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Traditional Chinese medicine — Determination of microorganisms in natural products

Médecine traditionnelle chinoise — Détermination des micro-organismes dans les produits naturels

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Foreword

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This document was prepared by Technical Committee ISO/TC 249, *Traditional Chinese medicine*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Natural products used in traditional Chinese medicine are widely used around the world due to their high medicinal values and mild side effects. It is a common phenomenon that natural products are contaminated by microorganisms which not only impact their quality and efficacy, but also restrict the international trade in them and related products. Although the Pharmacopeia of the People's Republic of China, the British Pharmacopoeia, the Japanese Pharmacopoeia, the European Pharmacopoeia and the United States Pharmacopeia have stipulated the microbial limits of natural products, there is no International Standard for microorganism detection methods, which adversely affects communication and trade between researchers and factories in different countries. Furthermore, microorganism levels on or in natural products usually exceed the maximum limit levels set by many international organizations and countries due to the lack of an International Standard.

Traditional Chinese medicine — Determination of microorganisms in natural products

1 Scope

This document specifies test methods to determine microorganisms in natural products. It is applicable only to natural products used in traditional Chinese medicine, including raw materials, herbal pieces and preparations.

2 Normative reference

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
sterility
state of being free from viable microorganisms

Note 1 to entry: In practice, no such absolute statement regarding the absence of microorganisms can be proven.

[SOURCE: ISO 11139:2018, 3.274]

3.2
microbial enumeration test
quantitative counting of mesophilic bacteria and fungi which may grow under aerobic conditions

4 Symbols and abbreviated terms

ATCC	American Type Culture Collection
CMCC	National Center for Medical Culture Collections
CIP	Collection de Bactéries de I'Institut Pasteur
IMI	International Mycological Institute
IP	Institut Pasteur
MPN	most-probable-number
NBRC	Biological Resource Center, National Institute of Technology and Evaluation
NCIMB	National Collection of Industrial and Marine Bacteria Ltd

NCPF	National Collection of Pathogenic Fungi
NCTC	National Collection of Type Cultures
TAMC	total aerobic microbial count
TYMC	total combined yeast and mould count

5 Test methods

5.1 General

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

5.2 Strains

The standardized stable suspensions of test strains as stated in Table 1 shall be used. Seed-lot culture maintenance techniques (seed-lot systems) shall be used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot.

Table 1 — Standard strains

Escherichia coli	ATCC 8739, NCIMB 8545, CIP 53.126 NBRC 3972 or CMCC (B) 44102
Pseudomonas aeruginosa	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275 or CMCC (B) 10104
Clostridium sporogenes	CMCC (B) 64941 or ATCC 11437 (NBRC 14293, NCIMB 12343, CIP 100651) or ATCC 19404 (NCTC 532 or CIP 79.03) or NBRC 14293
Staphylococcus Aureus	ATCC6538, NCIMB9518, CIP 4.83, NBRC 13276 or CMCC (B)26003
Bacillus subtilis	ATCC 6633, NCIMB 8054, CIP 52.62, NBRC 3134 or CMCC (B) 63501
Candida albicans	ATCC 10231, NCPF 3179, IP 48.72 or NBRC 1594 or CMCC (F) 98001
Aspergillus brasiliensis (Aspergillus niger)	ATCC 16404, IMI 149007, IP 1431.83 NBRC 9455 or CMCC (F) 98003
Salmonella paratyphi B	CMCC(B)50094, ATCC 14028 or, as an alternative, *Salmonella enteric* subsp. *enteric* serovar Abony such as NBRC 100797, NCTC 6017 or CIP 80.39
Shigella dysenteriae type 1	ATCC 11835, ATCC 9361, CMCC 51252

5.3 Test for sterility

5.3.1 General

The test is applied to raw materials, herbal pieces and preparations which are required to be sterile. However, a satisfactory result only indicates that no contaminating microorganism has been found in the sample examined under the conditions of the test. The acceptance criteria for microbiological quality of products to be tested shall be done according to A.1 in Annex A.

5.3.2 Culture media and incubation temperatures

Recommended media for the test may be prepared as shown in Annex B, or equivalent commercial media may be used provided that they conform to the growth promotion test.

The 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 can also detect aerobic bacteria. Soya-bean casein digest medium is suitable for the culture of both fungi and aerobic bacteria.

Fluid thioglycollate medium shall be incubated at 30 °C to 35 °C. For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycollate medium incubated at 20 °C to 25 °C may be used instead of soya-bean casein digest medium provided that it has been validated as described in the growth promotion test.

5.3.3 Growth promotion test for aerobes, anaerobes and fungi

Test each lot of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients. Suitable strains of microorganisms are indicated in Table 2.

Inoculate portions of fluid thioglycollate medium with a small number (not more than 100 cfu) of microorganisms, using a separate portion of medium for each of the following species of microorganism: *Clostridium sporogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Inoculate portions of alternative thioglycollate medium with a small number (not more than 100 cfu) of *Clostridium sporogenes*. Inoculate portions of soya-bean casein digest medium with a small number (not more than 100 cfu) of microorganisms, using a separate portion of medium for each of the following species of microorganism: *Aspergillus brasiliensis*, *Bacillus subtilis*, and *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 microorganisms used for inoculation are not more than five passages removed from the original master seed-lot.

Table 2 — Strains of the test microorganisms suitable for use in the growth promotion test and the method validation

Aerobic bacteria	*Staphylococcus aureus* ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276 or CMCC (B)26003
	Bacillus subtilis ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134 or CMCC (B) 63501
	Pseudomonas aeruginosa[a] ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275 or CMCC (B) 10104
Anaerobic bacterium	*Clostridium sporogenes*[b] ATCC 19404, CIP 79.3, NCTC 532, ATCC 11437, NBRC 14293 or CMCC (B) 64941
Fungi	*Candida albicans* ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594 or CMCC (F) 98001
	Aspergillus brasiliensis ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455 or CMCC (F) 98003

[a] An alternative microorganism in Aerobic bacteria is *Micrococcus luteus* (ATCC 9341).

[b] An alternative to *Clostridium sporogenes*, when a nonspore-forming microorganism is desired, is *Bacteroides vulgatus* (ATCC 8482).

5.3.4 Method suitability test

5.3.4.1 Membrane filtration

After transferring the contents of the container or containers to be tested to the membrane, add an inoculum of a small number of viable microorganisms (not more than 100 cfu) to the final portion of sterile diluent used to rinse the filter.

5.3.4.2 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 microorganisms (not more than 100 cfu) to the medium.

In both cases, use the same microorganisms as those described in 5.3.3. Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 5 days.

5.3.5 Test for sterility of the product to be examined

5.3.5.1 General

Unless otherwise specified elsewhere in this document or in the individual monograph, test the number of articles specified in Table 3. If the contents of each article are of sufficient quantity (see Table 3), they may be divided so that equal appropriate portions are added to each of the specified media. (Perform sterility testing employing two or more of the specified media.) If neither article contains sufficient quantities for each medium, use twice the number of articles indicated in Table 4.

The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture medium 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.

5.3.5.2 Membrane filtration

5.3.5.2.1 General

Use membrane filters having a nominal pore size not greater than 0,45 µm, in which the effectiveness to retain microorganisms has been established. Cellulose nitrate filters, for example, are used for aqueous, oily and weakly alcoholic solutions. Cellulose acetate filters, for example, are used for strongly alcoholic solutions. Specially adapted filters may be needed for certain products.

Sterility test 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 shall be 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.

5.3.5.2.2 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 diluents may contain suitable neutralizing substances, appropriate inactivating substances or both, 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 5.3.4 with the chosen sterile diluent; in any case, using not less than the quantities of the product to be examined prescribed in Table 3. 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 5.3.4. Do not exceed a washing cycle of five times 100 ml per filter, even if during the method suitability test 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.

5.3.5.2.3 Soluble solids

Use for each medium not less than the quantity prescribed in Table 3 of the product dissolved in a suitable solvent, such as the solvent provided with the preparation, water for injections, saline or a 1 g/l neutral solution of meat or casein peptone. Proceed with the test as described in 5.3.5.2.2 or aqueous solutions using a membrane appropriate to the chosen solvent.

5.3.5.2.4 Oils and oily solutions

Use for each medium not less than the quantity of the product prescribed in Table 3. 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 5.3.4, 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 in 5.3.5.2.2, and incubate at the same temperature for the same time.

5.3.5.2.5 Ointments and creams

Use for each medium not less than the quantities of the product prescribed in Table 3. Ointments in a fatty base and emulsions of the water-in-oil type may be diluted to 1 % in isopropyl myristate as described in 5.3.5.2.4 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 in 5.3.5.2.4.

5.3.5.2.6 Sterile aerosol products

For fluid products in pressurized aerosol form, freeze the containers in an alcohol-dry ice mixture of at least –20 °C for about 1 hour. If feasible, allow the propellant to escape before aseptically opening the container and transfer the contents to a sterile pooling vessel. Add 100 ml of fluid D (see Table B.2) to the pooling vessel and mix gently. Proceed as described in 5.3.5.2.2 or 5.3.5.2.4, whichever applies.

Table 3 — Minimum quantity to be used for each medium

Quantity per container	Minimum quantity to be used for each medium unless otherwise justified and authorized
Liquids	
— less than 1 ml	The entire contents of each container
— 1 ml to 40 ml	Half the contents of each container but not less than 1 ml
— greater than 40 ml and less than 100 ml	20 ml
— greater than 100 ml	10 % 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 entire 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 to 5 g	150 mg
Greater than 5 g	500 mg

Table 4 — 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 authorized
Parenteral preparations	
Not more than 100 containers	10 % or four containers, whichever is greater
More than 100 but not more than 500 containers	10 containers
More than 500 containers	2 % or 20 containers (10 containers for large-volume parenterals), whichever is less
Ophthalmic and other non-injectable	
Not more than 200 containers	5 % or two containers, whichever is greater
More than 200 containers	10 containers
If the product is presented in the form of single-dose containers, apply the scheme shown for preparations for parenteral use	
Bulk solid products	
Up to four containers	Each container
More than four containers but not more than 50 containers	20 % or four containers, whichever is greater
More than 50 containers	2 % or 10 containers, whichever is greater

5.3.5.3 Direct inoculation of the culture medium

5.3.5.3.1 General

Transfer the quantity of the preparation to be examined prescribed in Table 3 directly into the culture medium so that the volume of the product is not more than 10 % of the volume of the medium, unless otherwise prescribed.

If the product to be examined has antimicrobial activity, carry out the test after neutralizing this with a suitable neutralizing 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.

5.3.5.3.2 Oily liquids

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

5.3.5.3.3 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 microorganisms, keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

5.3.5.4 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.

The validity of the test is established according to 6.1.4. If the test is declared to be invalid, it is repeated with the same number of units as in the original test.

5.3.5.5 Minimum number of items to be tested

The minimum number of items to be tested in relation to the size of the batch is given in Table 4. If the batch size is unknown, use the maximum number of items prescribed. If the contents of one container are enough to inoculate the two media, this column gives the number of containers needed for both the media together.

5.4 Microbiological examination of non-sterile products: microbial enumeration tests

5.4.1 General

The test is applied to determinate whether the non-sterile products involving raw materials, herbal pieces and preparations conformed to the standard or not.

If the product to be examined has antimicrobial activity, this is as far as possible removed or neutralized. If neutralizers or inactivators are used in tests, their efficacy and their absence of toxicity for microorganisms shall be demonstrated. If surfactants are used for sample preparation, their absence of toxicity for microorganisms and their compatibility with any neutralizers or inactivators used shall be demonstrated.

The enumeration methods involve membrane filtration, the plate count method and the most-probable-number (MPN) method. Use the membrane filtration method or one of the plate-count methods, as directed. The MPN method is generally the least-accurate method for microbial counts; however, for certain product groups with very low bioburden it might 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 microorganisms. The method chosen shall allow testing of a sufficient sample size to judge conformity with the specification. The suitability of the chosen method shall be established.

The acceptance criteria for microbiological quality of products to be tested shall be done according to A.2 (Table A.1 to Table A.15) in Annex A.

5.4.2 Growth promotion test, suitability of the counting method and negative controls

5.4.2.1 General

The ability of the test to detect microorganisms in the product to be tested shall be established. Suitability shall be confirmed if a change in testing performance or a change in the product that may affect the outcome of the test is introduced.

5.4.2.2 Preparation of test strains

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

Table 5 — Preparation and use of test microorganisms

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

Use buffered sodium chloride–peptone solution pH 7,0 (see Table B.3) or phosphate buffer solution pH 7,2 (see Table B.1) to make test suspensions; to suspend *Aspergillus brasiliensis* spores, 0,05 % of polysorbate 80 may be added to the buffer. Use the suspensions within 2 hours, or within 24 hours if stored between 2 °C and 8 °C. As an alternative to preparing and then diluting a fresh suspension of vegetative cells of *Aspergillus brasiliensis* or *B. 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 °C to 8 °C for a validated period of time.

The preparation of media in Table 5 are shown in Table B.4 to Table B.8.

5.4.2.3 Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There shall be no growth of microorganisms. A negative control is also performed when testing the products as described in 5.4.3. A failed negative control requires an investigation.

5.4.2.4 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 or plates of soya-bean casein digest broth (see Table B.4) and soya-bean casein digest agar (see Table B.5) with a small number (not more than 100 cfu) of the microorganisms indicated in Table 5, using a separate portion or plate of medium for each. Inoculate plates of Sabouraud dextrose agar with a small number (not more than 100 cfu) of the microorganisms indicated in Table 5, using a separate plate of medium for each. Incubate according to the conditions described in Table 5.

For solid media, growth obtained shall not differ by a factor greater than two from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the microorganisms 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 microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

5.4.2.5 Suitability of the counting method

5.4.2.5.1 Preparation of the sample

The method for sample preparation depends on the physical characteristics of the product to be tested. If none of the following procedures can be demonstrated to be satisfactory, a suitable alternative procedure shall be developed.

1) 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 (see Table B.3), phosphate buffer solution pH 7,2 (see Table B.1) or soya-bean casein digest broth (see Table B.4). If necessary, adjust to a pH of 6 to 8. Further dilutions, where necessary, are prepared with the same diluent.

2) 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 (see Table B.3), phosphate buffer solution pH 7,2 (see Table B.1) or soya-bean casein digest broth (see Table B.4). A surface-active agent such as 1 g/l of polysorbate 80 may be added to assist the suspension of poorly wet table substances. If necessary, adjust to a pH of 6 to 8. Further dilutions, where necessary, are prepared with the same diluent.

3) 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 a sufficient quantity of the prewarmed chosen diluent to make a 1 in 10 dilution of the original product. Mix carefully, while 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.

4) 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.

5) Transdermal patches: remove the protective cover sheets (“release liners”) of the transdermal patches and place them, adhesive side upwards, on sterile glass or plastic trays. Cover the adhesive

surface with a suitable sterile porous material (e.g. 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 or lecithin. Shake the preparation vigorously for at least 30 min.

5.4.2.5.2 Inoculation and dilution

Add to the sample prepared as described in 5.4.2.5.1 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 % of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution factor of the prepared sample shall be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols shall 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.

5.4.2.5.3 Neutralization or removal of antimicrobial

The number of microorganisms recovered from the prepared sample, diluted as described in 5.4.2.5.2 and incubated following the procedure described in 5.4.2.5.4, is compared to the number of microorganisms recovered from the control preparation.

If growth is inhibited (reduction by a factor greater than two), 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;

4) a combination of measures 1) to 3).

Neutralizing agents may be used to neutralize the activity of antimicrobial agents (see Table 6). They may be added to the chosen diluent or the medium, preferably before sterilization. If used, their efficacy and their absence of toxicity for microorganisms shall be demonstrated by carrying out a blank with neutralizer and without product.

Table 6 — Common neutralizing agents for interfering substances

Interfering substance	Potential neutralizing method
Glutaraldehyde, mercurials	Sodium hydrogensulfite (sodium bisulfite)
Phenolics, alcohol, aldehydes, sorbate	Dilution
Aldehydes	Glycine
Quaternary Ammonium Compounds (QACs), parahydroxybenzoates (parabens), bis-biguanide	Lecithin
QACs, iodine, parabens	Polysorbate
Mercurials	Thioglycollate
Mercurials, halogens, aldehydes	Thiosulfate
EDTA (edetate)	Mg^{2+} or Ca^{2+} ions

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 microorganism.

However, it is possible that the product inhibits only some of the microorganisms specified herein, but does not inhibit others not included among the test strains or those for which the latter are not

representative. In that case, perform the test with the highest dilution factor compatible with microbial growth and the specific acceptance criterion.

5.4.2.5.4 Recovery of microorganisms

5.4.2.5.4.1 General

For each of the microorganisms listed in Table 5, separate tests are performed. Only microorganisms of the added test strain are counted.

5.4.2.5.4.2 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 microorganisms listed, one membrane filter is used.

Transfer a suitable quantity of the sample prepared as described in 5.4.2.5.1, 5.4.2.5.2 and 5.4.2.5.3 (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 the soya-bean casein digest agar (see Table B.5). For the determination of total combined yeast and mould count (TYMC), transfer the membrane to the surface of the Sabouraud's dextrose agar (see Table B.6). Incubate the plates as indicated in Table 5. Perform the counting.

5.4.2.5.4.3 Plate-count methods

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

1) Pour-plate method: for petri dishes 9 cm in diameter, add to the dish 1 ml of the sample prepared as described in 5.4.2.5.1, 5.4.2.5.2 and 5.4.2.5.3 and 15 ml to 20 ml of soya-bean casein digest agar (see Table B.5) or Sabouraud's dextrose agar (see Table B.6), both media maintained at not more than 45 °C. If larger petri dishes are used, the amount of agar medium is increased accordingly. For each of the microorganisms listed in Table 5, at least two petri dishes are used.

Incubate the plates as indicated in Table 5. Take the arithmetic mean of the counts per medium and calculate the number of cfu in the original inoculum.

2) Surface-spread method: for petri dishes 9 cm in diameter, add 15 ml to 20 ml of soya-bean casein digest agar (see Table B.5) or Sabouraud's dextrose agar (see Table B.6) at about 45 °C to each petri dish and solidify. If larger petri dishes are used, the volume of the agar is increased accordingly. Dry the plates, for example in a laminar-airflow cabinet or in an incubator. For each of the microorganisms listed in Table 4, at least two petri dishes are used. Spread a measured volume of not less than 0,1 ml of the sample, prepared as described in 5.4.2.5.1, 5.4.2.5.2 and 5.4.2.5.3. Incubate and count as directed for the pour-plate method.

5.4.2.5.4.4 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 three serial tenfold dilutions of the product as described in 5.4.2.5.1, 5.4.2.5.2 and 5.4.2.5.3. From each level of dilution, three aliquots of 1 g or 1 ml are used to inoculate three tubes with 9 ml to 10 ml of soya-bean casein digest broth (see Table B.4). If necessary, a surface-active agent such as polysorbate 80 or an inactivator of antimicrobial agents may be added to the medium. Thus, if three levels of dilution are prepared, nine tubes are inoculated.

Incubate all tubes at 30 °C to 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 in soya-bean casein digest agar (see Table B.5) for 1 to 2 days at the same temperature and use these results. From Table 7, determine the most probable number of microorganisms per g or ml of the product to be examined.

Table 7 — MPN values of microorganisms

Observed combinations of numbers of tubes g or ml of product per tube			**MPN per g or per ml of product**	**95 % confidence limits**
0,1	0,01	0,001		
0	0	0	< 3	0 to 9,4
0	0	1	3	0,1 to 9,5
0	1	0	3	0,1 to 10
0	1	1	6,1	1,2 to 17
0	2	0	6,2	1,2 to 17
0	3	0	9,4	3,5 to 35
1	0	0	3,6	0,2 to 17
1	0	1	7,2	1,2 to 17
1	0	2	11	4 to 35
1	1	0	7,4	1,3 to 20
1	1	1	11	4 to 35
1	2	0	11	4 to 35
1	2	1	15	5 to 38
1	3	0	16	5 to 38
2	0	0	9,2	1,5 to 35
2	0	1	14	4 to 35
2	0	2	20	5 to 38
2	1	0	15	4 to 38
2	1	1	20	5 to 38
2	1	2	27	9 to 94
2	2	0	21	5 to 40
2	2	1	28	9 to 94
2	2	2	35	9 to 94
2	3	0	29	9 to 94
2	3	1	36	9 to 94
3	0	0	23	5 to 94
3	0	1	38	9 to 104
3	0	2	64	16 to 181
3	1	0	43	9 to 181
3	1	1	75	17 to 199
3	1	2	120	30 to 360
3	1	3	160	30 to 380
3	2	0	93	18 to 360
3	2	1	150	30 to 380
3	2	2	210	30 to 400
3	2	3	290	90 to 990
3	3	0	240	40 to 990

Table 7 *(continued)*

Observed combinations of numbers of tubes g or ml of product per tube			MPN per g or per ml of product	95 % confidence limits
3	3	1	460	90 to 1 980
3	3	2	1 100	200 to 4 000
3	3	3	> 1 100	

5.4.3 Testing of products

5.4.3.1 Test sample

Unless otherwise directed, use 10 g or 10 ml of the product to be examined taken with the precautions referred to in 5.4.2.5.3. 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 are 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 g or ml (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 the sample quantity is limited or batch size is extremely small (i.e. less than 1 000 ml or 1 000 g), the amount tested shall be 1 % of the batch unless a lesser amount is prescribed or justified and authorized.

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 two units, or one 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.

5.4.3.2 Examination of the product

5.4.3.2.1 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 to be suitable as described in 5.4.2.4 and 5.4.2.5, transfer the appropriate amount to each of two 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 soya-bean casein digest agar. For the determination of TYMC, transfer the other membrane to the surface of Sabouraud's dextrose agar (see Table B.6). Incubate the plate of soya-bean casein digest agar (see Table B.5) at 30 °C to 35 °C for 3 days to 5 days and the plate of Sabouraud's dextrose agar (see Table B.6) at 20 °C to 25 °C for 5 days to 7 days. Calculate the number of cfu per g or per ml of product.

When examining transdermal patches, separately filter 10 % of the volume of the preparation described in 5.4.2.5.1 through each of two sterile filter membranes. Transfer one membrane to soya-bean casein digest agar (see Table B.5) for TAMC and the other membrane to Sabouraud's dextrose agar (see Table B.6) for TYMC.

5.4.3.2.2 Plate-count methods

5.4.3.2.2.1 Pour-plate method

Prepare the sample using a method that has been shown to be suitable as described in 5.4.2.4 and 5.4.2.5. Prepare for each medium at least two Petri dishes for each level of dilution. Incubate the plates of soya-bean casein digest agar (see Table B.5) at 30 °C to 35 °C for 3 days to 5 days and the plates of Sabouraud's dextrose agar (see Table B.6) at 20 °C to 25 °C for 5 days to 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 g or per ml of product.

5.4.3.2.2.2 Surface-spread method

Prepare the sample using a method that has been shown to be suitable as described in 5.4.2.4 and 5.4.2.5. Prepare at least two petri dishes for each medium and each level of dilution. For incubation and calculation of the number of cfu, proceed as directed for the pour-plate method.

5.4.3.2.3 MPN method

Prepare and dilute the sample using a method that has been shown to be suitable as described in 5.2.2.4 and 5.2.2.5. Incubate all tubes for 3 days to 5 days at 30 °C to 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 microorganisms per g or ml of the product to be examined from Table 7.

5.4.4 Interpretation of the results

The TAMC is considered to be equal to the number of cfu found using soya-bean casein digest agar (see Table B.5); if colonies of fungi are detected on this medium, they are counted as part of TAMC. The TYMC is considered to be equal to the number of cfu found using Sabouraud's dextrose agar (see Table B.6); 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's dextrose agar (see Table B.6) containing antibiotics may be used. If the count is carried out by the MPN method, the calculated value is TAMC.

The recommended solutions and media are described in Annex B.

5.5 Microbiological examination of non-sterile products: tests for specified microorganisms

5.5.1 General

The test is applied to determinate whether the non-sterile products involving raw materials, herbal pieces and preparations conform to the standard's microbial limit or not.

The requirements of neutralizers, inactivators and surfactants shall be harmonized with those in 5.4.1 and preparation of the sample shall be carried out as described in 5.4.

The acceptance criteria for microbiological quality of products to be tested shall be done according to A.2 (Table A.1 to Table A.15) in Annex A.

5.5.2 Growth-promoting and inhibitory properties of the media, suitability of the test and negative controls

5.5.2.1 General

The ability of the test to detect microorganisms in the product to be tested shall be established. Suitability shall be confirmed if a change in testing performance or a change in the product that may affect the outcome of the test is introduced.

5.5.2.2 Preparation of test strains

5.5.2.2.1 General

Use standardized stable suspensions of test strains as stated in 5.5.2.2.2. Seed-lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot.

5.5.2.2.2 Aerobic microorganisms

Aerobic strains for test are stated in Table 8. Grow each of the bacterial test strains separately in containers containing soya-bean casein digest broth or on soya-bean casein digest agar (see Table B.5) at 30 °C to 35 °C for 18 h to 24 h. Grow the test strain for *Candida albicans* separately on Sabouraud's dextrose agar (see Table B.6) or in Sabouraud's dextrose broth (see Table B.8) at 20 °C to 25 °C for 2 days to 3 days.

Table 8 — Aerobic strains for test

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	
Salmonella enteric subsp. *enterica* serovar Typhimurium	such as ATCC 14028
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 (see Table B.3) or phosphate buffer solution pH 7,2 (see Table B.1) to make test suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2 °C to 8 °C.

5.5.2.2.3 *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 (see Table B.17) for *Clostridia* at 30 °C to 35 °C for 24 h to 48 h. As an alternative to preparing and then diluting down a fresh suspension of vegetative cells of *Clostridia sporogenes*, a stable spore suspension is used for test inoculation. The stable spore suspension may be maintained at 2 °C to 8 °C for a validated period.

5.5.2.3 Negative control

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There shall be no growth of microorganisms. A negative control is also performed when testing the products as described in 5.5.3. A failed negative control requires an investigation.

5.5.2.4 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 9.

Table 9 — Growth promoting, inhibitory and indicative properties of media

Medium	Property	Test strains
Test for bile-tolerant gram-negative bacteria		
Enterobacteria enrichment broth mossel	Growth-promoting	*Escherichia coli* *Pseudomonas aeruginosa*
	Inhibitory	*Staphylococcus aureus*
Violet red bile glucose agar	Growth-promoting + indicative	*Escherichia coli* *Pseudomonas aeruginosa*
Test for *Escherichia coli*		
MacConkey broth	Growth-promoting	*Escherichia coli*
	Inhibitory	*Staphylococcus aureus*
MacConkey agar	Growth-promoting + indicative	*Escherichia coli*
Test for *Salmonella*		
Rappaport vassiliadis *Salmonella* enrichment broth	Growth-promoting	*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* or *Salmonella enterica* subsp. *enterica* serovar Abony
	Inhibitory	*Staphylococcus aureus*
Xylose, lysine, deoxycholate agar	Growth-promoting + indicative	*Salmonella enterica* subsp. *enteric* serovar *Typhimurium* or *Salmonella enterica* subsp. *enteric* serovar Abony
Test for *Pseudomonas aeruginosa*		
Cetrimide agar	Growth-promoting	*Pseudomonas aeruginosa*
	Inhibitory	*Escherichia coli*
Test for *Staphylococcus aureus*		
Mannitol salt agar	Growth-promoting + indicative	*Staphylococcus aureus*
	Inhibitory	*Escherichia coli*
Test for *Clostridia*		
Reinforced medium for *Clostridia*	Growth-promoting	*Clostridia sporogenes*
Columbia agar	Growth-promoting	*Clostridia sporogenes*
Test for *Candida albicans*		
Sabouraud's dextrose broth	Growth-promoting	*Candida. albicans*
Sabouraud's dextrose agar	Growth-promoting + indicative	*Candida. albicans*

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 microorganism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Clearly visible growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for growth-promoting properties, solid media: perform the surface-spread method (see 5.4.2), inoculating each plate with a small number (not more than 100 cfu) of the appropriate microorganism. Incubate at the specified temperature for not more than the shortest period of time specified in the test. Growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for inhibitory properties, liquid or solid media: inoculate the appropriate medium with at least 100 cfu of the appropriate microorganism. Incubate at the specified temperature for not less than the longest period of time specified in the test. No growth of the test microorganism occurs.

Test for indicative properties: perform the surface-spread method (see 5.4.2), inoculating each plate with a small number (not more than 100 cfu) of the appropriate microorganism. 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.

The preparation of media in Table 9 shall be done in accordance with Table B.6, and Table B.8 to Table B.18.

5.5.2.5 Suitability of the test method

For each new product to be tested, perform sample preparation as described in the relevant paragraph under 5.5.2. At the time of mixing, add each test strain in the prescribed growth medium.

Inoculate the test strains individually. The number of microorganisms is equivalent to not more than 100 cfu in the inoculated test preparation.

Perform the test as described in the relevant paragraph in 5.5.2 using the shortest incubation period prescribed.

The specified microorganisms shall be detected with the indication reactions as described in 5.5.2.

Any antimicrobial activity of the product necessitates a modification of the test procedure as described in 5.4.2.5.3.

For a given product, if the antimicrobial activity with respect to a microorganism for which testing is prescribed cannot be neutralized, then it shall be assumed that the inhibited microorganism will not be present in the product.

5.5.3 Testing of products

5.5.3.1 Bile-tolerant gram-negative bacteria

5.5.3.1.1 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 5.4.2.5.1, but using soya-bean casein digest broth (see Table B.4) as the chosen diluent, mix and incubate at 20 °C to 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).

5.5.3.1.2 Test for absence

Unless otherwise prescribed, use the volume corresponding to 1 g of the product, as prepared in 5.5.3.1.1 to inoculate enterobacteria enrichment broth-mossel (see Table B.9). Incubate at 30 °C to 35 °C for 24 h to 48 h. Subculture on plates of violet red bile glucose agar (see Table B.10).

Incubate at 30 °C to 35 °C for 18 h to 24 h. The product conforms with the test if there is no growth of colonies.

5.5.3.1.3 Quantitative test

Selection and subculture: inoculate suitable quantities of enterobacteria enrichment broth-mossell (see Table B.9) with the preparation as described in 5.5.3.1.1 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 °C to 35 °C for 24 h to 48 h. Subculture each of the cultures on a plate of violet red bile glucose agar (see Table B.10). Incubate at 30 °C to 35 °C for 18 h to 24 h.

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 determined from Table 10.

Table 10 — The probable number of bacterial

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 103
+	+	-	less than 103 and more than 102
+	-	-	less than 102 and more than 10
-	-	-	less than 10

5.5.3.2 *Escherichia coli*

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 5.4.2.5.1 and use 10 ml or the quantity corresponding to 1 g or 1 ml to inoculate a suitable amount (determined as described in 5.5.2.5) of soya-bean casein digest broth (see Table B.4), mix and incubate at 30 °C to 35 °C for 18 h to 24 h.

Selection and subculture: shake the container, transfer 1 ml of soya-bean casein digest broth (see Table B.4) to 100 ml of macconkey broth (see Table B.11) and incubate at 42 °C to 44 °C for 24 h to 48 h. Subculture on a plate of macconkey agar (see Table B.12) at 30 °C to 35 °C for 18 h to 72 h.

Interpretation: growth of colonies indicates the possible presence of *Escherichia coli*. This is confirmed by identification tests. The product conforms with the test if no colonies are present or if the identification tests are negative.

5.5.3.3 *Salmonella*

Sample preparation and pre-incubation: prepare the product to be examined as described in 5.4.2.5.1, and use the quantity corresponding to not less than 10 g or 10 ml to inoculate a suitable amount (determined as described in 5.5.2.5) of soya-bean casein digest broth (see Table B.4), mix and incubate at 30 °C to 35 °C for 18 h to 24 h.

Selection and subculture: transfer 0,1 ml of soya-bean casein digest broth (see Table B.4) to 10 ml of rappaport vassiliadis salmonella enrichment broth (see Table B.13) and incubate at 30 °C to 35 °C for 18 h to 24 h. Subculture on plates of xylose- lysine-deoxycholate agar (see Table B.14). Incubate at 30 °C to 35 °C for 18 h to 48 h.

Interpretation: the possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centres. This is confirmed by identification tests. The product conforms with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

5.5.3.4 *Pseudomonas aeruginosa*

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 5.4.2.5.1, and use 10 ml or the quantity corresponding

to 1 g or 1 ml to inoculate a suitable amount (determined as described in 5.5.2.5) of soya-bean casein digest broth (see Table B.4), and mix. When testing transdermal patches, filter the volume of sample corresponding to one patch of the preparation (see 5.4.2.5.1) through a sterile filter membrane, and place in 100 ml of soya-bean casein digest broth (see Table B.4). Incubate at 30 °C to 35 °C for 18 h to 24 h.

Selection and subculture: subculture on a plate of cetrimide agar (see Table B.15) and incubate at 30 °C to 35 °C for 18 h to 72 h.

Interpretation: growth of colonies indicates the possible presence of *Pseudomonas aeruginosa*. This is confirmed by identification tests. The product conforms with the test if colonies are not present or if the confirmatory identification tests are negative.

5.5.3.5 *Staphylococcus aureus*

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 5.4.2.5.1, and use 10 ml or the quantity corresponding to 1 g or 1 ml to inoculate a suitable amount (determined as described in 5.5.2.5) of soya-bean casein digest broth (see Table B.4), and homogenize. When testing transdermal patches, filter the volume of sample corresponding to one patch of the preparation (see 5.4.2.5.1) through a sterile filter membrane, and place in 100 ml of soya-bean casein digest broth (see Table B.4). Incubate at 30 °C to 35 °C for 18 h to 24 h.

Selection and subculture: subculture on mannitol salt agar (see Table B.16) and incubate at 30 °C to 35 °C for 18 h to 72 h.

Interpretation: the possible presence of *Staphylococcus aureus* is indicated by the growth of yellow or white colonies surrounded by a yellow zone. This is confirmed by identification tests. The product conforms with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

5.5.3.6 *Clostridia*

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 5.4.2.5.1. Divide the sample into two portions of at least 10 ml. Heat one portion at 80 °C for 10 min and cool rapidly. Do not heat the other portion.

Selection and subculture: each portion of product should be examined in a different plate. 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 5.5.2.5) of reinforced medium (see Table B.17) for *Clostridia*. Incubate under anaerobic conditions at 30 °C to 35 °C for 48 hours. After incubation, make subcultures from each container on columbia agar (see Table B.18) and incubate under anaerobic conditions at 30 °C to 35 °C for 48 h to 72 h.

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

5.5.3.7 *Candida albicans*

Sample preparation and pre-incubation: prepare the product to be examined as described in 5.4.2.5.1, and use 10 ml or the quantity corresponding to not less than 1 g or 1 ml to inoculate 100 ml of Sabouraud's dextrose broth (see Table B.8), and mix. Incubate at 30 °C to 35 °C for 3 days to 5 days.

Selection and subculture: subculture on a plate of Sabouraud's dextrose agar (see Table B.6) and incubate at 30 °C to 35 °C for 24 h to 48 h.

Interpretation: growth of white colonies may indicate the presence of *Candida albicans.* This is confirmed by identification tests. The product conforms with the test if such colonies are not present or if the confirmatory identification tests are negative.

5.5.3.8 *Shigella dysenteriae*

5.5.3.8.1 Direct inoculation of agar plates

Use two or three loopfuls of the herbal materials, preparations or products to be tested. Incubate plates at 35 °C to 37 °C for 18 h to 24 h.

Inoculate a general-purpose plating medium of low selectivity and one of moderate or high selectivity. Macconkey agar (see Table B.12) is recommended as a medium of low selectivity. Macconkey agar (see Table B.12) with 1 µg/ml of potassium tellurite has been reported to be particularly useful for *S. dysenteriae* type 1 (Sd1). Use a small inoculum. Incubate at 35 °C to 37 °C for 18 h to 24 h.

Xylose-lysine-desoxycholate (XLD) agar is recommended as a medium of moderate or high selectivity for isolation of *Shigella*. Desoxycholate citrate agar (DCA) is a suitable alternative. Do not use salmonella–shigella (SS) agar, as it often inhibits growth of Sd1.

Each new batch of medium should be controlled for quality before routine use by inoculating it with known reference strains and observing their growth and colony characteristics.

5.5.3.8.2 Identification of colonies on plating media

Colonies suspicious for *Shigella* appear as follows:

— Macconkey agar (see Table B.12): convex, colourless, 2 mm to 3 mm;

— XLD agar: red, smooth, 1 mm to 2 mm;

— DCA agar: colourless, translucent, 2 mm to 3 mm.

Identify well-separated colonies of typical appearance to be transferred from each of the plating media for further testing by making a mark on the bottom of the petri dish.

Whenever possible, a person experienced in the identification of *Shigella* should train laboratory workers who are unfamiliar with its identification.

5.5.3.8.3 Inoculation of Kligler iron agar

Pick three characteristic colonies from the plating media and inoculate into Kligler iron agar (KIA) as follows: stab the butt and then streak the slant with a zigzag configuration. Pay attention to proper labelling of the tubes. If screw-cap KIA tubes are used, make sure that the caps are loose. Incubate overnight. The following morning, examine the reactions in the KIA tubes. Tubes with possible signs of *Shigella* will have an acid (yellow) butt and an alkaline (red) slant. They will not produce gas (no bubbles or cracks in the agar) and will not produce hydrogen sulfide (no black along the stab line).

Triple sugar iron agar (TSI) can also be used for the identification of *Shigella*. It gives the same reactions as KIA.

6 Acceptance criterion of test methods

6.1 Acceptance criterion of test for sterility

6.1.1 Acceptance criterion of sterility in test for culture medium

Incubate portions of the media for 14 days. No growth of microorganisms occurs.

6.1.2 Acceptance criterion of growth promotion test of aerobes, anaerobes and fungi

Growth promotion tests shall be carried out according to 5.3.3, and the media are suitable if a clearly visible growth of the microorganisms occurs.

6.1.3 Acceptance criterion of method suitability test

If clearly visible growth of microorganisms 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 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.

6.1.4 Acceptance criterion of test for sterility

If no evidence of microbial growth is found, the product to be examined conforms with the test for sterility. If evidence of microbial growth is found, the product to be examined does not conform 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 is 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 microorganisms isolated from the test, the growth of this species or these species may be ascribed unequivocally to faults with respect to the material, the technique used in conducting the sterility test procedure or both.

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 conforms with the test for sterility. If microbial growth is found in the repeat test, the product examined does not conform with the test for sterility.

6.2 Acceptance criterion of microbial enumeration tests in microbiological examination of non-sterile products

6.2.1 Acceptance criterion of preparation of test strains in microbiological examination of non-sterile products: microbial enumeration tests

Test strains shall be prepared according to 5.4, and the test strains are those recommended in Table 5. Seed-lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot. The solution in 5.4.2.2 shall be sterile and ensure the standardized stable suspensions are alive.

If the preparation of test strains satisfies the standard in the previous paragraph, it is qualified and none of the standard can be ignored.

6.2.2 Acceptance criterion of negative control in microbiological examination of non-sterile products

Subclause 5.4.2.3 requires the use of the sterile solution as negative control. There shall be no microorganism in the negative control, otherwise the negative control is unavailable.

A negative control is also performed when testing the products as described in 5.4.3. A failed negative control requires an investigation.

6.2.3 Acceptance criterion of media suitability in microbiological examination of non-sterile products

For solid media, growth obtained shall not differ by a factor greater than two from the calculated value for a standardized inoculum. For a freshly prepared inoculum, growth of the microorganisms 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 microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

6.2.4 Acceptance criterion of the method suitability in microbiological examination of non-sterile products

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 two from the value of the control defined in 5.4.2.5.2 in the absence of product shall be obtained. When verifying the suitability of the MPN method, the calculated value from the inoculum shall be within 95 % confidence limits of the results obtained with the control.

6.2.5 Acceptance criterion of the validity of the results in microbiological examination of non-sterile products

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There shall be no growth of microorganisms. A negative control is also performed when testing the products as described in 5.4.3. A failed negative control requires an investigation.

6.3 Acceptance criterion of tests for specified microorganisms in microbiological examination of non-sterile products

6.3.1 Acceptance criterion of preparation of test strains in microbiological examination of non-sterile products: tests for specified microorganisms

The test strains are recommended in Table 9 in 5.5.2.4. Seed-lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot. The solution in Table 9 shall be sterile, and ensure that the standardized stable suspensions are alive.

If the preparation of test strains satisfies the standard in the previous paragraph, it is qualified.

6.3.2 Acceptance criterion of negative control in microbiological examination of non-sterile products: tests for specified microorganisms

Subclause 5.5.2.3 requires the sterile solution to be used as negative control. There shall be no microorganism in the negative control, otherwise the negative control is unavailable.

A negative control is also performed when testing the products as described in 5.5.3. A failed negative control requires an investigation.

6.3.3 Acceptance criterion of media suitability in microbiological examination of non-sterile products: tests for specified microorganisms

Test for growth-promoting properties, liquid media: clearly visible growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for growth-promoting properties, solid media: growth of the microorganism comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Test for inhibitory properties, liquid or solid media: no growth of the test microorganism occurs.

Test for indicative properties: colonies are comparable in appearance and indication reactions to those previously obtained with a previously tested and approved batch of medium.

6.3.4 Acceptance criterion of the method suitability in microbiological examination of non-sterile products: tests for specified microorganism

The specified microorganisms shall be detected with the indication reactions as described in 5.5.3, or the test is unavailable.

6.3.5 Acceptance criterion of the validity of the results in microbiological examination of non-sterile products

To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There shall be no growth of microorganisms. A negative control is also performed when testing the products as described in 5.5.3. A failed negative control requires an investigation.

Annex A
(normative)

Microbiological quality of natural products

A.1 Acceptance criteria of sterile natural products

Carry out the test for sterility in 5.3 to determine the natural products; if the result is valid, and the negative control and the production are without any microorganisms, the production conforms with this document.

A.2 Acceptance criteria for non-sterile natural products

A.2.1 General

Microbial examination of non-sterile products is performed according to the methods given in 5.4 and 5.5. Acceptance criteria are based on individual results or on the average of replicate counts when replicate counts are performed (e.g. direct plating methods). When an acceptance criterion for microbiological quality is prescribed, it is interpreted as follows:

10^1 cfu: maximum acceptable count = 20;

10^2 cfu: maximum acceptable count = 200;

10^3 cfu: maximum acceptable count = 2 000; and so forth.

The list is not necessarily exhaustive, and for a given preparation it may be necessary to test for other microorganisms depending on the nature of the starting materials and the manufacturing process. If it has been shown that none of the prescribed tests allow valid enumeration of microorganisms at the level prescribed, a validated method with a limit of detection as close as possible to the indicated acceptance criterion is used.

In addition to the microorganisms listed, the significance of other microorganisms recovered should be evaluated in terms of the following:

1) use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract);
2) nature of the product: its ability to support growth, the presence of adequate antimicrobial preservation;
3) method of application;
4) intended recipient: risk may differ from neonates, infants, the debilitated;
5) use of immunosuppressive agents, corticosteroids;
6) the presence of disease, wounds, organ damage.

Where warranted, a risk-based assessment of the relevant factors is conducted by personnel with specialized training in microbiology and interpretation of microbiological data. For raw materials, the assessment takes account of processing to which the product is subjected, the current technology of testing and the availability of materials of the desired quality.

A.2.2 The national and organizational acceptance criteria of microorganisms in natural products

Table A.1 — Acceptance criteria for herbal medicinal products in the British Pharmacopoeia

TAMC	Acceptance criterion: 10^7 cfu/g Maximum acceptable count: 50 000 000 cfu/g
TYMC	Acceptance criterion: 10^5 cfu/g Maximum acceptable count: 500 000 cfu/g
Escherichia coli	Acceptance criterion: 10^3 cfu/g
Salmonella	Absence (25 g)
Shigella	Absence (1 g)
NOTE Herbal medicinal products containing herbal drugs, with or without excipients, intended for the preparation of infusions and decoctions using boiling water (e.g. herbal teas, with or without added flavourings).	

Table A.2 — Acceptance criteria for herbal medicinal products in the British Pharmacopoeia

TAMC	Acceptance criterion: 10^4 cfu/g or cfu/ml Maximum acceptable count: 50 000cfu/g or cfu/ml
TYMC	Acceptance criterion: 10^2 cfu/g or cfu/ml Maximum acceptable count: 500 cfu/g or cfu/ml
Bile-tolerant gram-negative bacteria	Acceptance criterion: 10^2 cfu/g or cfu/ml
Escherichia coli	Acceptance criterion: Absence 1 g or 1 ml
Shigella	Absence (1 g)
Salmonella	Absence (25 g or 25 ml)
NOTE Herbal medicinal products containing, for example, extracts or herbal drugs, with or without excipients, where the method of processing (e.g. extraction) or, where appropriate, in the case of herbal drugs, of pre-treatment, reduces the levels of organisms to below those stated for this category.	

Table A.3 — Acceptance criteria for herbal medicinal products in the British Pharmacopoeia

TAMC	Acceptance criterion: 10^5 cfu/g or cfu/ml Maximum acceptable count: 500 000cfu/g or cfu/ml
TYMC	Acceptance criterion: 10^4 cfu/g or cfu/ml Maximum acceptable count: 50 000 cfu/g or cfu/ml
Bile-tolerant gram-negative bacteria	Acceptance criterion: 10^4 cfu/g or cfu/ml
Escherichia coli	Acceptance criterion: Absence (1 g or 1 ml)
Shigella	Absence (1 g)
Salmonella	Absence (25 g or 25 ml)
NOTE Herbal medicinal products containing, for example, extracts or herbal drugs, with or without excipients, where it can be demonstrated that the method of processing (e.g. extraction with low-strength ethanol or water that is not boiling, or low-temperature concentration) or, in the case of herbal drugs, of pre-treatment, would not reduce the level of organisms sufficiently to reach the criteria required in Table A.2.	

Table A.4 — Acceptance criteria for crude drugs and crude drug preparations in the Japanese Pharmacopoeia

Microorganisms	Category 1[a] cfu/g or cfu/ml	Category 2[b] cfu/g or cfu/ml
Aerobic bacteria	10^7	10^5
Moulds and yeasts	10^4	10^3
Enterobacteria and other gram-negative bacteria	—[c]	10^3
Escherichia coli	10^2	Not detected
Salmonella	Not detected	Not detected
Staphylococcus aureus	—	—
Shigella	—	—

[a] Category 1 includes crude drugs and crude drug preparations which are used for extraction by boiling water or to which boiling water is added before use.

[b] Category 2 includes crude drugs which are taken directly without extraction process and directly consumed crude drug preparations containing powdered crude drugs.

[c] The limits are not specified.

Table A.5 — Acceptance criteria for crude drugs and crude drug preparations in the Korean Pharmacopoeia

Microorganisms	Category 1[a] cfu/g or cfu/ml	Category 2[b] cfu/g or cfu/ml
Aerobic bacteria	10^7	10^5
Moulds and yeasts	10^4	10^3
Enterobacteria and other gram-negative bacteria	—[c]	10^3
Escherichia coli	10^2	Not detected
Salmonella	Not detected	Not detected
Staphylococcus aureus	—	—

NOTE Enterobacteria and other gram-negative bacteria, *Escherichia coli*, Salmonella and Staphylococcus aureus are mentioned as specified microorganisms, but it is also necessary to test other microorganisms such as certain species of Bacillus cereus, Clostridia, Pseudomonas, Burkholderia, Asperigillus and Enterobacter species depending on the origin of raw materials for crude drugs or the preparation method of crude drug preparations.

[a] Category 1 includes crude drugs and crude drug preparations which are used for extraction by boiling water or to which boiling water is added before use.

[b] Category 2 includes crude drugs which are taken directly without extraction process and directly consumed crude drug preparations containing powdered crude drugs.

[c] The limits are not specified.

Table A.6 — Microbial enumeration limits for raw materials in the Korean Pharmacopoeia

Microorganisms	Target limit cfu/g or cfu/ml
Aerobic bacteria	≤ 10^3
Fungi (moulds, yeasts)	≤ 10^2

Table A.7 — Acceptance criteria for microbiological quality of the raw material and excipients in the Pharmacopoeia of the People's Republic of China

	Total eerobic microbial count NMT cfu/g, cfu/ml	**Total combined yeast and mould count NMT** cfu/g, cfu/ml	**Special microorganism**
Raw material and excipients	10^3	10^2	—[a]
[a] The limits are not specified.			

Table A.8 — Acceptance criteria for microbiological quality of the extracts of traditional Chinese medicine and herbal pieces in the Pharmacopoeia of the People's Republic of China

	Total aerobic microbial count NMT cfu/g, cfu/ml or cfu/10 cm^2	**Total combined yeast and mould count NMT** cfu/g, cfu/ml or cfu/10 cm^2	**Special microorganism**
Extracts	10^3	10^2	—[a]
Herbal pieces	—	—	Absence of *Escherichia coli* (1 g or 1 ml) Absence of *Salmonella* (10 g or 10 ml) gram-negative bacteria < 10^4 (1 g)
[a] The limits are not specified.			

Table A.9 — Acceptance criteria for microbiological quality of herbs, processed herbs and herbal products in the Indian Pharmacopoeia

	Total aerobic microbial count NMT cfu/g, cfu/ml	**Total combined yeast and mould count NMT** cfu/g, cfu/ml	**Special microorganism**
Products to which boiling water is added before use	10^7	10^5	For oral use *Escherichia coli* – absent in 1 g or 1 ml *Salmonella and Shigella* – absent in 10 g or 10 ml Not more than 10^3 CFU of bile-tolerant gram-negative bacteria
Products to which boiling water is not added before use	10^7	10^5	For topical use *Staphylococcus aureus* – absent in 1 g or 1 ml *Pseudomonas aeruginosa* – absent in 1 g or 1 ml

Table A.10 — Acceptance criteria for microbiological quality of raw materials of natural origin in the Indian Pharmacopoeia

Raw materials of natural origin (plant, animal, or mineral)	Total aerobic microbial count NMT cfu/g, cfu/ml	Total combined yeast and mould count NMT cfu/g, cfu/ml	Special microorganism
Used in preparations for oral use	10^3	10^2	*Escherichia coli* – absent in 1 g or 1 ml *Salmonella and Shigella* – absent in 10 g or 10 ml
Used in preparations for cutaneous/ topical use			*Staphylococcus aureus* – absent in 1 g or 1 ml *Pseudomonas aeruginosa* – absent in 1 g or 1 ml
Used in preparations for vaginal /urethral use			*Staphylococcus aureus* – absent in 1 g or 1 ml *Pseudomonas aeruginosa* – absent in 1 g or 1 ml *Candida albicans* – absent in 1 g or 1 ml

Table A.11 — Acceptance criteria for microbiological quality of herbal materials, preparations and finished products in WHO

	Aerobic bacteria cfu/g	**Yeasts and moulds** cfu/g	*Escherichia coli* cfu/g	**Other enterobacteria** cfu/g	*Clostridia* cfu/g	*Salmonellae* cfu/g	*Shigella* cfu/g	**Mould propagules** cfu/g
Raw medicinal plant and herbal materials intended for further processing[a]	–[c]	–	10^4	–			Absence	10^5
Herbal materials that have been pretreated[b]	10^7	10^4	10^2	10^4	Absence	Absence	Absence	–
Other herbal materials for internal use	10^5	10^3	10^1	10^3	Absence	Absence	Absence	–
Herbal medicines to which boiling water is added before use	10^7	10^4	10^1	10^3	Absence	Absence	Absence	–
Other herbal medicines	10^5	10^3	Absence	10^3	Absence	Absence	Absence	–

[a] For contamination of raw medicinal plant and herbal materials intended for further processing (including additional decontamination by a physical or chemical process) the limits, adapted from the provisional guidelines established by an international consultative group, are given for untreated herbal material harvested under acceptable hygienic conditions.

[b] For herbal materials that have been pretreated (e.g. with boiling water as used for herbal teas and infusions) or that are used as topical dosage forms.

[c] The limits are not specified.

Table A.12 — Acceptance criteria for non-sterile traditional Chinese medicine preparations of natural origin in the Pharmacopoeia of the People's Republic of China

Route		Total aerobic microbial count NMT cfu/g cfu/ml or cfu/10 cm^2	Total combined yeast and mould count NMT cfu/g cfu/ml or cfu/10 cm^2	Special microorganism
Non-aqueous preparations for oral use	Without fermented soya beans and aflatoxin	10^4 pill 3×10^4	10^2	Absence of *Salmonella* (1 g or 1 ml) Absence of *Escherichia coli* (1 g or 1 ml) gram-negative bacteria < 100 (1 g/1 ml)
	With fermented soya beans and aflatoxin	10^5	5×10^2	
Aqueous preparations for oral use	Without fermented soya beans and aflatoxin	5×10^2	10^2	Absence of *Salmonella* (1 g or 1 ml) Absence of *Escherichia coli* (1 g or 1 ml) gram-negative bacteria < 100 (1 g/1 ml)
	With fermented soya beans and aflatoxin	10^3		
Non-aqueous preparations for local use	Trauma	10^3		Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml)
	Without trauma	10^4		
Aqueous preparations for local use	Trauma	10^2		Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml)
	Without trauma			

Table A.13 — Acceptance criteria for microbiological quality of the non-sterile substances for pharmaceutical use in the Japanese Pharmacopoeia, the United States Pharmacopoeia and the European Pharmacopoeia

	Total aerobic microbial count NMT cfu/g, cfu/ml	Total combined yeast and mould count NMT cfu/g, cfu/ml	Special microorganism
Substances for pharmaceutical use	10^3	10^2	—[a]

[a] The limits are not specified.

A.2.3 The acceptance criteria of microorganisms for non-sterile traditional Chinese medicine preparations in different pharmacopoeias

Table A.14 — Acceptance criteria of TAMC and TYMC for non-sterile traditional Chinese medicine preparations without natural origin in the Pharmacopoeia of the People's Republic of China, the Japanese Pharmacopoeia, the Korean Pharmacopoeia, the United States Pharmacopoeia, the European Pharmacopoeia and the Indian Pharmacopoeia

Route	Total aerobic microbial count NMT cfu/g, cfu/ml	Total combined yeast and mould count NMT cfu/g, cfu/ml	Differences
Non-aqueous preparations for oral use	10^3	10^2	
Aqueous preparations for oral use	10^2	10^1	TYMC: the acceptance criteria in KP is < = 50.
Oromucosal use Gingival use Nasal use	10^2	10^1	There are no acceptance criteria of products for oromucosal use and gingival use in KP and the acceptance criteria of products for nasal use is < = 50.
Auricular use Cutaneous use	10^2	10^1	There are no acceptance criteria of products for cutaneous use in KP and the acceptance criteria of products for auricular use is < = 50.
Inhalation use	10^2	10^1	TAMC: more rigorous requirements apply to liquid preparations for nebulization in JP, USP, EP, IP and BP. The acceptance criteria of liquid preparations for inhalation use in KP is < = 20 and powder is < = 10^2. TYMC: the acceptance criteria of liquid preparations for inhalation use in KP is < = 20 and powder is < = 50.
Vaginal use	10^2	10^1	TYMC: the acceptance criteria of products for vaginal use in KP is < = 50.
Rectal use Non-aqueous aqueous	10^3 10^2	10^2 10^2	TAMC: the acceptance criteria of aqueous products for rectal use in ChP is 10^2.

Table A.14 *(continued)*

Route	Total aerobic microbial count NMT cfu/g, cfu/ml	Total combined yeast and mould count NMT cfu/g, cfu/ml	Differences
Transdermal patches (limits for one patch including adhesive layer and backing)	102	10^2	TYMC: the acceptance criteria of products for transdermal patches in KP is < = 50.
Special Ph. Eur. provision for oral dosage forms containing raw materials of natural (animal, vegetal or mineral) origin for which antimicrobial pretreatment is not feasible and for which the competent authority accepts TAMC of the raw material exceeding 103 CFU/g or CFU/ml	10^4	10^2	This part is only included in EP and BP.

Table A.15 — Acceptance criteria of specified microorganisms for non-sterile traditional Chinese medicine preparations without natural origin

Route	Specified microorganisms	Pharmacopoeia						
		ChP	JP	KP	USP	EP	IP	BP
Non-aqueous preparations for oral use	*Escherichia coli*	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)
	Salmonella in preparations contain organ extracts	Absence (10 g or 10 ml)	–[a]	–	–	–	–	–
Aqueous preparations for oral use	*Escherichia coli*	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)
	Salmonella in preparations contain organ extracts	Absence (10 g or 10 ml)	–	–	–	–	–	–
Oromucosal use	*Escherichia coli*	Absence(1 g, 1 ml or 10cm^2)	–	–	–	–	–	–
	Staphylococcus aureus		Absence (1 g or 1 ml)	–	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)
	Pseudomonas aeruginosa							
Gingival use	*Escherichia coli*	Absence(1 g, 1 ml or 10 cm^2)	–	–	–	–	–	–
	Staphylococcus aureus		Absence (1 g or 1 ml)	–	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)
	Pseudomonas aeruginosa							
Nasal use	*Escherichia coli*	Absence(1 g, 1 ml or 10 cm^2)	–	–	–	–	–	–
	Staphylococcus aureus		Absence (1 g or 1 ml)	Absence	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)
	Pseudomonas aeruginosa							
Auricular use	*Staphylococcus aureus*	Absence(1 g, 1 ml or 10 cm^2)	Absence (1 g or 1 ml)	Absence	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)	Absence (1 g or 1 ml)
	Pseudomonas aeruginosa							

Key

BP The British Pharmacopoeia

ChP Pharmacopoeia of the People's Republic of China

EP The European Pharmacopoeia

IP The Indian Pharmacopoeia

JP The Japanese Pharmacopoeia

KP The Korean Pharmacopoeia

USP The United States Pharmacopoeia

[a] The limits are not specified.