium with a small number (not more than 100 CFU) of the following micro-organisms, using a separate portion of medium for each of the following species of micro-organism: *Clostridium sporogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

Inoculate portions of soya-bean casein digest medium with a small number (not more than 100 CFU) of the following micro-organisms, using a separate portion of medium for each of the following species of micro-organism: *Aspergillus niger*, *Bacillus subtilis, Candida albicans*.

Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi.

Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than five passages removed from the original master seed-lot.

The media are suitable if a clearly visible growth of the micro-organisms occurs

Table 4.06 -1 — *Strains of the test micro-organisms suitable for use in the Growth Promotion Test and the Method suitability Test*

Aerobic bacteria	
Staphylococcus aureus	ATCC 6538, NBRC 13276, CIP 4.83, NCTC 10788, NCIMB 9518
Bacillus subtilis	ATCC 6633, NBRC 3134, CIP 52.62, NCIMB 8054
Pseudomonas aeruginosa	ATCC 9027, NBRC 13275, NCIMB 8626, CIP 82.118
Anaerobic bacterium	
Clostridium sporogenes	ATCC 19404, NBRC 14293, CIP 79.3, NCTC 532 *or* ATCC 11437
Fungi	
Candida albicans	ATCC 10231, NBRC 1594, IP 48.72, NCPF 3179
Aspergillus niger	ATCC 16404, NBRC 9455, IP 1431.83, IMI 149007

4. Method suitability test

Carry out a test as described below under Test for sterility of the product to be examined using exactly the same methods except for the following modifications.

Membrane filtration

After transferring the content of the container or containers to be tested to the membrane add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the final portion of sterile diluent used to rinse the filter.

Direct inoculation

After transferring the contents of the container or containers to be tested to the culture medium add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the medium.

In both cases use the same micro-organisms as those described above under Growth promotion test of aerobes, anaerobes and fungi. Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 5 days.

If clearly visible growth of micro-organisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without further modification.

If clearly visible growth is not obtained in the presence of the product to be tested, visually comparable to that in the control vessels without product, the product possesses antimicrobial activity that has not been satisfactorily eliminated under the conditions of the test. Modify the conditions in order to eliminate the antimicrobial activity and repeat the method suitability test.

This method suitability is performed:

a) when the test for sterility has to be carried out on a new product;

b) whenever there is a change in the experimental conditions of the test.

The method suitability may be performed simultaneously with the Test for sterility of the product to be examined.

5. Test for sterility of the product to be examined

5.1. Introduction

The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits, that is, for filterable aqueous preparations, for alcoholic or oily preparations and for preparations miscible with or soluble in aqueous or oily solvents provided these solvents do not have an antimicrobial effect in the conditions of the test.

5. 2. Membrane filtration

Use membrane filters having a nominal pore size not greater than 0.45 µm whose effectiveness to retain micro-organisms has been established. Cellulose nitrate filters, for example, are used for aqueous, oily and weakly alcoholic solutions and cellulose acetate filters, for example, for strongly alcoholic solutions. Specially adapted filters may be needed for certain products, e.g. for antibiotics.

The technique described below assumes that membranes about 50 mm in diameter will be used. If filters of a different diameter are used the volumes of the dilutions and the washings should be adjusted accordingly. The filtration apparatus and membrane are sterilized by appropriate means. The apparatus is designed so that the solution to be examined can be introduced and filtered under aseptic conditions; it permits the aseptic removal of the membrane for transfer to the medium or it is suitable for carrying out the incubation after adding the medium to the apparatus itself.

Aqueous solutions

If appropriate, transfer a small quantity of a suitable, sterile diluent such as a 1 g / L neutral solution of meat or casein peptone pH 7.1 ± 0.2 onto the membrane in the apparatus and filter. The diluent may contain suitable neutralizing substances and/or appropriate inactivating substances for example in the case of antibiotics.

Transfer the contents of the container or containers to be tested to the membrane or membranes, if necessary after diluting to the volume used in the method suitability test with the chosen sterile diluent but in any case using not less than the quantities of the product to be examined prescribed in Table 4.06-2. Filter immediately. If the product has antimicrobial properties, wash the membrane not less than three times by filtering through it each time the volume of the chosen sterile diluent used in the method suitability test. Do not exceed a washing cycle of 5 times 100 mL per filter, even if during method suitability it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity. Transfer the whole membrane to the culture medium or cut it aseptically into two equal parts and transfer one half to each of two suitable media. Use the same volume of each medium as in the method suitability test. Alternatively, transfer the medium onto the membrane in the apparatus. Incubate the media for not less than 14 days.

Table 4.06-2 — Minimum quantity to be used for each medium

Quantity per container	Minimum quantity to be used for each medium unless otherwise justified and authorised
Liquids	
– less than 1 mL:	The whole contents of each container
– 1-40 mL:	Half the contents of each container but not less than 1 mL
– greater than 40 mL and not greater than 100 mL	20 mL
– greater than 100 mL :	10 per cent of the contents of the container but not less than 20 mL
Antibiotic liquids	1 mL
Insoluble preparations, creams and ointments to be suspended or emulsified	Use the contents of each container to provide not less than 200 mg
Solids	
– less than 50 mg	The whole contents of each container
– 50 mg or more but less than 300 mg	Half the contents of each container but not less than 50 mg
– 300 mg – 5 g	150 mg
– greater than 5 g	500 mg

Soluble solids

Use for each medium not less than the quantity prescribed in Table 4.06-2 of the product dissolved in a suitable solvent such as the solvent provided with the preparation, water for injection, saline or a 1 g / L neutral solution of meat or casein peptone and proceed with the test as described above for aqueous solutions using a membrane appropriate to the chosen solvent.

Oils and oily solutions

Use for each medium not less than the quantity of the product prescribed in Table 4.06-2. Oils and oily solutions of sufficiently low viscosity may be filtered without dilution through a dry membrane. Viscous oils may be diluted as necessary with a suitable sterile diluent such as isopropyl myristate shown not to have antimicrobial activity in the conditions of the test. Allow the oil to penetrate the membrane by its own weight then filter, applying the pressure or suction gradually. Wash the membrane at least three times by filtering through it each time about 100 mL of a suitable sterile solution such as 1 g / L neutral meat or casein peptone containing a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability of the test, for example polysorbate 80 at a concentration of 10 g / L. Transfer the membrane or membranes to the culture medium or media or vice versa as described above for aqueous solutions, and incubate at the same temperatures and for the same times.

Ointments and creams

Use for each medium not less than the quantities of the product prescribed in Table 4.06-2. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1 per cent in isopropyl myristate as described above, by heating, if necessary, to not more than 40 °C. In exceptional cases it may be necessary to heat to not more than 44 °C. Filter as rapidly as possible and proceed as described above for oils and oily solutions.

5.3. Direct inoculation of the culture medium

Transfer the quantity of the preparation to be examined prescribed in Table 4.06-2 directly into the culture medium so that the volume of the product is not more than 10 per cent of the volume of the medium, unless otherwise prescribed.

If the product to be examined has antimicrobial activity, carry out the test after neutralising this with a suitable neutralising substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate the concentrated medium may be added directly to the product in its container.

Oily liquids

Use media to which have been added a suitable emulsifying agent at a concentration shown to be appropriate in the method suitability of the test, for example polysorbate 80 at a concentration of 10 g / L.

Ointments and creams

Prepare by diluting to about 1 in 10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluent such as a 1 g / L neutral solution of meat or casein peptone. Transfer the diluted product to a medium not containing an emulsifying agent.

Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Shake cultures containing oily products gently each day. However when fluid thioglycollate medium is used for the detection of anaerobic micro-organisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

6. Observation and interpretation of results

At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1 mL)

of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days.

If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined.

The test may be considered invalid only if one or more of the following conditions are fulfilled:

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.

If the test is declared to be invalid it is repeated with the same number of units as in the original test.

If no evidence of microbial growth is found in the repeat test the product examined complies with the test for sterility. If microbial growth is found in the repeat test the product examined does not comply with the test for sterility.

7. Application of the test to parenteral preparations, ophthalmic and other non-injectable preparations required to comply with the test for sterility

When using the technique of membrane filtration, use, whenever possible, the whole contents of the container, but not less than the quantities indicated in Table 4.06-2, diluting where necessary to about 100 mL with a suitable sterile solution, such as 1 g / L neutral meat or casein peptone.

When using the technique of direct inoculation of media, use the quantities shown in Table 4.06-2, unless otherwise justified and authorised. The tests for bacterial and fungal sterility are carried out on the same sample of the product to be examined. When the volume or the quantity in a single container is insufficient to carry out the tests, the contents of two or more containers are used to inoculate the different media.

458 **8. Minimum number of items to be tested**

459 The minimum number of items to be tested in relation to the size of the batch is given in Table
460 4.06-3.

461 Table 4.06-3. Minimum number of items to be tested

Number of items in the batch*	Minimum number of items to be tested for each medium, unless otherwise justified and authorised**
Parenteral preparations	
– Not more than 100 containers	10 per cent or 4 containers whichever is the greater
– More than 100 but not more than 500 containers	10 containers
– More than 500 containers	2 per cent or 20 containers (10 containers for large-volume parenterals) whichever is the less
Ophthalmic and other non-injectable preparations	
– Not more than 200 containers	5 per cent or 2 containers whichever is the greater
– More than 200 containers	10 containers
– If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral use	
Bulk solid products	
– Up to 4 containers	Each container
– More than 4 containers but not more than 50 containers	20 per cent or 4 containers whichever is the greater
– More than 50 containers	2 per cent or 10 containers whichever is the greater

462 * If the batch size is not known, use the maximum number of items prescribed
463 **If the contents of one container are enough to inoculate the two media, this column gives the
464 number of containers needed for both the media together.
465