REVISED GENERAL CHAPTER

2.2.9. Microbial Contamination in Nonsterile Products

The following tests are designed for the estimation of number of viable aerobic microorganisms present, for detecting the presence of designated microbial species and acceptance criteria in pharmaceutical substances, nonsterile dosage forms, herbs, processed herbs and herbal products.

1. Total Aerobic Viable Count

Introduction

The tests described here after will allow enumeration of mesophilic bacteria and fungi that may grow under aerobic conditions.

These tests are not applicable to product containing viable microorganisms as active ingredients.

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

These tests should be carried out under conditions designed to avoid accidental contamination during the test. The precautions taken to avoid contamination must be such that they do not adversely affect any microorganisms that should be revealed in the test.

Before carrying out these tests for enumeration of the aerobic microorganisms in the test specimen, it should be confirmed that:

i) The media used in the tests, promote the growth of the respective microorganisms for which they are used.

ii) The test specimens to which the tests are applied do not themselves inhibit the microorganisms and the appropriateness of the methods used for enumeration of microorganism in presence of the product is determined.

Growth Promotion Test, Negative Control and Appropriateness of enumeration methods

Preparation of inoculum

Microorganisms used. *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404.

In order to prevent any phenotypic changes in the strains used, the organisms used in the test should not be more than 5 passages from the original culture. One passage is defined as inoculation and growth of the organisms from existing culture to a fresh medium.

Grow each of the bacterial test strains separately in Casein soyabean digest broth (Medium 1) or Casein soyabean digest agar (Medium 2) and incubate at 30º to 35º for 18 to 24 hours. Grow *Candida albicans* separately in Sabouraud dextrose broth (Medium 3) or Sabouraud dextrose agar with antibiotic (Medium 4) and incubate at 20º to 25º for 48 to 72 hours. Grow *Aspergillus brasiliensis* on Sabouraud dextrose agar with antibiotic at 20º to 25º for 5 to 7 days or until good sporulation. After incubation, prepare test suspension of each organism separately in *Buffered sodium chloride-peptone solution pH 7.0* or *Phosphate buffer solution pH 7.2*. To suspend spores of *Aspergillus brasiliensis* 0.05 per cent *polysorbate 80* may be added to the buffered solution. ~~Use test suspension of these organisms within 2 to 4 hours. The suspension may be stored at 2º to 8º for a validated period of time.~~ Use test suspension of these organisms within 2 hours, or within

24 hours if stored between 2º to 8º. 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 stored in suitable preserving medium for a validated period of time.

Growth promotion by media

Test each batch of the medium prepared either from the dehydrated medium or from the ingredients described for growth promotion. Use the already approved medium prepared from dehydrated medium or from the ingredients as a positive control.

Inoculation

Inoculate 10 ml of Casein soyabean digest broth (Medium 1) and plates of Casein soyabean digest agar (Medium 2) with not more than 100 CFU of each of the three above mentioned species of bacteria using separate tube and plate of medium for each and incubate at 30º to 35º for ~~18 to 24 hours~~ ≤3 days. Similarly inoculate plates of Casein soyabean digest agar (Medium 2) with not more than 100 CFU of *Candida albicans* and *Aspergillus brasiliensis* using separate plate of medium for each and incubate at 30º to 35º for ~~18 to 24 hours~~ ≤5 days. Inoculate plates of Sabouraud dextrose agar with antibiotic (Medium 4) using not more than 100 CFU of *Candida albicans* and *Aspergillus brasiliensis* and incubate at 20º to 25º for ≤5 days.

For comparison purpose, similarly inoculate and incubate the previously approved liquid and solid medium (as a positive control) along with the media under test.

~~**Growth**~~

Liquid media under test should be considered suitable if clearly visible growth obtained is comparable to that obtained on ~~the same medium,~~ previously tested and approved batch of medium occurs.

Growth obtained on a solid medium must not differ from the calculated CFU of the standardized inoculum by a factor greater than 2. For a freshly prepared inoculum, growth of the microorganism should be comparable to that obtained on the ~~same medium~~ previously tested and approved batch of medium occurs.

Negative control

To verify the test conditions, use the diluent, sterile *buffered sodium chloride-peptone solution pH 7.0* or *Phosphate buffer solution pH 7.2* as a negative control in place of the test organisms (inoculum). There should not be any growth of microorganisms in this control. If a negative control fails (microorganisms grow in the control), its cause should be investigated.

Appropriateness of enumeration methods in presence of product

~~Pre-treatment~~ Preparation of the sample

Use suitable alternative method if following methods are not applicable.

Water soluble product. ~~Dissolve 10g or dilute 10 ml of the preparation~~ Dissolve or dilute (usually a 1 in 10 dilution is prepared) product under examination, unless otherwise specified and justified, in *buffered sodium chloride-peptone solution pH 7.0*, *Phosphate buffer solution pH 7.2* or any other suitable medium shown to have no antimicrobial activity under the conditions of the test ~~and adjust the volume to 100 ml with the same~~. If necessary, adjust ~~the pH to about 7.0~~ to a pH of 6.0 to 8.0. If required, further dilutions are prepared with the same diluent.

Products insoluble in water (non-fatty). ~~Suspend 10 g or 10 ml of the preparation under examination,~~ Suspend the product to be examined (usually a 1 in 10 dilution is prepared) unless otherwise specified and justified, in *buffered sodium chloride-peptone solution pH 7.0, Phosphate buffer solution pH 7.2* or any other suitable medium shown to have no antimicrobial activity under the conditions of the test ~~and adjust the volume to 100 ml with the same~~. If necessary, divide the preparation under examination and homogenise mechanically. A suitable surface active agent such as 0.1 per cent w/v solution of *polysorbate 80* may be added to assist the suspension of poorly wettable substances. If necessary, adjust the pH of the suspension to ~~about 7~~ 6.0 to 8.0. If required, further dilutions are prepared with the same diluent.

Fatty products. ~~Homogenize 10 g or 10 ml of the preparation~~ Dissolve the product under examination in pre sterilized *isopropyl myristate* or unless otherwise specified and justified, with 5 g of sterile *polysorbate 20* or *polysorbate 80*. If necessary, heat to not more than 40º. Mix carefully while maintaining the temperature in waterbath. Add ~~85 ml~~ a sufficient quantity of *buffered sodium chloride peptone solution* pH 7.0, *Phosphate buffer solution pH 7.2* or any other suitable medium to make a 1 in 10 dilution of the original product which does not have any antimicrobial activity under the conditions of the test, heated to not more than 40º, if necessary. Maintain this temperature for the shortest time necessary for formation of an emulsion and in any case for not more than 30 minutes. ~~Adjust the volume to 100 ml with the same medium. If necessary, adjust the pH to about 7.0.~~ Further dilutions may be prepared using the same diluent containing a suitable concentration of sterile *polysorbate 80* or other non-inhibitory sterile surface-active reagent.

Fluids or solids in aerosol form~~. In sterile conditions,~~ Aseptically transfer the product into a membrane filter apparatus or to a sterile container for further sampling. Use either the total contents or a defined number of metered doses from each of the containers tested.

Transdermal patches. Remove the protective cover sheets 'release liners' of transdermal patches ~~using sterile forceps~~ aseptically and place them, adhesive side upwards, on sterile glass or plastic trays. Cover the adhesive surface with sterile gauze and transfer them to a suitable volume of *buffered sodium chloride-peptone solution* pH 7.0, *Phosphate buffer solution pH 7.2* or any other suitable medium containing inactivator such as *polysorbate 80, lecithin* or any other suitable inactivator. Shake the preparation vigorously for at least 30 minutes.

Inoculation and Dilution

Add to the sample prepared as mentioned above and to a control (only diluent without prepared sample) a sufficient volume of microbial suspension to obtain an inoculum of not more than 100 CFU. The volume of the inoculum should not exceed 1 per cent of the volume of the diluted product.

Carry out inoculation using each of the microorganisms separately, like this, with and without product under examination.

Use the lowest possible dilution factor of the prepared sample to demonstrate acceptable microbial recovery from the product. If the product has antimicrobial property, then the inoculum may be added after inactivation, dilution or filtration.

Inactivation of antimicrobial activity

If the test specimen is known to contain any of the below antimicrobial substances, then use the corresponding inactivating agent to neutralize the antimicrobial activity (Table 1). The inactivating agent may be added to the chosen diluent or the medium preferably before sterilization. Before using the inactivating agent, verify its efficiency against the antimicrobial

substance and also confirm that the inactivating agent does not have toxic effect on the organisms used in the test by carrying out a blank with the inactivator and without product. The inhibitory effect can be removed by increasing the volume of the diluent or culture media. If the inhibitory substances are water-soluble then membrane filtration method may be used. If inspite of incorporation of suitable inactivating agent and substantial increase in the volume of the diluent, it is still not possible to recover the viable culture from the product then it can be assumed that the failure to isolate the inoculated organisms may be due to microbicidal property of the product and the product is not likely to be contaminated with the microorganisms inoculated but can contain other organisms. Then carry out the tests with highest dilution factor compatible with microbial growth and the specific acceptance criteria.

Table 1-Antimicrobial substances with corresponding inactivating agents

Antimicrobial substances	Inactivator	Concentration
~~Phenolics,~~ Parahydroxy benzoate (Parabens)	Polysorbate 80	30 g per litre
Iodine, Quaternary ammonium compound (QACs)	Lecithin	3 g per litre
	Sodium lauryl sulphate	4 g per litre
Phenolics, Alcohol, Aldehydes, Sorbates	Dilution	–
Mercurial, halogens	Sodium thiosulphate	5 g per litre

Enumeration of aerobic organisms in presence of product

For each of the microorganisms listed, separate tests are performed. Only microorganisms of the added test strains are counted.

Membrane Filtration

Use membrane filters ~~50 mm in~~ of suitable diameter and having a nominal pore size of not greater than 0.45 µm the effectiveness of which in retaining bacteria has been established for the type of preparation under examination. 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 examined. Cellulose nitrate filters may be used for aqueous, oily and weakly alcoholic solutions and cellulose acetate filters for strongly alcoholic solutions. For each of the microorganisms listed, one membrane filter is used.

Transfer a suitable quantity of the sample prepared as described under ~~*pre-treatment*~~ *Preparation of the sample*, ~~*inoculum,*~~ *inoculation and dilution and inactivation of the antimicrobial activity* to the membrane filter, filter immediately. The prepared sample should contain 1 g of the product. However, if large numbers of microorganisms are expected in the sample, then lesser quantity may be taken.

Wash each membrane by ~~filtering through it~~ with an appropriate quantity of a suitable liquid such as *buffered sodium chloride- peptone solution pH 7.0.* For determination of total aerobic viable count (TAC), transfer the membrane filter to the surface of Casein soyabean digest agar (Medium 2) and incubate the plates at 30º to 35º for ≤3 days and ≤5 days for *Candida albicans* and *Aspergillus brasiliensis*. For total fungal count (TFC), transfer the membrane to the surface of Sabouraud dextrose agar with antibiotic (Medium 4) and incubate the plates at 20º to 25º for ≤5

days. Count the number of colonies that are formed and calculate the number of microorganisms per g or per ml of the product under examination.

Plate count methods

Pour-plate method. To a 9 cm diameter Petri dish add 1 ml of the sample prepared as described in ~~*pre-treatment*~~ *Preparation of the sample,* ~~*inoculum,*~~ *inoculation and dilution, and* ~~*inactivating agents if necessary*~~ *inactivation of the antimicrobial activity* and 15 ml of Casein soyabean digest agar (Medium 2) or Sabouraud dextrose agar with antibiotic (Medium 4), at not more than 45º. Use at least two Petri dishes for each of the test organisms. Incubate the plates of Casein soyabean digest agar (Medium 2) at 30º to 35º for ≤3 days and ≤5 days for *Candida albicans* and *Aspergillus brasiliensis*. Similarly incubate the plates of Sabouraud dextrose agar with antibiotic (Medium 4) at 20º to 25º for ≤5 days. Calculate the mean count on each medium and from that calculate the number of CFU.

Surface-spread method. Using Petri dishes of 9 cm diameter add 15 ml of Casein soyabean digest agar (Medium 2) for cultivation of aerobic microorganisms or Sabouraud dextrose agar with antibiotic (Medium 4) for cultivation of fungi, at about 45º, to each Petri dish and allow to solidify. Dry the plates, in ~~an LAF bench or in an incubator~~ a Laminar Air Flow. Spread a measured volume of not less than 0.1 ml of the sample prepared as described ~~earlier~~ above, over the surface of the medium. Use atleast two Petri dishes for each medium and each strain of test organism. For incubation and calculation of the number of colony forming units proceed as described in the pour-plate method.

Most probable number method

This method (originally known as multiple-tube or serial dilution method) is to be followed when no other method is available. The ~~procedure~~ precision and accuracy of the method is less than that of the membrane filtration method or the plate count methods. Unreliable results are obtained particularly for the enumeration of molds. Hence this method is carried out for the enumeration of TAC in situation when no other method is available. If use of this method is justified, then proceed as follows

Prepare a series of at least three subsequent tenfold dilutions of the product as described in ~~Pre-treatment~~ *Preparation of the sample,* ~~*inoculum,*~~ *inoculation and dilution,* ~~*inactivating agents if necessary,*~~ *inactivation of the antimicrobial activity*. From each level of dilution three aliquots of 1 g or 1 ml are used to inoculate three tubes with 9.0 ml of sterile Casein soyabean digest broth (Medium 1) If necessary, *polysorbate 80* or an inactivator of antimicrobial agents (Table 1) may be added to the medium. Thus, if three levels of dilution are prepared, 9 tubes are inoculated.

Incubate all the tubes for three days at 30º to 35º. Record for each level of dilution the number of tubes showing microbial growth. If detection of growth is difficult or uncertain owing to the nature of the product under examination, sub-culture in the same broth, or on a suitable agar medium such as Casein soyabean digest agar (Medium 2) for ~~18 to 24 hours~~ 24 to 48 hours at 30º to 35º. Determine the most probable number of bacteria per g or ml of the product from Table 2.

Result

The number of microorganisms recovered from the prepared sample, inoculated and processed as mentioned above is compared with that of the number of microorganisms recovered from the control (without test specimen) preparation.

i) If growth in presence of product is inhibited by a factor >2, it indicates that the product has antimicrobial property hence process the sample as described under inactivation of

antimicrobial activity and carry out the test as mentioned under *Enumeration of aerobic organisms in presence of product* and then interpret the result as mentioned under *Interpretation*.

ii) If growth is not inhibited, interpret the results as under

Interpretation

Membrane filtration or a plate-count method is considered suitable, if mean count of any of the test organisms do not differ by a factor >2 from the mean count of the control (in absence of the product). When verifying the validity of MPN method, calculated value from the inoculum must be within 95 per cent confidence limits of the results obtained with the control. If this criteria cannot be met for any one or more of the organisms tested with any of the described methods, the method and the test conditions that come closest to the criteria are used to test the product.

Table 2- Most-probable-number (MPN) values of microorganisms, 3 tubes at each level of dilution

Observed combinations of Numbers of tubes showing growth in each set			MPN per g or per ml of the product	95 per cent Confidence limits
Number of g or ml of product per tube				
0.1	0.01	0.001		
0	0	0	< 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	> 1100	-

Testing of Products

Sampling and amount used for the test

Sampling of the product must follow a well-defined sampling plan that takes into account the batch size, the characteristics of the product, the health hazards associated with highly contaminated products and the expected level of contamination. Unless otherwise stated, use 10 ml or 10 g ~~specimens~~ product for testing. 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 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.

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

For materials used as active ingredients where the batch size is less than 1 kg or 1 litre, the amount tested shall be 1 per cent of the batch-size. Unless a lesser amount is prescribed or justified and authorized.

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

Enumeration of aerobic microorganisms present in the product

Membrane Filtration

Prepare the sample using the method that has been shown to be appropriate as described in appropriateness of enumeration methods in presence of product. Transfer appropriate amount to each of the two membrane filters and filter immediately. Wash each filter following the procedure found to be suitable. For determination of total aerobic ~~microbial~~ viable count transfer one of the membrane filters to Casein soyabean digest agar (Medium 2). Incubate the plate at 30º to 35º for 3 to 5 days. For determination of total ~~yeast and mould~~ fungal count transfer the other membrane

to the surface of Sabouraud dextrose agar with antibiotic (Medium 4) and incubate at 20º to 25º for 5 to 7 days. Calculate the number of CFU per g or per ml of the product.

Plate count methods

Pour-plate method. Prepare the sample using a method that has shown to be suitable as described in *appropriateness of the enumeration methods in presence of product.* Stir the prepared sample in the tubes/flasks vigorously on a vortex mixer for few minutes. Each dilution of the product sample should be stirred like this. Use atleast two plates of Casein soyabean digest agar for each dilution. Incubate the plates at 30º to 35º for 3 to 5 days. Similarly for total fungal count use atleast two plates of Sabouraud dextrose agar with antibiotic for each dilution and incubate at 20º to 25º for 5 to 7 days. Then select the plates corresponding to a given dilution and showing the highest number of colonies but less than 250 of aerobic microorganisms and 25 colonies for fungi. Calculate the mean count and number of CFU per g or per ml of the product.

Surface-spread method. Prepare the sample using a method that has been shown to be suitable as described in Appropriateness of the enumeration methods in presence of product and further process it as mentioned in the pour-plate method above. Spread 0.1 ml of the dilution. Use atleast two plates for each medium and each of the dilutions. Incubate, select and calculate the number of CFU as mentioned in *pour plate method.*

Most probable number method

Prepare and dilute the sample using a method that has been shown to be appropriate as described in the *Growth promotion test* and *Appropriateness of the enumeration method.* Incubate all tubes at 30º to 35º for ~~at least 3 days~~ 3 to 5 days. For each level of dilution note down the number of tubes showing microbial growth. Subculture if required as described before. Determine the most probable number of microorganisms per g or per ml of the product as per Table 2.

Interpretation of the results

The total aerobic viable count (TAC) is considered to be equal to the number of CFU found on Casein soyabean digest agar. If colonies of fungi are detected on this medium, they are counted as part of TAC. The total fungal count (TFC) is considered to be equal to the number of CFU found using Sabouraud dextrose agar with antibiotic.

Acceptance criteria for microbiological quality should be interpreted as follows:

10^1 CFU: maximum acceptable count 20

10^2 CFU: maximum acceptable count 200

10^3 CFU: maximum acceptable count 2000, and so forth…

2. Tests for specified microorganisms

The tests described below will allow determination of the absence of, or limited occurrence of specified microorganisms that may be detected under the conditions described.

These tests are carried out to find out whether a substance or a preparation complies with an established specification for microbiological quality.

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

Following procedures are carried out as described under *Total aerobic viable count* ~~in *Microbial contamination in nonsterile products* (2.2.9)~~.

i) The preparation of sample.

ii) Inactivation of antimicrobial property, if present, in the product.

iii) Confirmation that the surface active substances if used, are not toxic for the test-organisms and are compatible with the inactivating agent.

Growth promotive and selective properties of the media and validity of the test

Test each batch of the medium prepared either from the dehydrated medium or from the ingredients described for growth promotion. Use the already tested and approved medium prepared from dehydrated medium or from the ingredients as a positive control. Verify the properties of relevant media as described in Table 3.

Preparation of the inoculum

Microorganisms used. *Staphylococus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Salmonella enterica spp. entrica* serotype *typhimurium* ATCC 14028 or *Salmonella enterica* spp. *entrica* serotype *abony* NCTC 6017, *Shigella boydii* ATCC 8700 or NCTC 12985, *Candida albicans* ATCC 10231, *Clostridium sporogenes* ATCC 11437 or ATCC 19404.

In order to prevent any phenotypic changes in the strains used, the organisms used in the test should not be more than 5 passages made from the original culture. One passage is defined as inoculation and growth of the organisms from existing culture to a fresh medium.

Grow each of the aerobic bacterial strains separately in Casein soyabean digest broth (Medium 1) or Casein soyabean digest agar (Medium 2) and incubate them at 30º to 35º for 18 to 24 hours. Grow *Candida albicans* in Sabouraud dextrose broth (Medium 3) or Sabouraud dextrose agar with antibiotic (Medium 4) and incubate at 20º to 25º for ~~at least~~ 48 to 72 hours.

After incubation, re-suspend the growth of each of the organisms separately in *buffered sodium chloride-peptone solution pH 7.0* *or Phosphate buffer solution pH 7.2*. ~~Use suspension of these organisms within 2 to 4 hours. The suspension may be stored at 2º to 8º for a validated period of time.~~ Use test suspension of these organisms within 2 hours, or within 24 hours if stored between 2º to 8º.

Grow *Clostridium sporogenes* ATCC 11437 *or* ATCC 19404 under anaerobic conditions in Reinforced medium for *Clostridia* (Medium 15) at 30º to 35º for 24 to 48 hours. Instead of vegetative cells a spore suspension may be used for inoculation. The stable spore suspension stored in suitable preserving medium for a validated period of time.

Negative control. To test the sterility of the medium and the diluent, use the diluent, *buffered sodium chloride-peptone solution pH 7.0* or *Phosphate buffer solution pH 7.2* (without organisms) as a negative control. There should not be any growth of microorganisms in this control. If a negative control fails (microorganisms grow in the control), its cause should be investigated.

Tests for growth promotion by liquid media. Inoculate 10 ml of the appropriate medium with not more than 100 CFU of the appropriate test microorganisms. Incubate at the specified temperature for ~~the time specified in the test.~~ not more than the shortest period of time specified in the test. Clearly visible growth of the microorganism comparable to that obtained with previously approved batch of medium occurs.

Tests for growth promotion by solid media. Using the surface spread method as described under *Total* ~~*viable*~~ *aerobic* *viable* *count* ~~*in Microbial contamination in nonsterile products* (2.2.9)~~, inoculate each plate with not more than 100 CFU of appropriate microorganism. Incubate at the specified temperature for ~~the time specified in the test.~~ not more than the shortest period of the

time specified in the test. Growth obtained on the medium should be comparable to that on the ~~same~~ medium previously approved.

Tests for growth inhibition in liquid or solid media. Inoculate the appropriate medium with not less than 100 CFU of the appropriate microorganism. Incubate at the specified temperature for ~~the time specified in the test~~ not less than the longest period of time specified in the test. There should not be any growth of the microorganism on this medium.

Tests for indicative properties. Using the surface spread method as described under *Total aerobic viable count* ~~in *Microbial contamination in nonsterile products* (2.2.9)~~. Inoculate each plate with not more than 100 CFU of appropriate microorganism. ~~Incubate at the specified temperature for the time specified in the test.~~ Incubate at the specified temperature for a period of time within the range specified in the test. Colony morphology and indication reactions should be similar to that obtained with the previously approved batch of medium.

Validity of the test method. For each new product to be tested prepare a sample as described below in the pertinent paragraph under *Testing of Products*. At the time of mixing add each test organism in the prescribed growth medium. Inoculate not more than 100 CFU of the test organisms individually. Carry out the test as described under *Testing of Products* using the shortest incubation period prescribed. The specified microorganism must be detected with the colony morphology and indication reaction as described under *Testing of Products*.

If the product has antimicrobial activity then it should be inactivated as mentioned in *inactivation of antimicrobial activity* under *appropriateness of enumeration method in presence of product*. If the antimicrobial activity of a given product cannot be inactivated then it is assumed that the inhibited microorganism will not be present in the product.

Testing of Products

Bile-Tolerant Gram-Negative Bacteria

Preparation of Sample and Pre-Incubation. Using Casein soyabean digest broth (Medium 1) as a diluent, make 1 in 10 dilution of ~~more~~ not less than 1 g of the product to be examined as mentioned under *Total aerobic viable count* ~~in *Microbial contamination in nonsterile products* (2.2.9)~~. Mix well and keep at 20º to 25º for about 2 to 5 hours to resuscitate the organisms.

Test for detection of organisms. From above prepared sample, take a volume corresponding to 1 g of the product and inoculate in Enterobacteria Enrichment Broth – Mossel (Medium 5). Incubate at 30º to 35º for 24 to 48 hours. Subculture on plates of Violet red bile glucose agar (Medium 6). Incubate at 30º to 35º for 18 to 24 hours**.**

The product passes the test if there is no growth of colonies of gram negative bacteria.

Table 3-Growth promoting, inhibitory and indicative properties of media

Test/Medium	Property	Test Strains
Test for bile tolerant Gram-negative bacteria		
Enterobacteria Enrichment Broth Mossel	Growth promoting	*E. coli, P. aeruginosa*
	Inhibitory	*S. aureus*
Violet Red Bile Glucose Agar	Growth promoting +	*E. coli*
	Indicative	*P. aeruginosa*
Test for *Escherichia coli*		
MacConkey Broth	Growth promoting	*E. coli*
	Inhibitory	*S. aureus*

MacConkey Agar	Growth promoting + Indicative	*E. coli*
Test for *Salmonella*		
Rappaport Vassiliadis Salmonella Enrichment Broth	Growth promoting	*Salmonella enterica spp. enterica* serotype *typhimurium or Salmonella enterica spp. enterica* serotype *abony*
	Inhibitory	*S. aureus*
Wilson and Blair's BBSAgar*	Growth promoting + Indicative	*Salmonella enteric spp. enterica* serotype *typhimurium or Salmonella enterica spp. enterica* serotype *abony*
	Inhibitory	*E. coli, Shigella boydii*
Test for *Shigella*		
GNMedium	Growth promoting	*Shigella boydii*
	Inhibitory	*S. aureus*
Xylose Lysine Deoxycholate Agar	Growth promoting + Indicative	*Shigella boydii*
	Indicative	*E. coli*
Test for *Pseudomonas aeruginosa*		
CetrimideAgar	Growth promoting	*P. aeruginosa*
	Inhibitory	*E. coli*
Test for *Staphylococus aureus*		
Mannitol Salt Agar	Growth promoting + Indicative	*S. aureus*
	Inhibitory	*E. coli*
Test for *Clostridia*		
Reinforced Medium for *Clostridia*	Growth promoting	*Cl. sporogenes*
Columbia Agar	Growth promoting	*Cl. sporogenes*
Test for *Candida albicans*		
Sabouraud Dextrose Broth or Agar	Growth promoting	*C. albicans*

* Xylose Lysine Deoxycholate Agar may be used.

Quantitative evaluation. From the above mentioned prepared sample take a volume corresponding to 0.1 g, 0.01 g and 0.001 g (or 0.1 ml, 0.01 ml, and 0.001 ml) of the product in suitable quantity of Enterobacteria enrichment Broth–Mossel (Medium 5). Incubate at 30º to 35º for 24 to 48 hours. Subculture each of the cultures on a plate of Violet red bile glucose agar with dextrose (Medium 6). Incubate at 30º to 35º for 18 to 24 hours. Growth of well developed reddish colonies of Gram negative bacteria is considered positive. Note the smallest quantity of the product that gives the positive result and the largest quantity that gives the negative result. Determine from Table 4 the most probable number of bacteria.

Table 4–Quantitative determination of most probable number of bacteria

Results for each quantity of product			Probable number of bacteria per g 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

Escherichia coli

Using Casein soyabean digest broth (Medium 1) as a diluent make 1 in 10 dilution of ~~more~~ not less than 1 g of the product as mentioned under *Total aerobic viable count* ~~in *Microbial contamination in nonsterile products* (2.2.9)~~ and use 10 ml or the quantity corresponding to 1 g or 1 ml of the product to inoculate a suitable amount (determined as under *Validity of the Test method)* of Casein soyabean digest broth, incubate at 30º to 35º for 18 to 24 hours.

After incubation shake the broth and transfer 1 ml to 100 ml of MacConkey broth (Medium 7). Incubate at 42º to 44º for 24 to 48 hours. Subculture on a plate of MacConkey agar (Medium 8) and incubate at 30º to 35º for 18 to 72 hours. Growth of pink, non-mucoid colonies indicates the possible presence of *Escherichia coli*. This should be confirmed by identification test.

If there is no growth of such type of colonies, or the identification tests are negative it indicates absence of *E. coli* and the product passes the test.

Salmonella

Prepare a sample from the product as mentioned under *Total aerobic viable count* ~~in *Microbial contamination in nonsterile products* (2.2.9)~~ and use the quantity corresponding to not less than10 g or 10 ml of the product to inoculate a suitable amount (determined as under *Validity of the Test method)* of Casein soyabean digest broth incubate at 30º to 35º for 18 to 24 hours.

After incubation shake the broth and transfer 0.1 ml to 10 ml of Rappaport Vassiliadis Salmonella enrichment broth (Medium 9) and incubate at 30º to 35º for ~~24 to 48 hours~~ 18 to 24 hours. Subculture on a plate of Wilson and Blair's BBS Agar. (Medium 10) and incubate at 30º to 35º for ~~24 to 48 hours~~ 18 to 48 hours. Green colonies with black center develop and in 48 hours the colonies become uniformly black. Colonies surrounded by a dark zone and metallic sheen indicates possibility of presence of *Salmonella*. If sub cultured on plates of Xylose lysine deoxycholate agar (Medium 12) and incubate at 30º to 35º for ~~24 to 48 hours~~ 18 to 48 hours. Well developed, red colonies with or without black centers indicates possibility of *Salmonella.* This should be confirmed by identification tests.

If there is no growth of such type of colonies, or identification tests are negative it indicates absence of *Salmonella* and the product passes the test.

Shigella

Prepare a sample from the product to be examined as mentioned under *Total aerobic viable count* ~~in *Microbial contamination in nonsterile products* (2.2.9)~~ and use the quantity corresponding to not less than10 g or 10 ml of the product to inoculate a suitable amount (determined as under *Validity of the Test method)* of Casein soyabean digest broth incubate at 30º to 35º for 18 to 24 hours.

After incubation shake the ~~growth~~ broth and transfer 1 ml to 100 ml of GN Broth (Medium 11) and incubate at 30º to 35º for 24 to 48 hours. Subculture on a plate of Xylose lysine deoxycholate medium (Medium 12). Incubate at 30º to 35º for 24 to 48 hours. A red colored translucent colony without black centre indicates possibility of presence of *Shigella*. This should be confirmed by identification tests.

If there is no growth of such colonies or if identification tests are negative, it indicates absence of *Shigella* and the product passes the test.

Pseudomonas aeruginosa

Using Casein soyabean digest broth as a diluent make 1 in 10 dilution of ~~more~~ not less than 1 g of the product as mentioned in *Total aerobic viable count* ~~under *Microbial contamination in nonsterile products* (2.2.9)~~ and use 10 ml or the quantity corresponding to 1 g or 1 ml of the product to inoculate a suitable amount (determined as under *Validity of the Test method)* of Casein soyabean digest broth incubate at 30º to 35º for 18 to 24 hours. Subculture on a plate of Cetrimide agar (Medium 13) and incubate at 30º to 35º for 18 to 72 hours. A greenish color colony indicates the possibility of presence of *Pseudomonas aeruginosa*. This should be confirmed by identification tests.

If there is no growth of such type of colonies, or identification tests are negative it indicates absence of *P. aeruginosa* and the product passes the test.

Staphylococcus aureus

Using Casein soyabean digest broth as a diluent make 1 in 10 dilution of ~~more~~ not less than 1 g of the product as mentioned in *Total aerobic viable count* ~~under *Microbial contamination in nonsterile products* (2.2.9)~~ and use 10 ml or the quantity corresponding to 1 g or 1 ml of the product to inoculate a suitable amount (determined as under *Validity of the Test method)* of Casein soyabean digest broth incubate at 30º to 35º for 18 to 24 hours.

Sub-culture on a plate of Mannitol salt agar (Medium 14) incubate at 30º to 35º for 18 to 72 hours. Yellow or white colonies with yellow zones indicate the possibility of presence of *S. aureus*. This should be confirmed by identification tests.

If there is no growth of such type of colonies, or the identification tests are negative it indicates absence of *S. aureus* and the product passes the test.

Clostridia

~~Prepare a sample from the product under examination~~ Using Casein soyabean digest broth as a diluent make 1 in 10 dilution (with a minimum total volume of 20 ml) of not less than 2 g or 2 ml of the product as mentioned under *Total aerobic viable count* ~~in *Microbial contamination in nonsterile products* (2.2.9)~~ Take two equal portions of at least 10 ml corresponding to 1 g or 1 ml of the product and heat one portion 80º for 10 minutes and cool rapidly. Do not heat the other portion. ~~Transfer 10 ml of each of the homogenised portions to two containers containing 100 ml of Reinforced medium for *Clostridia* (Medium 15).~~ Transfer 10 ml or the quantity corresponding

to 1g or 1 ml of the product to be examined of both portions to inoculate suitable amounts (determined as under *Validity of the Test method)* of Reinforced medium for *Clostridia* (Medium 15). Incubate under anaerobic conditions at 30º to 35º for 48 hours. After incubation, make sub-culture from each container on Columbia agar (Medium 16) and incubate under anaerobic conditions at 30º to 35º for 48 hours to 72 hours.

The occurence of anaerobic growth of Gram-positive bacilli with or without endospores giving a negative catalase test indicates possibility of presence of *Clostridia.*

If no anaerobic growth of microorganisms is detected on Columbia agar or identification test is negative, it indicates absence of *Clostridia* and the product passes the test.

Candida albicans

Prepare a sample from the product to be examined as mentioned in *Total aerobic viable count* ~~under *Microbial contamination* (2.2.9) *in nonsterile products*~~ and use the quantity corresponding to not less than1 g or 1 ml of the product to inoculate a suitable amount (determined as under *Validity of the Test method)* of Sabouraud dextrose broth (Medium 3) and incubate at 30º to 35º for 3 to 5 days.

Subculture on a plate of Sabouraud dextrose agar with antibiotic (Medium 4) and incubate at 30º to 35º for 24 to 48 hours. Growth of cream coloured colonies may indicate the possibility of presence of *C. albicans,* This is confirmed by identification tests.

If such colonies are not present, or the identification tests are negative, *C. albicans* is absent and the product passes the test.

Recommended Solutions and Culture Media

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

Buffered sodium chloride-peptone solution pH 7.0

Potassium dihydrogen phosphate	3.6	g
Disodium hydrogen phosphate dihydrate	7.2	g
Sodium chloride	4.3	g
Peptone (meat or casein)	1.0	g
Purified water	1000	ml

0.1 per cent to 1.0 per cent w/v polysorbate 20 or polysorbate 80 may be added, if required. Sterilise.

Stock Buffer Solution- Transfer 34 g of potassium hydrogen phosphate to a 1000 ml volumetric flask, dissolve in 500 ml od Purified water, adjust with sodium hydroxide to a pH of 7.2 ± 0.2, add purified water to volume and mix. Dispense in containers and sterilize. Store in temperature between 2º to 8º.

Phosphate Buffer solution pH 7.2- Prepare a mixture of purified water and stock buffer solution (800:1 v/v), and sterilize.

Medium 1. Casein soyabean digest broth

Pancreatic digest of casein	17.0 g
Papaic digest of soyabean	3.0 g

Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Dextrose monohydrate	2.5 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 7.3 ± 0.2.

Medium 2. Casein soyabean digest agar

Pancreatic digest of casein	15.0 g
Papaic digest of soyabean meal	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 7.3 ± 0.2.

Medium 3. Sabouraud dextrose broth

~~Peptones (meat and casein)~~ Mixture of Peptone and Tryptone (1:1)	10.0 g
Dextrose monohydrate	20.0 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 5.6 ± 0.2.

Medium 4. Sabouraud dextrose agar with antibiotic(s)

~~Peptones (meat and casein)~~ Mixture of Peptone and Tryptone (1:1)	10.0 g
Dextrose monohydrate	40.0 g
Agar	15.0 g
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 5.6 ±0.2. Sterilise immediately before use, add 0.1 g of benzylpenicillin sodium/potassium and 0.1 g of tetracycline/tetracycline hydrochloride or alternatively add 50 mg of chloramphenicol per liter of medium as sterile solutions.

Medium 5. Enterobacteria enrichment broth-Mossel

Pancreatic digest of gelatin	10.0 g
Dextrose 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	15.0mg
Purified water	1000 ml

Adjust the pH after heating 7.2 ± 0.2. Heat at 100º for 30 minutes and cool immediately.

Medium 6. 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
Dextrose monohydrate	10.0	g
Agar	15.0	g
Neutral red	30.0	mg
Crystal violet	2.0	mg
Purified water	1000	ml

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

Medium 7. MacConkey broth

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

Adjust the pH so that after sterilisation it is 7.3 ± 0.2.

Medium 8. MacConkey agar

Pancreatic digest of gelatin	17.0	g
Peptones (meat and casein, equal parts)	3.0	g
Lactose	10.0	g
Sodium chloride	5.0	g
Bile salts	1.5	g
Agar	13.5	g
Neutral red	30.0	mg
Crystal violet	1.0	mg
Purified water	1000	ml

Adjust the pH so that after sterilisation it is 7.1 ± 0.2. Boil the mixture of solids and water for 1 minute to ~~effect solution~~ dissolve the medium completely.

Medium 9. Rappaport Vassiliadis Salmonella Enrichment broth

Soya peptone	4.5	g
Magnesium chloride hexahydrate	29.0	g
Sodium chloride	8.0	g
Dipotassium phosphate	0.4	g
Potassium dihydrogen phosphate	0.6	g
Malachite green	0.036	g
Purified water	1000	ml

Dissolve, warm slightly. Sterilise at a temperature not exceeding 115º ~~for 30 minutes~~ using a validated cycle. pH 5.2 ± 0.2 after autoclaving.

Medium 10. Wilson and Blair's BBS Agar

Solution (i) Bismuth Sulphite Glucose Phosphate mixture

Bismuth ammonio - citrate scales	30.0 g
Sodium sulphite	100.0g.
Disodium hydrogen phosphate	100.0 g
Glucose	50.0 g
Purified water	1000 ml

Ascpticaly dissolve the Bismuth Ammonio – Citrate Scales in 250 ml of boiling water and the Sodium Sulphite in 500 ml of boiling water. Mix the solutions and while mixture is boiling add the Disodium Hydrogen Phosphate. When the mixture is cool, add the glucose previously dissolved in 250 ml boiling water and cool. This mixture can then be kept for few months.

Solution (ii) Iron citrate brilliant green mixture

Ferric citrate, brown scales	2.0 g
Brilliant green	0.25g
Purified water	225 ml

With sterile precautions mix a solution of ferric citrate in 200 ml water with the solution of brilliant green in 25 ml water. This mixture can then be kept for few months.

Solution (iii) Nutrient Agar Solution

Peptone	1.0 g
Beef Extract	1.0 g
Sodium Chloride	0.5 g
Agar	2.0 g
Purified water	100 ml

Mix thoroughly, sterilised and cool to 45^{o} to 50^{o}.

Complete Medium

Sterile Nutrient agar	100 ml
Bismuth sulphite glucose phosphate mixture	20 ml
Iron citrate brilliant green mixture	4.5 ml

Mix aseptically 20 volume of solution (i) and 4.5 volume of solution (ii) with 100 volume of Solution (iii) previously melted of Sterile Nutrient agar and cool to a temperature 60^{o} and pour.

Medium 11. GN Broth

Polypeptone peptone	20.0 g
Glucose	1.0 g
Sodium citrate	2.0 g
Sodium deoxycholate	0.5 g
Di-potassium hydrogen phosphate	4.0 g
Mono potassium dihydrogen phosphate	1.5 g
Sodium chloride	5.0 g

Purified water	1000 ml

Adjust the pH so that after heating it is 7.0 ± 0.2. Mix and allow to stand for 15 minutes. With continuous stirring, bring gently to the boil and maintain at boiling point ~~until solution is complete~~ till completely dissolved.

Medium 12. 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	80.0 mg
Agar	13.5 g
Sodium deoxycholate	2.5 g
Sodium thiosulphate	6.8 g
Ferric ammonium citrate	800 mg
Purified water	1000 ml

Adjust the pH so that after sterilisation it is 7.4 ± 0.2. Heat to boiling, cool to 50º and pour into petri dishes. Do not heat in an autoclave.

Medium 13. Cetrimide agar

Pancreatic digest of gelatin	20.0 g
Magnesium chloride	1.4 g
Potassium sulphate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Glycerin	10.0 g
Purified water	1000 ml

Heat to boiling for 1 minute with shaking. Adjust the pH so that after sterilisation it is ~~7.0 to 7.4~~ 7.0 ± 0.2.

Medium 14. Mannitol Salt Agar Medium

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	25.0 mg
Purified water	1000 ml

~~Mix. Heat with frequent agitation and boil~~ Heat to boiling for 1 minute with shaking to ~~effect solution~~ dissolve the medium completely. Adjust the pH after sterilisation to 7.4 ± 0.2.

Medium 15. Reinforced medium for Clostridia

Beef extract	10.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Soluble starch	1.0 g
Dextrose 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, and dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilisation it is about 6.8 ± 0.2.

Medium 16. 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 15.0g
Purified water	1000 ml

Hydrate the agar, dissolve by heating to boiling with continuous stirring. If necessary, adjust the pH so that after sterilisation it is 7.3 ± 0.2. Sterilise, allow to cool to 45º to 50º; add, where necessary, Gentamicin sulphate corresponding to 20 mg of gentamicin base and pour into petri dishes.

Acceptance Criteria for Microbiological Quality of Non Sterile Pharmaceutical Substances and Non Sterile Doses Forms.

This provides acceptance criteria for microbiological quality of non sterile substances of pharmaceutical use and non sterile dosage forms, unless otherwise specified in the monograph.

If microorganisms are present in a pharmaceutical preparation, they can reduce or inactivate the therapeutic activity of the product or can adversely affect the health of the patient. Hence pharmaceutical preparations should have low bio-burden and they should not have specified microorganisms which are harmful.

Microbial examination of non-sterile products is performed according to the methods given above. Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use and non sterile pharmaceutical products based upon the Total Aerobic Viable Count (TAC) and the Total Fungal Count (TFC) are given in Tables 5 and 6 respectively.

The significance of microorganisms in nonsterile pharmaceutical products should be evaluated in terms of the use of the product, the nature of the product and the potential hazard to the user. This includes the list of specified microorganisms for which acceptance criteria are set. This list is not exhaustive and depending on the nature of the ingredients and manufacturing process it may be necessary to test for other microorganisms. The importance of the microorganisms isolated from the product may be evaluated in terms of the following.

i. The nature of the product, its ability to support growth, the type and the quantity of the antimicrobial preservatives added.

ii. Use of product. Hazard varies according to the place of application (on intact skin or on wound) as well as route of administration (eye, nose, oral).

iii. Use of immunosuppressive agents, corticosteroids, etc.

iv. Intended recipient. Risk may vary for neonates, adults and the debilitated.

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

Table 5 – Acceptance criteria for microbiological quality of nonsterile substances for pharmaceutical use

	TAC (CFU per g or per ml)	TFC (CFU per g or per ml)
Substances for pharmaceutical use	10^3	10^2

Table 6- Acceptance criteria for microbiological quality of nonsterile dosage forms

Route of Administration	TAC (CFU per g or per ml)	TFC (CFU per g or per ml)	Specified Microorganisms
Non-aqueous preparations for oral use	10^3	10^2	*Escherichia coli* – absent in 1 g or 1 ml
Aqueous preparations for oral use	10^2	10^1	*Escherichia coli* – absent in 1 g or 1 ml
Rectal use	10^3	10^2	----

Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	*Staphylococcus aureus* – absent in 1 g or 1 ml *Pseudomonas aeruginosa* – absent in 1 g or 1 ml
Vaginal use	10^2	10^1	*Pseudomonas aeruginosa* – absent in 1 g or 1 ml *Staphylococcus aureus* – absent in 1 g or 1 ml *Candida albicans* – absent in 1 g or 1 ml
Inhalation use (special requirements apply to Liquid preparations for nebulisation)	10^2	10^1	*Staphylococcus aureus* – absent in 1 g or 1 ml *Pseudomonas aeruginosa* – absent in 1 g or 1 ml Bile-tolerant Gram-negative bacteria – absent in 1 g or 1 ml
Transdermal patches (limits for one patch including adhesive layer and backing)	10^2	10^1	*Staphylococcus aureus* – absent in ~~1 g or 1 ml~~ per patch *Pseudomonas aeruginosa* – absent in ~~1 g or 1 ml~~ per patch

Acceptance Criteria for Microbiological Quality of Herbal Medicinal Products for Oral Use

This provides acceptance criteria for microbiological quality of herbal medicinal products for oral use, unless otherwise specified in the monograph. The significance of microorganisms in herbal products should be evaluated in terms of the use of the product, the nature of the product and the potential hazard to the user. This includes the list of specified microorganisms for which acceptance criteria are set. This list is not exhaustive and depending on the nature of the ingredients and manufacturing process it may be necessary to test for other microorganisms.

Microbial examination of non sterile products is performed according to the methods given above. Acceptance criteria for microbiological quality of herbal medicinal products for oral use based upon the total aerobic viable count (TAC) and the total fungal count (TFC) are given in Table 7A, 7B and 7C respectively. Acceptance criteria are based on individual results or on the average of replicate counts when replicate counts are performed.

REVISED GENERAL CHAPTER

Table 7 A – Herbal medicinal products containing herbal drugs, with or without excipients, intended for the preparation of infusions and decoctions using boiling water (for example herbal teas, with or without added flavour)

TAC	Acceptance criterion: 10^7 CFU per g
TFC	Acceptance criterion: 10^5 CFU per g
Escherichia coli	Acceptance criterion: 10^3 CFU per g
Salmonella *Shigella*	Absent in 10 g Absent in 10 g

Table 7 B – Herbal medicinal products containing, for example, extracts and/or herbal drugs, with or without excipients, where the method of processing (for example, 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

TAC	Acceptance criterion: 10^4 CFU per g or CFU per ml
TFC	Acceptance criterion: 10^2 CFU per g or CFU per ml
Bile-tolerant Gram-negative bacteria	Acceptance criterion: 10^2 CFU per g or CFU per ml
Escherichia coli	Absent in 1 g or 1 ml
Salmonella	Absent in 10 g or 10 ml
Shigella	Absent in 10 g or 10 ml

Table 7 C – Herbal medicinal products containing, for example, extracts and/or herbal drugs, with or without excipients, where it can be demonstrated that the method of processing (for example, 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 under 7 B

TAC	Acceptance criterion: 10^5 CFU per g or CFU per ml
TFC	Acceptance criterion: 10^4 CFU per g or CFU per ml
Bile-tolerant Gram-negative bacteria	Acceptance criterion: 10^4 CFU per g or CFU per ml
Escherichia coli	Absent in 1 g or 1 ml
Salmonella	Absent in 10 g or 10 ml
Shigella	Absent in 10 g or 10 ml

It is recognized that for some herbal medicinal products the criteria given above under 7A, 7B or 7C for TAC, TFC and bile tolerant gram-negative bacteria can not be met because of the typical level of microbial contamination. Higher acceptance criteria may be applied on the basis of risk assessment that takes account of qualitative and quantitative characterization of the bioburden and the intended use of the medicinal product.