

JP16 table of errata

December 25, 2012

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		4.05 <u>Microbiological Examination of Non-sterile Products</u> 103	4.05 <u>Microbial Limit Test</u> 103

Preface まえがき

Page	Line	Correction	Error
iii right	↓ 1	General Tests; Official Monographs;	General Tests, <u>Processes and Apparatus</u> ; Official Monographs;
iii right	↓ 6	The articles in Official Monographs,	The articles in <u>General Rules for Preparations</u> , Official Monographs,
v left	↓ 9	4.05 <u>Microbiological Examination of Non-sterile Products</u>	4.05 <u>Microbial Limit Test</u>
vi right	↑ 13	Imipramine Hydrochloride <u>Tablets</u>	Imipramine Hydrochloride
x left	↓ 19	Imipramine Hydrochloride <u>Tablets</u>	Imipramine Hydrochloride

General rules for preparations 製剤総則

Page	Line	Correction	Error
7 right	↓ 16	they are applied to the test of <u>Microbiological Examination of Non-sterile Products</u> <4.05>, if necessary.	they are applied to the test of <u>Microbial Limit Test</u> <4.05>, if necessary.

1.08 Nitrogen Determination 窒素定量法

Page	Line	Correction	Error
31 right	↓ 8	by passing <u>steam</u> through it	by passing <u>stream</u> through it
31 right	↓ 14	<u>close</u> the clamp attached to the rubber tubing G	<u>lose</u> the clamp attached to the rubber tubing G

4.01 Bacterial Endotoxins Test エンドトキシン試験法

Page	Line	Correction	Error
93 left	↑ 5	maximum <u>bolus</u> dose of product per kg body mass.	maximum dose of product per kg body mass.

4.05 Microbiological Examination of Non-sterile Products 微生物限度試験法

Page	Line	Correction	Error
103 - 114		<u>Microbiological Examination of Non-sterile Products</u> (See Attachment I)	<u>Microbial Limit Test</u> (The rest is omitted.)

4.06 Sterility Test 無菌試験法

Page	Line	Correction	Error
114 left	↓ 20	The test is applied to <u>substances</u> , preparations or articles	The test is applied to <u>active pharmaceutical ingredients</u> , preparations or articles
114 left	↑ 16	<u>Soya-bean</u> casein digest medium is suitable for	<u>Soybean</u> casein digest medium is suitable for
114 right	↑ 26	<u>Soya-bean</u> casein digest medium	<u>Soybean</u> casein digest medium
114 right	↑ 25	<u>Pancreatic digest of casein</u>	<u>Casein peptone</u>
114 right	↑ 24	<u>Papaic digest of soya-bean meal</u>	<u>Soybean peptone</u>
114 right	↑ 9	<u>Soya-bean</u> casein digest medium is to be incubated at	<u>Soybean</u> casein digest medium is to be incubated at

9.41 Reagents, Test Solutions 試薬・試液

Page	Line	Correction	Error
248 right	↓ 19	magnesium sulfate <u>heptahydrate</u>	magnesium sulfate <u>hexahydrate</u>

Amphotericin B アムホテリシン B

Page	Line	Correction	Error
381 left	↓ 30	in 1.5 <u>Preparation of agar base layer plates</u> under the Cylinder plate method	in 1.5 under the Cylinder plate method

Beraprost Sodium ベラプロストナトリウム

Page	Line	Correction	Error
438 right	↑ 19	is <u>less than</u> 0.1%	is <u>not exceeding</u> 0.1%

Beraprost Sodium Tablets ベラプロストナトリウム錠

Page	Line	Correction	Error
439 right	↑ 7	Internal standard solution - A mixture of water and a <u>solution of</u> 4-isopropylphenol in methanol	Internal standard solution - A mixture of water and 4-isopropylphenol in methanol
440 right	↓ 10	<u>A</u> fluorophotometer	<u>An</u> fluorophotometer

Corn Starch トウモロコシデンプン

Page	Line	Correction	Error
678 left	↑ 12	<u>Examined under</u> a microscope <5.01>	<u>Under</u> a microscope <5.01>
678 left	↑ 9	with diameters of <u>2 - 23 μm</u>	with diameters <u>ranging from about 2 μm to about 23 μm</u>
678 left	↑ 7	with diameters of <u>25 - 35 μm</u>	with diameters <u>ranging from about 25 μm to about 35 μm</u>

Elcatonin エルカトニン

Page	Line	Correction	Error
773 right	↓ 17	$s^2 = \{ y^2 - (Y/f) \} / n$	$s^2 = _ \{ y^2 - (Y - f) \} / n$

Ethanol エタノール

Page	Line	Correction	Error
803 left	↓ 8	35 cm per <u>second</u>	35 cm per <u>minute</u>

Anhydrous Ethanol 無水エタノール

Page	Line	Correction	Error
804 left	↓ 6	35 cm per <u>second</u>	35 cm per <u>minute</u>

Insulin Human (Genetical Recombination) ヒトインスリン (遺伝子組換え)

Page	Line	Correction	Error
963 left	↑ 11	GIVEQCCTSI CSLYQLENYC <u>N</u> FVNQHLCGSH LVEALYLVCG ERGFFYTP <u>K</u> T	GIVEQCCTSI CSLYQLENYC <u>N</u> FVNQHLCGSH LVEALYLVCG ERGFFYTP <u>L</u> T

Isopropanol イソプロパノール

Page	Line	Correction	Error
988 right	↓ 15	20 mL of potassium dichromate <u>TS</u>	20 mL of potassium dichromate

Lysozyme Hydrochloride リゾチーム塩酸塩

Page	Line	Correction	Error
1050 left	↓ 3	<pre> KVPGRC¹ELAA AMKRHGLDNY RGYSLGNWVC AAKFESNFNT QATNRNTDGS TDYGI¹LQINS R¹WCNDGRTP GSRNLCN¹IPC SALLSSE¹ITA SVNCAKKIVS DGNMNAWVA WRNRC¹KT¹DV QAWIRG¹CRL • xHCl </pre>	<pre> KVPGRC¹ELAA AMKRHGLDNY RGYSLGNWVC AAKFESNFNT QATNRNTDGS TDYGI¹LQINS R¹WCNDGRTP GSRNLCN¹IPC SALLSSE¹ITA SVNCAKKIVS DGNMNAWVA WRNRC¹KT¹DV QAWIRG¹CRL • xHCl </pre>

Macrogol 20000 マクロゴール 20000

Page	Line	Correction	Error
1053 left	↑ 16	practically insoluble in methanol, in ethanol (95), in petroleum benzine and in macrogol 400.	practically insoluble in methanol, in ethanol (95), <u>in dehydrated diethyl ether</u> , in petroleum benzine and in macrogol 400.

Methylcellulose メチルセルロース

Page	Line	Correction	Error
1096 right	↓ 9	(Deleted)	<u>Cellulose, methyl ether</u>

Methyl Parahydroxybenzoate パラオキシ安息香酸メチル

Page	Line	Correction	Error
1103 left	↑ 3	[99-76-3]	[98-76-3]

Povidone ポビドン

Page	Line	Correction	Error
1275 left	↓ 18	a powdered mixture of 33 g of potassium <u>sulfate</u>	a powdered mixture of 33 g of potassium <u>slfate</u>

Sodium Risedronate Hydrate リセドロン酸ナトリウム水和物

Page	Line	Correction	Error
1412 right	↑ 25	add 1 drop each of sodium <u>sulfide</u> TS	add 1 drop each of sodium <u>sulfate</u> TS
1413 left	↓ 17	in 3 mL of <u>0.2 mol/L</u> sodium hydroxide TS,	in 3 mL of <u>0.2 mL/L</u> sodium hydroxide TS,
1413 left	↑ 30	with <u>0.2 mol/L</u> sodium hydroxide TS.	with <u>0.2 mL/L</u> sodium hydroxide TS.
1413 right	↓ 8	dissolve in 1.5 mL of <u>0.2 mol/L</u> sodium hydroxide TS,	dissolve in 1.5 mL of <u>0.2 mL/L</u> sodium hydroxide TS,
1413 right	↑ 15	with <u>0.2 mol/L</u> sodium hydroxide TS.	with <u>0.2 mL/L</u> sodium hydroxide TS.

Tiaramide Hydrochloride Tablets チアラミド塩酸塩錠

Page	Line	Correction	Error
1497 left	↓ 3	To 1 tablet of Tiaramide Hydrochloride Tablets add <u>3V/5 mL</u> of 0.1 mol/L <u>hydrochloric acid TS</u> , shake for 60 minutes. <u>Add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 1 mg of tiaramide (C₁₅H₁₈ClN₃O₃S), and filter.</u>	To 1 tablet of Tiaramide Hydrochloride Tablets add <u>a volume of 0.1 mol/L hydrochloric acid TS</u> , equivalent to 3/5 volume of V mL which makes a solution so that each mL contains about 1 mg of tiaramide (C ₁₅ H ₁₈ ClN ₃ O ₃ S), shake for 60 minutes, then <u>add 0.1 mol/L hydrochloric acid TS to make exactly VmL</u> , and filter.

Wheat Starch コムギデンプン

Page	Line	Correction	Error
1574 left	↑ 23	Examine under a microscope <5.01> using a mixture of water and <u>glycerin</u> (1:1)	Examine under a microscope <5.01> using a mixture of water and <u>glycerinol</u> (1:1)

Mentha Oil ハッカ油

Page	Line	Correction	Error
1689 right	↑ 7	Amount (mg) of menthol (C ₁₀ H ₂₀ O) = $M_S \times Q_T / Q_S \times 1/5$	Amount (mg) of menthol (C ₁₀ H ₂₀ O) = $M_S \times Q_T / Q_S$

Ultraviolet-visible Reference Spectra: Dexamethasone 参照紫外可視吸収スペクトル デキサメタゾン

Page	Line	Correction	Error
2017	middle	<u>240.0 (Scale)</u>	<u>220.0 (Scale)</u>

Media Fill Test (Process Simulation) 培地充てん試験(プロセスシミュレーション)

Page	Line	Correction	Error
2207 right	↑ 5	When strains listed in the <u>Microbiological Examination of Non-sterile Products <4.05></u>	When strains listed in the <u>Microbial Limit Test <4.05></u>

Microbial Attributes of Non-sterile Pharmaceutical Products 非無菌医薬品の微生物学的品質特性

Page	Line	Correction	Error
2209 left	↓ 18	the methods given in the <u>Microbiological Examination of Non-sterile Products <4.05></u>	the methods given in the <u>Microbial Limit Test <4.05></u>
2209 right	↑ 23	When the “ <u>Microbiological Examination of Non-sterile Products <4.05></u> ” is applied to a	When the “ <u>Microbial Limit Test <4.05></u> ” is applied to a

Microbiological Evaluation of Processing Areas for Sterile Pharmaceutical Products 無菌医薬品製造区域の微生物評価試験法

Page	Line	Correction	Error
2212 right	↑ 2	Methods described in the <u>Microbiological Examination of Non-sterile Products</u> .	Methods described in the <u>Microbial Limit Test</u> .
2213 Table 3	↑ 7	*1 If necessary, antibiotics may be added to media in an appropriate concentration (see <u>Microbiological Examination of Non-sterile Products</u>).	*1 If necessary, antibiotics may be added to media in an appropriate concentration (see <u>Microbial Limit Test</u>).
2214 right	↑ 15	antimi-crobal substances" in the <u>Microbiological Examination of Non-sterile Products <4.05></u> .	antimi-crobal substances" in the <u>Microbial Limit Test <4.05></u> .
2214 right	↑ 11	See <u>Microbiological Examination of Non-sterile Products</u> .	See <u>Microbial Limit Test</u> .
2214 right	↑ 8	See <u>Microbiological Examination of Non-sterile Products</u> .	See <u>Microbial Limit Test</u> .
2214 right	↑ 5	See <u>Microbiological Examination of Non-sterile Products</u> .	See <u>Microbial Limit Test</u> .
2215 right	↓ 15	See <u>Microbiological Examination of Non-sterile Products</u> .	See <u>Microbial Limit Test</u> .
2215 right	↓ 17	See <u>Microbiological Examination of Non-sterile Products</u> .	See <u>Microbial Limit Test</u> .

Preservatives-Effectiveness Tests 保存効力試験法

Page	Line	Correction	Error
2216 right	↑ 23	on the Pour Plate Methods in “ <u>Microbiological Examination of Non-sterile Products</u> ”.	on the Pour Plate Methods in “ <u>Microbial Limit Tests</u> ”.
2216 right	↑ 11	by the Membrane Filtration Method in “ <u>Microbiological Examination of Non-sterile Products</u> ”.	by the Membrane Filtration Method in “ <u>Microbial Limit Tests</u> ”.

Quality Control of Water for Pharmaceutical Use 製薬用水の品質管理

Page	Line	Correction	Error
2250 right	↓ 3	the procedure prescribed in the <u>Microbiological Examination of Non-sterile Products <4.05></u>	the procedure prescribed in the <u>Microbial Limit Test <4.05></u>

4.05 Microbiological Examination of Non-sterile Products

This chapter includes microbial enumeration tests and tests for specified micro-organisms. For the test, use a mixture of several portions selected at random from the bulk or from the contents of a sufficient number of containers. If test specimens are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard.

I. Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests

These tests are harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

The tests described hereafter will allow quantitative enumeration of mesophilic bacteria and fungi which may grow under aerobic conditions.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results as stated below.

The methods are not applicable to products containing viable micro-organisms as active ingredients.

Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

1. General Procedures

Carry out the determination under conditions designed to avoid extrinsic microbial contamination of the product to be examined. The precautions taken to avoid contamination must be such that they do not affect any micro-organisms which are to be revealed in the test.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized. If inactivators are used for this purpose their efficacy and their absence of toxicity for micro-organisms must be demonstrated.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated.

2. Enumeration Methods

Use the membrane filtration method, or the plate-count methods, as prescribed. The most probable number (MPN) method is generally the least accurate method for microbial counts, however, for certain product groups with very low bioburden, it may be the most appropriate method.

The choice of a method is based on factors such as the nature of the product and the required limit of micro-organisms. The method chosen must allow testing of

a sufficient sample size to judge compliance with the specification. The suitability of the chosen method must be established.

3. Growth Promotion Test, Suitability of the Counting Method and Negative Controls

The ability of the test to detect micro-organisms in the presence of product to be tested must be established.

Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

3.1. Preparation of test strains

Use standardized stable suspensions of test strains or prepare as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot. Grow each of the bacterial and fungal test strains separately as described in Table 4.05-I-1.

Use *buffered sodium chloride-peptone solution pH 7.0* or *phosphate buffer solution pH 7.2* to make test suspensions; to suspend *Aspergillus brasiliensis* spores, 0.05 per cent of polysorbate 80 may be added to the buffer. Use the suspensions within 2 hours or within 24 hours if stored at 2 - 8°C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Aspergillus brasiliensis* or *Bacillus subtilis*, a stable spore suspension is prepared and then an appropriate volume of the spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 - 8°C for a validated period of time.

3.2. Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described under 4. Testing of Products. A failed negative control requires an investigation.

3.3. Growth promotion of the media

Test each batch of ready-prepared medium and each batch of medium, prepared either from dehydrated medium or from the ingredients described.

Inoculate portions/plates of *casein soya bean digest broth* and *casein soya bean digest agar* with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 4.05-I-1, using a separate portion/plate of medium for each. Inoculate plates of *Sabouraud-dextrose agar* with a small number (not more than 100 CFU) of the micro-organisms indicated in Table 4.05-I-1, using a separate plate of medium for each. Incubate in the conditions described in Table 4.05-I-1.

For solid media, growth obtained must not differ by a factor greater than 2 from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs. Liquid media are suitable if clearly visible growth of the micro-organisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Attachment1

4.05 Microbiological Examination of Non-sterile Products / General test

Table 4.05-I-1 Preparation and use of test micro-organisms

Micro-organism	Preparation of test strain	Growth promotion		Suitability of counting method in the presence of the product	
		Total aerobic microbial count	Total yeasts and moulds count	Total aerobic microbial count	Total yeasts and moulds count
<i>Staphylococcus aureus</i> such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276	Casein soya bean digest agar or casein soya bean digest broth 30 - 35°C 18 - 24 hours	Casein soya bean digest agar and casein soya bean digest broth 100 CFU 30 - 35 °C 3 days		Casein soya bean digest agar/MPN casein soya bean digest broth 100 CFU 30 - 35 °C 3 days	
<i>Pseudomonas aeruginosa</i> such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275	Casein soya bean digest agar or casein soya bean digest broth 30 - 35°C 18 - 24 hours	Casein soya bean digest agar and casein soya bean digest broth 100 CFU 30 - 35 °C 3 days		Casein soya bean digest agar/MPN casein soya bean digest broth 100 CFU 30 - 35 °C 3 days	
<i>Bacillus subtilis</i> such as ATCC 6633, NCIMB 8054, CIP 52.62 or NBRC 3134	Casein soya bean digest agar or casein soya bean digest broth 30 - 35°C 18 - 24 hours	Casein soya bean digest agar and casein soya bean digest broth 100 CFU 30 - 35 °C 3 days		Casein soya bean digest agar/MPN casein soya bean digest broth 100 CFU 30 - 35 °C 3 days	
<i>Candida albicans</i> such as ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594	Sabouraud-dextrose agar or Sabouraud-dextrose broth 20 - 25°C 2 - 3 days	Casein soya bean digest agar 100 CFU 30 - 35 °C 5 days	Sabouraud-dextrose agar 100 CFU 20 - 25°C 5 days	Casein soya bean digest agar 100 CFU 30 - 35 °C 5 days MPN: not applicable	Sabouraud-dextrose agar 100 CFU 20 - 25°C 5 days
<i>Aspergillus brasiliensis</i> such as ATCC 16404, IMI 149007, IP 1431.83 or NBRC 9455	Sabouraud-dextrose agar or potato-dextrose agar 20 - 25°C 5 - 7 days, or until good sporulation is achieved	Casein soya bean digest agar 100 CFU 30 - 35 °C 5 days	Sabouraud-dextrose agar 100 CFU 20 - 25°C 5 days	Casein soya bean digest agar 100 CFU 30 - 35 °C 5 days MPN: not applicable	Sabouraud-dextrose agar 100 CFU 20 - 25°C 5 days

3.4. Suitability of the counting method in the presence of product

3.4.1. Preparation of the sample

The method for sample preparation depends on the physical characteristics of the product to be tested. If none of the procedures described below can be demonstrated to be satisfactory, an alternative procedure must be developed.

(i) Water-soluble products: Dissolve or dilute (usually a 1 in 10 dilution is prepared) the product to be examined in buffered sodium chloride-peptone solution pH 7.0, phosphate buffer solution pH 7.2 or casein soya bean digest broth. If necessary adjust to pH 6 - 8. Further dilutions, where necessary, are prepared with the same diluent.

(ii) Non-fatty products insoluble in water: Suspend the product to be examined (usually a 1 in 10 dilution is

prepared) in buffered sodium chloride-peptone solution pH 7.0, phosphate buffer solution pH 7.2 or casein soya bean digest broth. A surface-active agent such as 1 g/L of polysorbate 80 may be added to assist the suspension of poorly wetttable substances. If necessary adjust to pH 6 - 8. Further dilutions, where necessary, are prepared with the same diluent.

(iii) Fatty products: Dissolve in isopropyl myristate, sterilized by filtration or mix the product to be examined with the minimum necessary quantity of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent, heated if necessary to not more than 40°C, or in exceptional cases to not more than 45°C. Mix carefully and if necessary maintain the temperature in a water-bath. Add sufficient of the pre-warmed chosen diluent to make a 1 in 10 dilution of

the original product. Mix carefully whilst maintaining the temperature for the shortest time necessary for the formation of an emulsion. Further serial tenfold dilutions may be prepared using the chosen diluent containing a suitable concentration of sterile polysorbate 80 or another non-inhibitory sterile surface-active reagent.

(iv) Fluids or solids in aerosol form: Aseptically transfer the product into a membrane filter apparatus or a sterile container for further sampling. Use either the total contents or a defined number of metered doses from each of the containers tested.

(v) Transdermal patches: Remove the protective cover sheets ("release liner") of the transdermal patches and place them, adhesive side upwards, on sterile glass or plastic trays. Cover the adhesive surface with sterile porous material, for example sterile gauze, to prevent the patches from sticking together, and transfer the patches to a suitable volume of the chosen diluent containing inactivators such as polysorbate 80 and/or lecithin. Shake the preparation vigorously for at least 30 minutes.

3.4.2. Inoculation and dilution

Add to the sample prepared as described above (3.4.1.) and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than 100 CFU. The volume of the suspension of the inoculum should not exceed 1 per cent of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution factor of the prepared sample must be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols must be developed. If inhibition of growth by the sample cannot otherwise be avoided, the aliquot of the microbial suspension may be added after neutralization, dilution or filtration.

3.4.3. Neutralization/removal of antimicrobial activity

The number of micro-organisms recovered from the prepared sample diluted as described in 3.4.2. and incubated following the procedure described in 3.4.4., is compared to the number of micro-organisms recovered from the control preparation.

If growth is inhibited (reduction by a factor greater than 2), then modify the procedure for the particular enumeration test to ensure the validity of the results. Modification of the procedure may include, for example, (1) an increase in the volume of the diluent or culture medium, (2) incorporation of a specific or general neutralizing agents into the diluent, (3) membrane filtration or (4) a combination of the above measures.

Neutralizing agents. Neutralizing agents may be used to neutralize the activity of antimicrobial agents (Table 4.05-I-2). They may be added to the chosen diluent or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for micro-organisms must be demonstrated by carrying out a blank with neutralizer and without product.

If no suitable neutralizing method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This

information serves to indicate that the article is not likely to be contaminated with the given species of the micro-organism. However, it is possible that the product only inhibits some of the micro-organisms specified herein, but does not inhibit others not included amongst the test strains or for which the latter are not representative. Then, perform the test with the highest dilution factor compatible with microbial growth and the specific acceptance criterion.

3.4.4. Recovery of micro-organism in the presence of product

For each of the micro-organisms listed in Table 4.05-I-1, separate tests are performed. Only micro-organisms of the added test strain are counted.

3.4.4.1. Membrane filtration

Use membrane filters having a nominal pore size not greater than 0.45 μm . The type of filter material is chosen in such a way that the bacteria-retaining efficiency is not affected by the components of the sample to be investigated. For each of the micro-organisms listed in Table 4.05-I-1, one membrane filter is used.

Transfer a suitable amount of the sample prepared as described under 3.4.1. to 3.4.3. (preferably representing 1 g of the product, or less if large numbers of CFU are expected) to the membrane filter, filter immediately and rinse the membrane filter with an appropriate volume of diluent.

For the determination of total aerobic microbial count (TAMC), transfer the membrane filter to the surface of *casein soya bean digest agar*. For the determination of total combined yeasts/moulds count (TYMC) transfer the membrane to the surface of *Sabouraud-dextrose agar*. Incubate the plates as indicated in Table 4.05-I-1. Perform the counting.

3.4.4.2. Plate-count methods

Perform plate-count methods at least in duplicate for each medium and use the mean count of the result.

(i) Pour-plate method: For Petri dishes 9 cm in diameter, add to the dish 1 mL of the sample prepared as described under 3.4.1. to 3.4.3. and 15 - 20 mL of *casein soya bean digest agar* or *Sabouraud-dextrose agar*, both media being at not more than 45°C. If larger Petri dishes are used, the amount of agar medium is increased accordingly. For each of the micro-organisms listed in Table 4.05-I-1, at least 2 Petri dishes are used.

Incubate the plates as indicated in Table 4.05-I-1. Take the arithmetic mean of the counts per medium and calculate the number of CFU in the original inoculum.

(ii) Surface-spread method: For Petri dishes 9 cm in diameter, add 15 - 20 mL of *casein soya bean digest agar* or *Sabouraud-dextrose agar* at about 45°C to each Petri dish and allow to solidify. If larger Petri dishes are used, the volume of the agar is increased accordingly. Dry the plates, for example in a laminar-air-flow cabinet or in an incubator. For each of the micro-organisms listed in Table 4.05-I-1, at least 2 Petri dishes are used. Spread a measured volume of not less than 0.1 mL of the sample prepared as described under 3.4.1. to 3.4.3. over the surface of the medium. Incubate and count as prescribed under 3.4.4.2.(i).

Table 4.05-I-2 Common neutralizing agents for interfering substances

Interfering substance	Potential neutralizing method
Glutaraldehyde, Mercurials	Sodium hydrogen sulfite (Sodium bisulfite)
Phenolics, Alcohol, Aldehydes, Sorbate	Dilution
Aldehydes	Glycine
Quaternary Ammonium Compounds (QACs), Parahydroxybenzoates (Parabens), Bis-biguanides	Lecithin
QAC, Iodine, Parabens	Polysorbate
Mercurials	Thioglycollate
Mercurials, Halogens, Aldehydes	Thiosulfate
EDTA (edetate)	Mg or Ca ions

3.4.4.3. Most-probable-number (MPN) method

The precision and accuracy of the MPN method is less than that of the membrane filtration method or the plate-count method. Unreliable results are obtained particularly for the enumeration of moulds. For these reasons the MPN method is reserved for the enumeration of TAMC in situations where no other method is available. If the use of the method is justified, proceed as follows.

Prepare a series of at least 3 serial tenfold dilutions of the product as described under 3.4.1. to 3.4.3.. From each level of dilution, 3 aliquots of 1 g or 1 mL are used to inoculate 3 tubes with 9 - 10 mL of *casein soya bean digest broth*. If necessary a surface-active agent such as polysorbate 80, or an inactivator of antimicrobial agents may be added to the medium. Thus, if 3 levels of dilution are prepared 9 tubes are inoculated.

Incubate all tubes at 30 - 35°C for not more than 3 days. If reading of the results is difficult or uncertain owing to the nature of the product to be examined, subculture in the same broth, or *casein soya bean digest agar*, for 1 - 2 days at the same temperature and use these results. Determine the most probable number of micro-organisms per gram or milliliter of the product to be examined from Table 4.05-I-3.

3.5. Results and interpretation

When verifying the suitability of the membrane filtration method or the plate-count method, a mean count of any of the test organisms not differing by a factor greater than 2 from the value of the control defined in 3.4.2. in the absence of the product must be obtained. When verifying the suitability of the MPN method the calculated value from the inoculum must be within 95 per cent confidence limits of the results obtained with the control.

If the above criteria cannot be met for one or more of the organisms tested with any of the described methods, the method and test conditions that come closest to the criteria are used to test the product.

4. Testing of Products

4.1. Amount used for the test

Unless otherwise prescribed, use 10 g or 10 mL of the product to be examined taken with the precautions referred to above. For fluids or solids in aerosol form, sample 10 containers. For transdermal patches, sample 10 patches.

The amount to be tested may be reduced for active substances that will be formulated in the following conditions: the amount per dosage unit (e.g. tablet, capsule, injection) is less than or equal to 1 mg or the amount per gram or milliliter (for preparations not presented in dose units) is less than 1 mg. In these cases, the amount of sample to be tested is not less than the amount present in 10 dosage units or 10 g or 10 mL of the product.

For materials used as active substances where sample quantity is limited or batch size is extremely small (i.e. less than 1000 mL or 1000 g), the amount tested shall be 1 per cent of the batch unless a lesser amount is prescribed or justified and authorised.

For products where the total number of entities in a batch is less than 200 (e.g. samples used in clinical trials), the sample size may be reduced to 2 units, or 1 unit if the size is less than 100.

Select the sample(s) at random from the bulk material or from the available containers of the preparation. To obtain the required quantity, mix the contents of a sufficient number of containers to provide the sample.

4.2. Examination of the product

4.2.1 Membrane filtration

Use a filtration apparatus designed to allow the transfer of the filter to the medium. Prepare the sample using a method that has been shown suitable as described in section 3 and transfer the appropriate amount to each of 2 membrane filters and filter immediately. Wash each filter following the procedure shown to be suitable.

For the determination of TAMC, transfer one of the membrane filters to the surface of *casein soya bean digest agar*. For the determination of TYMC, transfer the other membrane to the surface of *Sabouraud-dextrose agar*. Incubate the plate of *casein soya bean digest agar* at 30 - 35°C for 3 - 5 days and the plate of *Sabouraud-dextrose agar* at 20 - 25°C for 5 - 7 days. Calculate the number of CFU per gram or per milliliter of product.

When examining transdermal patches, filter 10 per cent of the volume of the preparation described under 3.4.1. separately through each of 2 sterile filter membranes. Transfer one membrane to *casein soya bean digest agar* for TAMC and the other membrane to *Sabouraud-dextrose agar* for TYMC.

Attachment1

4.05 Microbiological Examination of Non-sterile Products / General test

Table 4.05-I-3 Most-probable-number values of micro-organisms

Observed combinations of numbers of tubes showing growth in each set			MPN per g or per mL of product	95 per cent confidence limits
Number of g or mL of product per tube				
0.1	0.01	0.001		
0	0	0	Less than 3	0 – 9.4
0	0	1	3	0.1 – 9.5
0	1	0	3	0.1 – 10
0	1	1	6.1	1.2 – 17
0	2	0	6.2	1.2 – 17
0	3	0	9.4	3.5 – 35
1	0	0	3.6	0.2 – 17
1	0	1	7.2	1.2 – 17
1	0	2	11	4 – 35
1	1	0	7.4	1.3 – 20
1	1	1	11	4 – 35
1	2	0	11	4 – 35
1	2	1	15	5 – 38
1	3	0	16	5 – 38
2	0	0	9.2	1.5 – 35
2	0	1	14	4 – 35
2	0	2	20	5 – 38
2	1	0	15	4 – 38
2	1	1	20	5 – 38
2	1	2	27	9 – 94
2	2	0	21	5 – 40
2	2	1	28	9 – 94
2	2	2	35	9 – 94
2	3	0	29	9 – 94
2	3	1	36	9 – 94
3	0	0	23	5 – 94
3	0	1	38	9 – 104
3	0	2	64	16 – 181
3	1	0	43	9 – 181
3	1	1	75	17 – 199
3	1	2	120	30 – 360
3	1	3	160	30 – 380
3	2	0	93	18 – 360
3	2	1	150	30 – 380
3	2	2	210	30 – 400
3	2	3	290	90 – 990
3	3	0	240	40 – 990
3	3	1	460	90 – 1980
3	3	2	1100	200 – 4000
3	3	3	More than 1100	

4.2.2. Plate-count methods

(i) Pour-plate method: Prepare the sample using a method that has been shown to be suitable as described in section 3. Prepare for each medium at least 2 Petri dishes for each level of dilution. Incubate the plates of *casein soya bean digest agar* at 30 - 35°C for 3 - 5 days and the plates of *Sabouraud-dextrose agar* at 20 - 25°C for 5 - 7 days. Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC. Take the arithmetic mean per culture medium of

the counts and calculate the number of CFU per gram or per milliliter of product.

(ii) Surface-spread method: Prepare the sample using a method that has been shown to be suitable as described in section 3. Prepare at least 2 Petri dishes for each medium and each level of dilution. For incubation and calculation of the number of CFU proceed as described for the pour-plate method.

4.2.3. Most-probable-number method

Prepare and dilute the sample using a method that has

been shown to be suitable as described in section 3. Incubate all tubes for 3 - 5 days at 30 - 35°C. Subculture if necessary, using the procedure shown to be suitable. Record for each level of dilution the number of tubes showing microbial growth. Determine the most probable number of micro-organisms per gram or milliliter of the product to be examined from Table 4.05-I-3.

4.3. Interpretation of the results

The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU found using *casein soya bean digest agar*; if colonies of fungi are detected on this medium, they are counted as part of TAMC. The total combined yeasts/mould count (TYMC) is considered to be equal to the number of CFU found using *Sabouraud-dextrose agar*; if colonies of bacteria are detected on this medium, they are counted as part of TYMC. When the TYMC is expected to exceed the acceptance criterion due to the bacterial growth, *Sabouraud-dextrose agar* containing antibiotics may be used. If the count is carried out by the MPN method the calculated value is the TAMC.

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

- 10¹ CFU: maximum acceptable count = 20,
 - 10² CFU: maximum acceptable count = 200,
 - 10³ CFU: maximum acceptable count = 2000,
- and so forth.

The recommended solutions and media are described in II. *Tests for specified micro-organisms*.

II. Microbiological Examination of Non-sterile Products: Tests for Specified Micro-organisms

These tests are harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

The tests described hereafter will allow determination of the absence or limited occurrence of specified micro-organisms which may be detected under the conditions described.

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality. When used for such purposes follow the instructions given below, including the number of samples to be taken and interpret the results as stated below.

Alternative microbiological procedures, including automated methods may be used, provided that their equivalence to the Pharmacopoeial method has been demonstrated.

1. General Procedures

The preparation of samples is carried out as described in I. *Microbial enumeration tests*.

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized as described in I. *Microbial enumeration tests*.

If surface-active substances are used for sample preparation, their absence of toxicity for micro-organisms and their compatibility with inactivators used must be demonstrated as described in I. *Microbial enumeration tests*.

2. Growth Promoting and Inhibitory Properties of the Media, Suitability of the Test and Negative Controls

The ability of the test to detect micro-organisms in the presence of the product to be tested must be established. Suitability must be confirmed if a change in testing performance, or the product, which may affect the outcome of the test is introduced.

2.1. Preparation of test strains

Use standardized stable suspensions of test strains or prepare as stated below. Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the original master seed-lot.

2.1.1. Aerobic micro-organisms

Grow each of the bacterial test strains separately in containers containing *casein soya bean digest broth* or on *casein soya bean digest agar* at 30 – 35°C for 18 - 24 hours. Grow the test strain for *Candida albicans* separately on *Sabouraud-dextrose agar* or in *Sabouraud-dextrose broth* at 20 - 25°C for 2 - 3 days.

Staphylococcus aureus such as ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276,

Pseudomonas aeruginosa such as ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275,

Escherichia coli such as ATCC 8739, NCIMB 8545, CIP 53.126 or NBRC 3972,

Salmonella enterica subsp. *enterica* serovar Typhimurium such as ATCC 14028
or, as an alternative,

Salmonella enterica subsp. *enterica* serovar Abony such as NBRC 100797, NCTC 6017 or CIP 80.39,

Candida albicans such as ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594.

Use *buffered sodium chloride-peptone solution pH 7.0* or *phosphate buffer solution pH 7.2* to make test suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2 - 8°C.

2.1.2. Clostridia

Use *Clostridium sporogenes* such as ATCC 11437 (NBRC 14293, NCIMB 12343, CIP 100651) or ATCC 19404 (NCTC 532 or CIP 79.3). Grow the clostridial test strain under anaerobic conditions in *reinforced medium for Clostridia* at 30 - 35°C for 24 - 48 hours. As an alternative to preparing and then diluting down a fresh suspension of vegetative cells of *Cl. sporogenes*, a stable spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 - 8°C for a validated period.

2.2. Negative control

To verify testing conditions a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of micro-organisms. A negative control is also performed when testing the products as described under 3. A failed negative control requires an investigation.

2.3. Growth promotion and inhibitory properties of the media

Test each batch of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients.

Verify suitable properties of relevant media as described in Table 4.05-II-1.

Attachment1

4.05 Microbiological Examination of Non-sterile Products / General test

Table 4.05-II-1 Growth promoting, inhibitory and indicative properties of media

Medium	Property	Test strains
Test for bile-tolerant gram-negative bacteria		
<i>Enterobacteria enrichment broth -Mossel</i>	Growth promoting	<i>E. coli</i> <i>P. aeruginosa</i>
	Inhibitory	<i>S. aureus</i>
<i>Violet red bile glucose agar</i>	Growth promoting + Indicative	<i>E. coli</i> <i>P. aeruginosa</i>
Test for <i>Escherichia coli</i>		
<i>MacConkey broth</i>	Growth promoting	<i>E. coli</i>
	Inhibitory	<i>S. aureus</i>
<i>MacConkey agar</i>	Growth promoting + Indicative	<i>E. coli</i>
Test for <i>Salmonella</i>		
<i>Rappaport Vassiliadis Salmonella enrichment broth</i>	Growth promoting	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium or <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony
	Inhibitory	<i>S. aureus</i>
<i>Xylose, lysine, deoxycholate agar</i>	Growth promoting + Indicative	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium or <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony
	Indicative	<i>E. coli</i>
Test for <i>Pseudomonas aeruginosa</i>		
<i>Cetrimide agar</i>	Growth promoting	<i>P. aeruginosa</i>
	Inhibitory	<i>E. coli</i>
Test for <i>Staphylococcus aureus</i>		
<i>Mannitol salt agar</i>	Growth promoting+ Indicative	<i>S. aureus</i>
	Inhibitory	<i>E. coli</i>
Test for Clostridia		
<i>Reinforced medium for Clostridia</i>	Growth promoting	<i>Cl. sporogenes</i>
<i>Columbia agar</i>	Growth promoting	<i>Cl. sporogenes</i>
Test for <i>Candida albicans</i>		
<i>Sabouraud-dextrose broth</i>	Growth promoting	<i>C. albicans</i>
<i>Sabouraud-dextrose agar</i>	Growth promoting + Indicative	<i>C. albicans</i>

(i) Test for growth promoting properties, liquid media: inoculate a portion of the appropriate medium with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

(ii) Test for growth promoting properties, solid media: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate

micro-organism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the micro-organism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

(iii) Test for inhibitory properties, liquid or solid media: inoculate the appropriate medium with at least 100 CFU of the appropriate micro-organism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the test micro-organism occurs.

(iv) Test for indicative properties: perform surface-spread method, inoculating each plate with a small number (not more than 100 CFU) of the appropriate micro-organism. Incubate at the specified temperature for a period of time within the range specified in the test. Colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

2.4. Suitability of the test method

For each product to be tested perform sample preparation as described in the relevant paragraph in section 3. Add each test strain at the time of mixing, in the prescribed growth medium. Inoculate the test strains individually. Use a number of micro-organisms equivalent to not more than 100 CFU in the inoculated test preparation.

Perform the test as described in the relevant paragraph in section 3 using the shortest incubation period prescribed.

The specified micro-organisms must be detected with the indication reactions as described in section 3.

Any antimicrobial activity of the product necessitates a modification of the test procedure (see 3.4.3. of I. *Microbial enumeration tests*).

If for a given product the antimicrobial activity with respect to a micro-organism for which testing is prescribed cannot be neutralized, then it is to be assumed that the inhibited micro-organism will not be present in the product.

3. Testing of Products

3.1. Bile-tolerant gram-negative bacteria

3.1.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. *Microbial enumeration tests*, but using *casein soya bean digest broth* as the chosen diluent, mix and incubate at 20 - 25°C for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 hours but not more than 5 hours).

3.1.2. Test for absence

Unless otherwise prescribed use the volume corresponding to 1 g of the product, as prepared in 3.1.1. to inoculate *enterobacteria enrichment broth-Mossel*. Incubate at 30 - 35°C for 24 - 48 hours. Subculture on plates of *violet red bile glucose agar*. Incubate at 30 - 35°C for 18 - 24 hours.

The product complies with the test if there is no growth of colonies.

3.1.3. Quantitative test

3.1.3.1. Selection and subculture

Inoculate suitable quantities of *enterobacteria enrichment broth-Mossel* with the preparation as described under 3.1.1. and/or dilutions of it containing respectively 0.1 g, 0.01 g and 0.001 g (or 0.1 mL, 0.01 mL and 0.001 mL) of the product to be examined. Incubate at 30 - 35°C for 24 - 48 hours. Subculture each of the cultures on a plate of *violet red bile glucose agar*. Incubate at 30 - 35°C for 18 - 24 hours.

3.1.3.2. Interpretation

Growth of colonies constitutes a positive result. Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result. Determine from Table 4.05-II-2 the probable number of

bacteria.

3.2. *Escherichia coli*

3.2.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. *Microbial enumeration tests* and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of *casein soya bean digest broth*, mix and incubate at 30 - 35°C for 18 - 24 hours.

3.2.2. Selection and subculture

Shake the container, transfer 1 mL of *casein soya bean digest broth* to 100 mL of *MacConkey broth* and incubate at 42 - 44°C for 24 - 48 hours. Subculture on a plate of *MacConkey agar* at 30 - 35°C for 18 - 72 hours.

3.2.3. Interpretation

Growth of colonies indicates the possible presence of *E. coli*. This is confirmed by identification tests.

The product complies with the test if no colonies are present or if the identification tests are negative.

3.3. *Salmonella*

3.3.1. Sample preparation and pre-incubation

Prepare the product to be examined as described in I. *Microbial enumeration tests* and use the quantity corresponding to not less than 10 g or 10 mL to inoculate a suitable amount (determined as described under 2.4.) of *casein soya bean digest broth*, mix and incubate at 30 - 35°C for 18 - 24 hours.

3.3.2. Selection and subculture

Transfer 0.1 mL of *casein soya bean digest broth* to 10 mL of *Rappaport Vassiliadis Salmonella enrichment broth* and incubate at 30 - 35°C for 18 - 24 hours. Subculture on plates of *xylose, lysine, deoxycholate agar*. Incubate at 30 - 35°C for 18 - 48 hours.

3.3.3. Interpretation

The possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centers. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

3.4. *Pseudomonas aeruginosa*

3.4.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. *Microbial enumeration tests* and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of *casein soya bean digest broth* and mix. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in I. *Microbial enumeration tests* (3.4.1.) through a sterile filter membrane and place in 100 mL of *casein soya bean digest broth*. Incubate at 30 - 35°C for 18 - 24 hours.

3.4.2. Selection and subculture

Subculture on a plate of *cetrimide agar* and incubate at 30 - 35°C for 18 - 72 hours.

3.4.3. Interpretation

Growth of colonies indicates the possible presence of *P. aeruginosa*. This is confirmed by identification tests.

Table 4.05-II-2 Interpretation of results

Results for each quantity of product			Probable number of bacteria per gram or mL of product
0.1 g or 0.1 mL	0.01 g or 0.01 mL	0.001 g or 0.001 mL	
+	+	+	more than 10 ³
+	+	-	less than 10 ³ and more than 10 ²
+	-	-	less than 10 ² and more than 10
-	-	-	less than 10

The product complies with the test if colonies are not present or if the confirmatory identification tests are negative.

3.5. *Staphylococcus aureus*

3.5.1. Sample preparation and pre-incubation

Prepare a sample using a 1 in 10 dilution of not less than 1 g of the product to be examined as described in I. *Microbial enumeration tests* and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described under 2.4.) of *casein soya bean digest broth* and homogenize. When testing transdermal patches, filter the volume of sample corresponding to 1 patch of the preparation described in I. *Microbial enumeration tests* (3.4.1.) through a sterile filter membrane and place in 100 mL of *casein soya bean digest broth*. Incubate at 30 - 35°C for 18 - 24 hours.

3.5.2. Selection and subculture

Subculture on a plate of *mannitol salt agar* and incubate at 30 - 35°C for 18 - 72 hours.

3-5-3 Interpretation

The possible presence of *S. aureus* is indicated by the growth of yellow/white colonies surrounded by a yellow zone. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

3.6. Clostridia

3.6.1. Sample preparation and heat treatment

Prepare a sample using a 1 in 10 dilution (with a minimum total volume of 20 mL) of not less than 2 g or 2 mL of the product to be examined as described in I. *Microbial enumeration tests*. Divide the sample into two portions of at least 10 mL. Heat 1 portion at 80°C for 10 minutes and cool rapidly. Do not heat the other portion.

3.6.2. Selection and subculture

Use 10 mL or the quantity corresponding to 1 g or 1 mL of the product to be examined of both portions to inoculate suitable amounts (determined as described under 2.4.) of *reinforced medium for Clostridia*. Incubate under anaerobic conditions at 30 - 35°C for 48 hours. After incubation, make subcultures from each container on *Columbia agar* and incubate under anaerobic conditions at 30 - 35°C for 48 - 72 hours.

3.6.3. Interpretation

The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction

indicates the presence of Clostridia. This is confirmed by identification tests.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

3.7. *Candida albicans*

3.7.1. Sample preparation and pre-incubation

Prepare the product to be examined as described in I. *Microbial enumeration tests* and use 10 mL or the quantity corresponding to not less than 1 g or 1 mL to inoculate 100 mL of *Sabouraud-dextrose broth* and mix. Incubate at 30 - 35°C for 3-5 days.

3.7.2. Selection and subculture

Subculture on a plate of *Sabouraud-dextrose agar* and incubate at 30 - 35°C for 24 - 48 hours.

3.7.3. Interpretation

Growth of white colonies may indicate the presence of *C. albicans*. This is confirmed by identification tests.

The product complies with the test if such colonies are not present or if the confirmatory identification tests are negative.

The following section is given for information.

4. Recommended Solutions and Culture Media

The following solutions and culture media have been found satisfactory for the purposes for which they are prescribed in the test for microbial contamination in the Pharmacopoeia. Other media may be used provided that their suitability can be demonstrated.

(i) Phosphate buffer solution pH 7.2

Prepare a mixture of purified water and stock buffer solution (800:1 V/V) and sterilize.

Stock buffer solution. Transfer 34 g of potassium dihydrogen phosphate to a 1000 mL volumetric flask, dissolve in 500 mL of purified water, adjust to pH 7.2 ± 0.2 with sodium hydroxide, add purified water to volume and mix. Dispense in containers and sterilize. Store at a temperature of 2 - 8°C.

(ii) Buffered sodium chloride-peptone solution pH 7.0

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dehydrate	7.2 g
	(equivalent to 0.067 mol phosphate)

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Sodium chloride 4.3 g
 Peptone (meat or casein) 1.0 g
 Purified water 1000 mL
 Sterilize in an autoclave using a validated cycle.

(iii) *Casein soya bean digest broth*

Pancreatic digest of casein 17.0 g
 Papaic digest of soya bean 3.0 g
 Sodium chloride 5.0 g
 Dipotassium hydrogen phosphate 2.5 g
 Glucose monohydrate 2.5 g
 Purified water 1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

(iv) *Casein soya bean digest agar*

Pancreatic digest of casein 15.0 g
 Papaic digest of soya bean 5.0 g
 Sodium chloride 5.0 g
 Agar 15.0 g
 Purified water 1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

(v) *Sabouraud-dextrose agar*

Dextrose 40.0 g
 Mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1) 10.0 g
 Agar 15.0 g
 Purified water 1000 mL

Adjust the pH so that after sterilization it is 5.6 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

(vi) *Potato dextrose agar*

Infusion from potatoes 200 g
 Dextrose 20.0 g
 Agar 15.0 g
 Purified water 1000 mL

Adjust the pH so that after sterilization it is 5.6 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

(vii) *Sabouraud-dextrose broth*

Dextrose 20.0 g
 Mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1) 10.0 g
 Purified water 1000 mL

Adjust the pH so that after sterilization it is 5.6 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

(viii) *Enterobacteria enrichment broth-Mossel*

Pancreatic digest of gelatin 10.0 g
 Glucose monohydrate 5.0 g
 Dehydrated ox bile 20.0 g
 Potassium dihydrogen phosphate 2.0 g
 Disodium hydrogen phosphate dihydrate 8.0 g
 Brilliant green 15 mg
 Purified water 1000 mL

Adjust the pH so that after heating it is 7.2 ± 0.2 at 25°C. Heat at 100°C for 30 minutes and cool immediately.

(ix) *Violet red bile glucose agar*

Yeast extract 3.0 g

Pancreatic digest of gelatin 7.0 g
 Bile salts 1.5 g
 Sodium chloride 5.0 g
 Glucose monohydrate 10.0 g
 Agar 15.0 g
 Neutral red 30 mg
 Crystal violet 2 mg
 Purified water 1000 mL

Adjust the pH so that after heating it is 7.4 ± 0.2 at 25°C. Heat to boiling; do not heat in an autoclave.

(x) *MacConkey broth*

Pancreatic digest of gelatin 20.0 g
 Lactose monohydrate 10.0 g
 Dehydrated ox bile 5.0 g
 Bromocresol purple 10 mg
 Purified water 1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

(xi) *MacConkey agar*

Pancreatic digest of gelatin 17.0 g
 Peptones (meat and casein) 3.0 g
 Lactose monohydrate 10.0 g
 Sodium chloride 5.0 g
 Bile salts 1.5 g
 Agar 13.5 g
 Neutral red 30 mg
 Crystal violet 1 mg
 Purified water 1000 mL

Adjust the pH so that after sterilization it is 7.1 ± 0.2 at 25°C. Boil for 1 minute with constant shaking then sterilize in an autoclave using a validated cycle.

(xii) *Rappaport Vassiliadis Salmonella Enrichment broth*

Soya peptone 4.5 g
 Magnesium chloride hexahydrate 29.0 g
 Sodium chloride 8.0 g
 Dipotassium hydrogen phosphate 0.4 g
 Potassium dihydrogen phosphate 0.6 g
 Malachite green 36 mg
 Purified water 1000 mL

Dissolve, warming slightly. Sterilize in an autoclave using a validated cycle, at a temperature not exceeding 115°C. The pH is to be 5.2 ± 0.2 at 25°C after heating and autoclaving.

(xiii) *Xylose, lysine, deoxycholate agar*

Xylose 3.5 g
 L-Lysine 5.0 g
 Lactose monohydrate 7.5 g
 Sucrose 7.5 g
 Sodium chloride 5.0 g
 Yeast extract 3.0 g
 Phenol red 80 mg
 Agar 13.5 g
 Sodium deoxycholate 2.5 g
 Sodium thiosulfate 6.8 g
 Ammonium iron (III) citrate 0.8 g
 Purified water 1000 mL

Adjust the pH so that after heating it is 7.4 ± 0.2 at 25°C.

Attachment 1

4.05 Microbiological Examination of Non-sterile Products / General test

Heat to boiling, cool to 50°C and pour into Petri dishes. Do not heat in an autoclave.

(xiv) *Cetrimide agar*

Pancreatic digest of gelatin	20.0 g
Magnesium chloride	1.4 g
Dipotassium sulfate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Purified water	1000 mL
Glycerol	10.0 mL

Heat to boiling for 1 minute with shaking. Adjust the pH so that after sterilization it is 7.2 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

(xv) *Mannitol salt agar*

Pancreatic digest of casein	5.0 g
Peptic digest of animal tissue	5.0 g
Beef extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Agar	15.0 g
Phenol red	25 mg
Purified water	1000 mL

Heat to boiling for 1 minute with shaking. Adjust the pH so that after sterilization it is 7.4 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

(xvi) *Reinforced medium for Clostridia*

Beef extract	10.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Soluble starch	1.0 g
Glucose monohydrate	5.0 g
Cysteine hydrochloride	0.5 g
Sodium chloride	5.0 g
Sodium acetate	3.0 g
Agar	0.5 g
Purified water	1000 mL

Hydrate the agar, dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilization it is about 6.8 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle.

(xvii) *Columbia agar*

Pancreatic digest of casein	10.0 g
Meat peptic digest	5.0 g
Heart pancreatic digest	3.0 g
Yeast extract	5.0 g
Maize Starch	1.0 g
Sodium chloride	5.0 g
Agar, according to gelling power	10.0 g to 15.0 g
Purified water	1000 mL

Hydrate the agar, dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°C. Sterilize in an autoclave using a validated cycle. Allow to cool to 45 - 50°C; add, where necessary, gentamicin sulfate corresponding to 20 mg of gentamicin base and pour into Petri dishes.

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August 26, 2013

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Page	Line	Correction	Error
		Test for Trace Amounts of Aluminum in <u>Total</u> Parenteral Nutrition (TPN) Solutions2146	Test for Trace Amounts of Aluminum in <u>Trans</u> Parenteral Nutrition (TPN) Solutions2146

2.59 Test for Total Organic Carbon 有機体炭素試験法

Page	Line	Correction	Error
79 right	↓ 21	when using a solution of <u>0.806 mg/L sodium dodecylbenzenesulfonate</u> as the sample.	when using a solution of <u>sodium dodecylbenzenesulfonate</u> (theoretical value of <u>total organic carbon</u> in this solution is <u>0.806 mg/L</u>) as the sample.

9.41 Reagents, Test Solutions 試薬・試液

Page	Line	Correction	Error
197 right	↑ 22	Perform the test with 10 µL of this solution as directed in the Purity (4) under Ursodeoxycholic Acid	Perform the test with 10 µL of this solution as directed in the Purity (Z) under Ursodeoxycholic Acid
199 right	↑ 18	Proceed with 10 µL of this solution as directed in the Purity (4) under Ursodeoxycholic Acid:	Proceed with 10 µL of this solution as directed in the Purity (Z) under Ursodeoxycholic Acid:
256 left	↓ 13	Molybdenum (VI) oxide MoO ₃	Molybdenum (III) oxide MoO ₃
256 left	↓ 19	Molybdenum (VI) oxide-citric acid TS To 54 g of molybdenum (VI) oxide and	Molybdenum (III) oxide-citric acid TS To 54 g of molybdenum (III) oxide and
256 left	↑ 25	See molybdenum (VI) oxide	See molybdenum (III) oxide
256 left	↑ 23	See molybdenum (VI) oxide-citric acid TS.	See molybdenum (III) oxide-citric acid TS.
267 left	↑ 21	15.20 mL of 0.2 mol/L sodium hydroxide <u>TS</u> ,	15.20 mL of 0.2 mol/L sodium hydroxide <u>VS</u> ,
267 right	↓ 1	29.54 mL of 0.2 mol/L sodium hydroxide <u>TS</u> ,	29.54 mL of 0.2 mol/L sodium hydroxide <u>VS</u> ,
267 right	↓ 17	34.7 mL of 0.2 mol/L sodium hydroxide <u>TS</u> ,	34.7 mL of 0.2 mol/L sodium hydroxide <u>VS</u> ,
267 right	↓ 21	39.50 mL of 0.2 mol/L sodium hydroxide <u>TS</u> ,	39.50 mL of 0.2 mol/L sodium hydroxide <u>VS</u> ,
267 right	↓ 29	46.1 mL of 0.2 mol/L sodium hydroxide <u>TS</u> ,	46.1 mL of 0.2 mol/L sodium hydroxide <u>VS</u> ,

Benzyl Alcohol ベンジルアルコール

Page	Line	Correction	Error
433 left	↑ 31	the peak area of benzaldehyde obtained with the sample solution is not more than the <u>difference</u> between the peak areas of	the peak area of benzaldehyde obtained with the sample solution is not more than the <u>deference</u> between the peak areas of
433 left	↑ 28	the peak area of cyclohexylmethanol with the sample solution is not more than the <u>difference</u> between the peak areas of	the peak area of cyclohexylmethanol with the sample solution is not more than the <u>deference</u> between the peak areas of
433 right	↓ 10	the peak area of cyclohexylmethanol with the sample solution is not more than the <u>difference</u> between the peak areas of	the peak area of cyclohexylmethanol with the sample solution is not more than the <u>deference</u> between the peak areas of

Betamethasone Sodium Phosphate ベタメタゾンリン酸エステルナトリウム

Page	Line	Correction	Error
449 left	↓ 3	Determine the infrared absorption spectrum of Betamethasone Sodium Phosphate, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Betamethasone Sodium Phosphate RS	Determine the infrared absorption spectrum of Betamethasone Sodium Phosphate, <u>previously dried</u> , as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of <u>previously dried</u> Betamethasone Sodium Phosphate RS

Microcrystalline Cellulose 結晶セルロース

Page	Line	Correction	Error
601 left	↓ 4	the <u>difference</u> between these conductivities is	the <u>deference</u> between these conductivities is

Fursultiamine Hydrochloride フルスルチアミン塩酸塩

Page	Line	Correction	Error
874 left	↓ 11	calculated on the <u>anhydrous</u> basis.	calculated on the <u>dried</u> basis.

Glimepiride Tablets グリメピリド錠

Page	Line	Correction	Error
887 right	↑ 17	Dissolve 0.5 g of sodium dihydrogen phosphate <u>dihydrate</u> in 500 mL of water,	Dissolve 0.5 g of sodium dihydrogen phosphate <u>dehydrate</u> in 500 mL of water,

Iopamidol イオパミドール

Page	Line	Correction	Error
971 right	↑ 6	add 5 mL of 1 mol/L sulfuric acid <u>TS</u>	add 5 mL of 1 mol/L sulfuric acid

Limaprost Alfadex リマプロスト アルファデクス

Page	Line	Correction	Error
1037 left	↓ 15	Separately, weigh accurately about 3 mg of Limaprost RS, <u>dissolve in exactly 5 mL of the internal standard solution, add 5 mL of water,</u>	Separately, weigh accurately about 3 mg of Limaprost RS, <u>dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution,</u>

Sodium Bicarbonate 炭酸水素ナトリウム

Page	Line	Correction	Error
1393 left	↓ 23	35 mL of water and 1 drop of <u>ammonia</u> TS,	35 mL of water and 1 drop of <u>ammonium</u> TS,

Spiramycin Acetate スピラマイシン酢酸エステル

Page	Line	Correction	Error
1424 left	↓ 8	(3R,4S,5S,6R,8R,9R,10E,12E,15R)-3-Acetoxy-5-[4-O-acetyl-2,6-dideoxy-3-C-methyl- α -L-ribo-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino- β -D-glucopyranosyloxy]-9-(2,3,4,6-tetradecoxy-4-dimethylamino- β -D- <i>erythro</i> -hexopyranosyloxy)-6-formylmethyl-4-methoxy-8-methylhexadeca-10,12-dien-15-olide [87111-42-0]	(3R,4R,5S,6R,8R,9R,10E,12E,15R)-3-Acetoxy-5-[4-O-acetyl-2,6-dideoxy-3-C-methyl- α -L-ribo-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino- β -D-glucopyranosyloxy]-9-(2,3,4,6-tetradecoxy-4-dimethylamino- β -D- <i>erythro</i> -hexopyranosyloxy)-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide
1424 left	↓ 16	(3R,4S,5S,6R,8R,9R,10E,12E,15R)-5-[4-O-Acetyl-2,6-dideoxy-3-C-methyl- α -L-ribo-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino- β -D-glucopyranosyloxy]-9-(2,3,4,6-tetradecoxy-4-dimethylamino- β -D- <i>erythro</i> -hexopyranosyloxy)-6-formylmethyl-4-methoxy-8-methyl-3-propanoyloxyhexadeca-10,12-dien-15-olide [112501-15-2]	(3R,4R,5S,6R,8R,9R,10E,12E,15R)-5-[4-O-Acetyl-2,6-dideoxy-3-C-methyl- α -L-ribo-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino- β -D-glucopyranosyloxy]-9-(2,3,4,6-tetradecoxy-4-dimethylamino- β -D- <i>erythro</i> -hexopyranosyloxy)-6-formylmethyl-9-hydroxy-4-methoxy-8-methyl-3-propanoyloxyhexadeca-10,12-dien-15-olide [74014-51-0, Spiramycin Acetate]

Triclofos Sodium トリクロホスナトリウム

Page	Line	Correction	Error
1532 left	↑ 13	add 50 mL of molybdenum (VI) oxide-citric acid TS	add 50 mL of molybdenum (III) oxide-citric acid TS

Wood Creosote 木クレオソート

Page	Line	Correction	Error
1577 right	↓ 25	Wood Creosote contains not less than <u>23</u> %,	Wood Creosote contains not less than <u>23.0</u> %

Test for Trace Amounts of Aluminum in Total Parenteral Nutrition (TPN) Solutions 中心静脈栄養剤中の微量アルミニウム試験法

Page	Line	Correction	Error
2146 left	↑ 5	<u>Total</u> Parenteral Nutrition (TPN)	<u>Trans</u> Parenteral Nutrition (TPN)
2146 left	↑ 3	<u>Total</u> parenteral nutrition solutions (TPNs)	<u>Trans</u> parenteral nutrition solutions (TPNs)

Water to be used in the Tests of Drugs 医薬品等の試験に用いる水

Page	Line	Correction	Error
2253 right	↓ 20	Test for Trace Amounts of Aluminum in <u>Total</u> Parenteral Nutrition (TPN) Solutions	Test for Trace Amounts of Aluminum in <u>Trans</u> Parenteral Nutrition (TPN) Solutions

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March 25, 2014

9.41 Reagents, Test Solutions 試薬・試液

Page	Line	Correction	Error
167 left	↑ 27	0.05 g of <u>murexide</u> -sodium chloride indicator,	0.05 g of <u>mulexide</u> -sodium chloride indicator,
167 left	↑ 4	0.05 g of <u>murexide</u> -sodium chloride indicator	0.05 g of <u>mulexide</u> -sodium chloride indicator
197 right	↑ 23	<i>Purity</i> Related substances - Dissolve 25 mg of chenodeoxycholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 250 mL, <u>and use this solution as the sample solution. Perform the test with this solution as directed under the Thin-layer Chromatography<2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Dry the plate at 120 °C for 30 minutes, spray evenly a solution of phosphomolybdic acid <i>n</i>-hydrate in ethanol (95) (1 in 5) immediately, and heat at 120 °C for 2 or 3 minutes: any spot other than the principal spot at the <i>R_f</i> value of about 0.4 does not appear.</u>	<i>Purity</i> Related substances - Dissolve 25 mg of chenodeoxycholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 250 mL. <u>Perform the test with 10 μL of this solution as directed in the Purity (4) under Ursodeoxycholic Acid: any spot other than the principal spot at the <i>R_f</i> value of about 0.4 does not appear.</u>
199 right	↑ 20	<i>Purity</i> Related substances - Dissolve 25 mg of <u>cholic acid</u> for thin-layer chromatography in acetone to make exactly 250 mL and use this solution as the sample solution. Perform the test with this solution as directed under the <u>Thin-layer Chromatography<2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Dry the plate at 120 °C for 30 minutes, spray evenly a solution of phosphomolybdic acid <i>n</i>-hydrate in ethanol (95) (1 in 5) immediately, and heat at 120 °C for 2 or 3 minutes: any spot other than the principal spot, having <i>R_f</i> about 0.1, does not appear.</u>	<i>Purity</i> Related substances - Dissolve 25 mg in acetone to make exactly 250 mL. <u>Proceed with 10 μL of this solution as directed in the Purity (4) under Ursodeoxycholic Acid: any spot other than the principal spot, having <i>R_f</i> about 0.1, does not appear.</u>
205 right	↓ 12	Crystal violet $C_{25}H_{30}ClN_3 \cdot 9H_2O$	Crystal violet $C_{25}H_{30}CN_3 \cdot 9H_2O$
234 right	↓ 7	Hydrazine sulfate See <u>hydrazinium sulfate</u> . Hydrazinium sulfate $N_2H_6SO_4$ [K 8992, Special class]	Hydrazine sulfate See <u>hydrazinium sulfate</u> . Hydrazinium sulfate $N_4H_6SO_4$ [K 8992, Special class]
245 left	↓ 6	Kinin-releasing activity per mg (μg bradykinin equivalent/mg) = $B_K \times 0.96$	Kinin-releasing activity per mg (μg bradykinin equivalent/mg) = $B_K \times 0.0096$

Ofloxacin オフロキサシン

Page	Line	Correction	Error
1182 left	↓ 10	Not <u>more</u> than 0.2%	Not <u>less</u> than 0.2%

Pyridoxine Hydrochloride ピリドキシン塩酸塩

Page	Line	Correction	Error
1323 right	↑ 20	Spray evenly a solution of sodium carbonate in diluted ethanol (<u>99.5</u>) (3 in 10) (1 in 20)	Spray evenly a solution of sodium carbonate in diluted ethanol (3 in 10) (1 in 20)