

Table 3 Minimum quantity to be used for each medium

Product to be tested	Quantity per container	Minimum quantity to be used
Liquids	Less than 1 ml	The whole contents of each container
	Not less than 1 ml but not greater than 40 ml	Half the content of each container, but not less than 1 ml
	Greater than 40 ml, but not greater than 100 ml	20 ml
	Greater than 100 ml	10% of the content of each container, but not less than 20 ml
Solids	Less than 50 mg	The whole contents of each container
	50 mg or more but less than 300 mg	Half the contents of each container but not less than 50 mg
	300 mg or more but not greater than 5 g	150 mg
	Greater than 5 g	500 mg Half the contents of each container(biologics)
Bulk or final bulk of biologics		Half the contents of each container
Medical devices	Surgical dressing/cotton/gauze(in packages)	100 mg or 1 cm×3 cm per package
	Sutures and other individually packaged single-use material	The whole device ^①
	The disposable medical apparatus with pathways (infusion bag)	Half the internal surface area
	Other medical devices	The whole device ^① (cut into pieces or disassembled)

Note:①For extremely large devices, more than 2000 ml of medium may be used to achieve complete immersion of those portions.

1105 Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests

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

The tests are designed primarily to determine whether nonsterile pharmaceutical products including raw materials and excipients comply with an established specification for microbiological quality. When used for such purposes, follow the instructions given below, including the number of samples to be taken and interpret the results as stated below. The methods are not applicable to products containing viable microorganisms as active ingredients unless otherwise specified.

The conditions of the microbial enumeration test should comply with the requirement of microbiological examination. Strictly sterile operation must be employed to avoid extrinsic microbial contamination of the product. The precautions taken to avoid contamination must not affect any microorganisms which are to be revealed in the tests. Clean air area, work surfaces, and the environments should be monitored regularly.

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

If surface-active substances are used for sample preparation, their absence of toxicity for microorganisms and their compatibility with any neutralizers or inactivators used must be demonstrated.

Enumeration Methods

Plate method, membrane filtration method and the most-probable-number (MPN) method can be all used for microbial enumeration test. The MPN method is generally the least accurate method for microbial counts, however, for certain product groups with very low bioburden, it may be the most appropriate method.

The choice of a method is based on factors such as the nature of the product and the required limit of microorganisms. The method chosen must allow testing of a sufficient sample size to judge compliance with the specification. The suitability of the chosen method must be established.

Growth Promotion of the Counting Media and Suitability of the Counting Method

The growth promotion of the media used in enumeration tests must be confirmed.

Method suitability tests should be carried out for the microbial counting method to confirm the method used is suitable for the microbial count in the product to be examined.

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

Table 1 Preparation and use of test microorganisms

Microorganism	Preparation of test strain	Growth promotion		Suitability of counting method	
		Total aerobic microbial count	Total molds and yeasts count	Total aerobic microbial count	Total molds and yeasts count
<i>Staphylococcus aureus</i> [CMCC (B) 26003]	Soybean-casein digest agar or Soybean-casein digest broth 30-35°C 18-24 h	Soybean-casein digest agar or Soybean-casein digest broth 30-35°C ≤100 cfu 30-35°C ≤ 3 days	/	Soybean-casein digest agar or Soybean-casein digest broth (MPN) 30-35°C ≤100 cfu 30-35°C ≤ 3 days	/
<i>Pseudomonas aeruginosa</i> [CMCC (B) 10104]	Soybean-casein digest agar or Soybean-casein digest broth 30-35°C 18-24 h	Soybean-casein digest agar or Soybean-casein digest broth 30-35°C ≤100 cfu 30-35°C ≤ 3 days	/	Soybean-casein digest agar or Soybean-casein digest broth (MPN) 30-35°C ≤100 cfu 30-35°C ≤ 3 days	/
<i>Bacillus subtilis</i> [CMCC (B) 63501]	Soybean-casein digest agar or Soybean-casein digest broth 30-35°C 18-24 h	Soybean-casein digest agar or Soybean-casein digest broth 30-35°C ≤100 cfu 30-35°C ≤ 3 days	/	Soybean-casein digest agar or Soybean-casein digest broth (MPN) 30-35°C ≤100 cfu 30-35°C ≤ 3 days	/
<i>Candida albicans</i> [CMCC (F) 98001]	Sabouraud dextrose agar or Sabouraud-dextrose broth 20-25°C 2-3 days	Soybean-casein digest agar 30-35°C ≤100 cfu 30-35°C ≤ 5 days	Sabouraud dextrose agar 20-25°C ≤100 cfu 30-35°C ≤ 5 days	Soybean-casein digest agar (MPN: Not applicable) 30-35°C ≤100 cfu 30-35°C ≤ 5 days	Sabouraud dextrose agar 20-25°C ≤100 cfu 30-35°C ≤ 5 days
<i>Aspergillus niger</i> [CMCC (F) 98003]	Sabouraud dextrose agar or Potato dextrose agar 20-25°C 5-7 days, or until good sporulation is achieved	Soybean-casein digest agar 30-35°C ≤100 cfu 30-35°C ≤ 5 days	Sabouraud dextrose agar 20-25°C ≤100 cfu 30-35°C ≤ 5 days	Soybean-casein digest agar (MPN: Not applicable) 30-35°C ≤100 cfu 30-35°C ≤ 5 days	Sabouraud dextrose agar 20-25°C ≤100 cfu 30-35°C ≤ 5 days

Note: When Rose bengal agar is needed for the total number of molds and yeasts test, growth promotion of the media must be confirmed according to Sabouraud dextrose agar.

Preparation of test strains and suspensions

Test strains The viable microorganisms used for inoculation are not more than 5 Passages (the freeze-dried strains obtained from Type Culture Collection are defined as Passage 0). Use appropriate culture maintenance techniques to preserve the test strains to keep their biological characteristics. Test strains used in medium growth promotion test and counting method suitability test are summarized in Table 1.

Preparation of microbial suspension Inoculate the test strains as the incubation conditions described in Table 1. Suspend fresh cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Candida albicans* in sterile buffered sodium chloride-peptone solution pH 7.0 or 0.9% sterile sodium chloride solution to make test suspensions of suitable concentrations. Add appropriate volume of sterile buffered sodium chloride-peptone solution pH 7.0 or 0.9% sterile sodium chloride solution which both containing 0.05% (ml/ml) polysorbate 80 in the fresh cultures of *Aspergillus niger* to elute the spores. Then, suck out the spore suspension by appropriate method and place into a sterile test tube. Prepare suitable concentrations of spore suspensions of *Aspergillus niger* with sterile buffered sodium chloride-peptone solution pH 7.0, or 0.9% sterile sodium chloride solution which both containing 0.05% (ml/ml) polysorbate 80.

Use the suspensions within 2 h if stored at ambient temperature. Use within 24 h if stored at 2-8°C. The stable spore suspension of *Aspergillus niger* may be maintained at 2-8°C and used within the validated period of time.

Negative control

To verify testing conditions, a negative control should be performed. There must be no growth of microorganisms. A failed negative control requires an investigation.

Growth promotion of the media

The media used for microbial counting, such as ready-prepared medium, the medium prepared either from dehydrated medium or from the ingredients described, should be validated for growth promotion.

Inoculate tubes/plates of Soybean-casein digest agar or Soybean-casein digest broth or inoculate plates of Sabouraud dextrose agar with not more than 100 cfu of the microorganisms indicated in Table 1 and incubate under the conditions described in Table 1. Use 2 tubes or 2 plates for each test strain in duplicate. At the same time, perform the tests using corresponding reference media in the same way as to the media tested.

The ratio between average numbers of colonies on the solid test media and reference media ranges in 0.5-2, and the colonial morphology on test media should be consistent with the reference media. The test microorganisms in the tubes of

liquid test media should grow well compared to the microorganisms in the tubes of reference media.

Suitability of the counting method

1. Preparation of the sample

The method for sample preparation depends on the physical and biological characteristics of the product to be tested. If a heating procedure is required during preparation, the sample solution should be heated evenly. The temperature should not exceed 45°C. The time interval between preparation of the sample and addition of the test medium should exceed 1 h. The common methods used for sample solution preparation are shown as follows. If none of the procedures described below can be demonstrated to be satisfactory, a suitable alternative procedure must be developed.

Water-soluble products Dissolve or dilute the product to be examined in sterile Buffered sodium chloride-peptone solution pH 7.0, Phosphate buffer solution pH 7.2, or Soybean-casein digest broth to prepare a 1 : 10 dilution. If necessary, adjust to a pH of 6-8. Further serial 10-fold dilutions, where necessary, are prepared with the same diluent. The stock solution of water-soluble liquid preparation can also be used as the sample solution.

Non-fatty products insoluble in water Suspend the product to be examined in Buffered sodium chloride-peptone solution pH 7.0, Phosphate buffer solution pH 7.2, or Soybean-casein digest broth to prepare a 1 : 10 dilution. A surface-active agent such as 0.1% (ml/ml) polysorbate 80 may be added to assist the suspension of poorly dispersible substances. If necessary, adjust to a pH of 6-8. Further serial 10-fold dilutions, where necessary, are prepared with the same diluent.

Fatty products Dissolve the product to be examined in sterile isopropyl myristate, or mix the product to be examined with the minimum necessary quantity of sterile polysorbate 80 or other noninhibitory sterile surface-active reagent. Temperature of the surface-active agent should be not more than 40°C or in exceptional cases, not more than 45°C. Mix carefully and if necessary maintain the temperature in a water-bath. Add sufficient of the pre-warmed chosen diluent to make a 1 : 10 dilution of the original product. Mix carefully, while maintaining the temperature for the shortest time necessary for the formation of emulsion. Further serial 10-fold dilutions may be prepared using the chosen diluent containing a suitable concentration of sterile polysorbate 80 or another non-inhibitory surface-active reagent.

Film products Cut the product to be examined into pieces, add sterile buffered sodium chloride-peptone solution pH 7.0, Phosphate buffer solution pH 7.2, or Soybean-casein digest broth, then soak and shake to prepare a 1 : 10 dilution. If necessary, adjust to pH of 6-8. Further serial 10-fold dilutions, where necessary, are prepared with the same diluent.

Enteric-coated and colonic-coated products Place the product to be examined in sterile buffered phosphate solution pH 6.8 (for enteric-coated products) or sterile buffered phosphate solution pH 7.6 (for colonic-coated products), maintain in a 45°C water-bath, and shake to dissolve, to prepare a 1 : 10 dilution. Further serial 10-fold dilutions, where necessary, are prepared with the same diluent.

Aerosol products Freeze the product to be examined at -20°C or other suitable temperature for 1 h. Take out the product and disinfect the position to be opened or valve rapidly. Put the container in upright position, and drill a hole in the

appropriate position matching the valve with a sterile steel cone or a needle-like device. The size and depth of each hole of container should be as consistent as possible. There should be no obvious propellant thrown when pulling out the steel cone. Rotate the container gently to release the propellant slowly. Special equipment can also be used to release the propellant. When the release of the propellant finished, aseptically open the container and transfer the residual contents to a sterile container, mix, and rinse the inner of the container with flushing solution if necessary. Other suitable methods can also be used for sampling.

Patches and Cataplasms Remove the protective cover sheets 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 a chosen diluent containing surfactants, such as polysorbate 80 or lecithin. Shake the preparation vigorously for at least 30 minutes. Further serial 10-fold dilutions, where necessary, are prepared with the same diluent.

2. Inoculation and dilution

Inoculate and dilute the sample prepared according to Table 1 and the following requirements, to prepare samples for microbial recovery test. 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 must be used for the test.

(1) **Inoculum group** Add the microbial suspension into the sample prepared as described above and mix. The quantity of microorganisms in 1 ml sample or solution filtered by each membrane filter should be not more than 100 cfu.

(2) **Product group** Perform the same examination as the inoculum group using the diluent of prepared sample instead of the microbial suspension.

(3) **Control group** Add the microbial suspension into corresponding diluent without neutralizer, inactivator and surfactants instead of the sample and perform the same examination as the inoculum group.

If it is not possible to choose the lowest possible dilution factor of the prepared sample to perform suitability test due to antimicrobial activity or poor solubility of the sample, further appropriate protocols must be developed to treat the sample. If inhibition of growth by the sample cannot be avoided by other methods, the aliquot of the microbial suspension may be added after neutralization, dilution, or filtration.

3. Neutralization or removal of antimicrobial activity

After inoculation, obtain the microbial count following the procedure described in following *Recovery of microorganism in the presence of product*. If the number of colonies of the inoculum group minus that of the product group is less than 50% of the number of colonies of the control group, the following procedures should be carried out to remove the antimicrobial activity of samples.

- (1) An increase in the volume of the diluents or medium.
- (2) Incorporation of an appropriate neutralizing or inactivating agents.

Neutralizing or inactivating agents may be used to neutralize or remove the activity of antimicrobial agents (see Table 2). They may be added to the chosen diluents or the medium preferably before sterilization. If used, their efficacy and their absence of toxicity for microorganisms must be

demonstrated by carrying out a blank with neutralizer or inactivator and without product. The ratio of the number of colonies of the neutralizer or inactivator group and the number of colonies of the control group should fall in the range of 0.5-2.

Table 2 Common neutralizing or inactivating agents/methods for interfering substances

Interfering substance	Potential neutralizing or inactivating agents/method
Glutaraldehyde, mercurials	Sodium hydrogen sulfite
Phenolics, alcohol, aldehydes, sorbate	Dilution
Aldehydes	Glycine
Quaternary ammonium compounds (QCA1), parahydroxybenzoates (parabens), bisbiguanides	Lecithin
QACs, iodine, parabens	Polysorbate
Mercurials	Thioglycollate
Mercurials, mercuric compounds, aldehydes	Thiosulfate
EDTA, Quinolones	Mg or Ca ions
Sulfonamides	Para-aminobenzoates
β-Lactam antibiotics	β-Lactamase

(3) Membrane filtration.

(4) A combination of the above measures.

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. Therefore, the test should be performed with a higher dilution factor in accordance with microbial growth and the specific acceptance criterion, provided they had no interference with the interpretation of test results. If the suitability of the method meets the requirements, this dilution factor should serve as the lowest dilution factor to perform the tests of the product.

4. Recovery of microorganism in the presence of product

For each of the microorganisms listed in Table 1, separate tests are performed. The plate method, membrane filtration method, or the MPN method can be used in the test.

(1) Plate-count methods The plate-count methods consist of the pour-plate method and the surface-spread method. For each of the microorganisms listed in Table 1, perform plate methods at least in duplicate for each medium and calculate the mean count of the result.

Pour-plate method For Petri dishes 90mm in diameter, add into the dish 1ml of the sample prepared as described under *Preparation of the Sample, Inoculation and Dilution, and Neutralization or Removal of Antimicrobial Activity* and 15-20 ml of Soybean-casein digest agar or Sabouraud dextrose agar, both media maintained at not more than 45°C. Mix, solidify, and incubate upside down. If larger Petri dishes are used, the amount of agar medium is increased accordingly. Incubate the plates as indicated in Table 1 and perform the counting. The numbers of microorganisms in Product group

and the control group should be determined with the same method. Calculate the arithmetic mean count of each group.

Surface-spread method For Petri dishes 90mm in diameter, add an appropriate amount (generally 15-20 ml) of Soybean-casein digest agar or Sabouraud dextrose agar at about 45°C to each Petri dish and allow to solidify. Dry the surface of medium with appropriate methods. If larger Petri dishes are used, the volume of the agar medium is increased accordingly. Spread a measured volume of not less than 0.1 ml of the sample, prepared as directed under *Preparation of the Sample, Inoculation and Dilution, and Neutralization or Removal of Antimicrobial Activity* over the surface of the medium. Incubate the plates as indicated in Table 1 and perform the counting. The numbers of microorganisms in product group and the control group should be determined by the same method. Calculate the arithmetic mean count of each group.

(2) Membrane filtration Use membrane filters having a nominal pore size not greater than 0.45 µm, and generally 50mm in diameter. If membrane filters of different diameters are used, the volume of rinse should be adjusted accordingly. 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. Filters and membranes should be sterilized by appropriate methods before use. The integrity of the membrane should be ensured before and after filtration. For water-soluble products, it is suggested to filter a small amount of rinsing fluid to wet the membrane before filtrating the sample. For fatty products, the membrane and filter should be dried thoroughly before use. For the maximum filtration efficiency of the membrane, attention should be paid to maintaining the entire surface of the membrane covered by the sample solution and the rinsing liquid. If the membrane needed to be rinsed after filtrating the sample, the volume of rinsing liquid is 100 ml per membrane for each time generally. To avoid the microorganisms on the membrane being injured, the total volume should not exceed 500ml generally and the maximum is 1000 ml.

Add a suitable quantity of the sample prepared as described under *Preparation of the Sample, Inoculation and Dilution, and Neutralization or Removal of Antimicrobial Activity* (preferably representing 1 g, 1 ml or 10 cm² of the product, or less if large numbers of microorganisms are expected) into an appropriate volume of diluents, mix, filter, and rinse the membrane with rinsing liquid.

For the determination of total aerobic microbial count (TAMC), transfer the membrane to the surface of Soybean-casein digest agar with the surface containing microorganisms upside; For the determination of total combined yeasts/molds count (TYMC) transfer the membrane to the surface of Sabouraud dextrose agar with the surface containing microorganisms upside. Incubate the plates as indicated in Table 1. Perform the counting. For each of the test strains and media listed in Table 1, at least one membrane filter is used. The numbers of microorganisms in product group and the control group should be determined with the same method.

(3) Most-Probable-Number (MPN) Method The precision and accuracy of the MPN Method is less than that of the Plate-count Method or the Membrane Filtration method. For these reasons, the MPN Method is only reserved for the enumeration of sample TAMC in situations where no other method is available and it is not applicable to the enumeration of molds. If the use of the method is justified, proceed as follows.

Prepare a series of at least 3 serial 10-fold dilutions of the product as described under *Preparation of the Sample*, *Inoculation and Dilution*, and *Neutralization or Removal of Antimicrobial Activity*. From each level of dilution, 3 aliquots of 1 ml are used to inoculate 3 tubes with 9-10 ml of Soybean-casein digest broth. The inoculum group should be determined with the same method. If necessary, a surfactant, or a neutralizer or an inactivator may be added into the medium.

Incubate all tubes at 30-35°C for not more than 3 days. Observe the growth of microorganisms in each tube daily. 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 Soybean-casein digest agar, for 1-2 days at the same conditions and observe the growth of microorganisms. Determine the most probable number of TAMC per gram or millilitre or 10 cm² of the product to be examined from Table 3.

5. Interpretation of results

When verifying the suitability of the membrane filtration method or the plate-count method, the ratio of the count of the inoculum group minus that of the product group to the count of the control group should be in the range of 0.5-2. When verifying the suitability of the MPN method, the calculated value from the inoculum group must be within 95% confidence limits of the results obtained with the control group. If the recovery of each test strain meets the requirement, the count method could be used to examine the total aerobic microbial count (TAMC) and total yeasts and molds count (TYMC).

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

Testing of Products

Amount used for the Test The amount used for the test refers to the quantity (g, ml or cm²) of the product to be examined for one test. Generally, no less than 2 minimum packing of units the product to be examined should be taken randomly. Then mix and take specified amount of sample to test. Unless otherwise specified, the common amount used for the test is 10 g or 10 ml of the product to be examined. For pellicles, patches and cataplasms form, sample 100 cm². Samples should be taken from more than 2 minimum packing units. For big honey pill, samples should not be less than 4 pills. For pellicles, patches and cataplasms form, sample should not be less than 4 patches.

The amount to be tested may be reduced for expensive medicines and micro-packaging drugs. If the amount of API per dosage unit (e.g., tablet, capsule) is less than or equal to 1 mg, or the amount per g or ml of API (for preparations not presented in dose units) is less than 1 mg, the amount used for the test is not less than 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 1000 ml or 1000 g), the amount tested shall be 1% of the batch unless otherwise specified, risk assessment is required when the amount is less. For products where the total number of entities in a batch is less than 200, the sample size may be reduced to two units, or one unit if the size is less than 100.

Examination of the product

Examine the total aerobic microbial count (TAMC) and total yeast and mold counts (TYMC) according to the counting methods confirmed in *Suitability of the counting method*.

Table 3 Most-probable-number values of microorganisms

Numbers of tubes showing growth			MPN of TAMC	95% Confidence limits	
Number of g or ml or 10 cm ² of product per tube			MPN/g or ml or 10 cm ²	Lower limit	Upper limit
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		

Note: When the amounts listed in the table switch to 1 g (or ml, 10 cm²), 0.1 g (or ml, 10 cm²), and 0.01 g (or ml, 10 cm²), the figures in this table should be correspondingly reduced by 10 times; when switch to 0.01 g (or ml, 10 cm²), 0.001 g (or ml, 10 cm²), and 0.0001 g (or ml, 10 cm²), the number should be multiplied by 10 times. Other situation could be done by analogy.

Soybean-casein digest agar or Soybean-casein digest broth is used for the total aerobic microbial count (TAMC). Sabouraud dextrose agar is used for the total yeasts and molds count (TYMC).

Negative Control Test

Negative control test is conducted by replacing the sample preparation with diluents according to the confirmed examination method. There should be no growing microorganisms. A failed negative control requires an investigation.

1. Plate-count methods

The plate-count methods consist of the pour-plate method and the surface-spread method. Unless otherwise specified, take specified amount of sample, prepare the sample, plate the sample and count the organisms in the product according to a method that has been confirmed per *suitability of the counting method*. Prepare for each medium at least 2 Petri dishes for each level of dilution.

Incubation and enumeration Unless otherwise specified, incubate the plates of Soybean-casein digest agar at 30–35°C for 3–5 days and the plates of Sabouraud dextrose agar at 20–25°C for 5–7 days. Observe the growth of microorganisms. Count all the growing colonies and report. For plates with colonies spreading into pieces should not be counted. After counting, calculate the arithmetic mean count for each level dilution and report in accordance with the *reporting criteria of microbial counts*. If both counts at the same level of dilution are not less than 15, the difference between the two Petri dishes should be not more than 1-fold.

Reporting criteria of microbial counts For the total aerobic microbial count (TAMC) choose a dilution less than 300 cfu. For total yeasts/molds count (TYMC), choose a dilution less than 100 cfu. Both levels of dilution described above are the basis of microbial counts of reporting. Calculate the number of microorganisms contained in 1 g, 1 ml or 10 cm² of products based on the highest mean colony count. Present microbial counts of reporting to 2 significant figures.

If no colony grows in the Petri dishes of each dilution, or only the lowest level of dilution grows less than 1 colony in average, the colony count should be reported as <1 multiplied by the lowest dilution factor.

2. Membrane filtration

Unless otherwise specified, prepare the sample using a method that has been confirmed per *Suitability of the Counting Method*. Transfer the appropriate amount of sample equivalent to 1 g, 1 ml or 10 cm² of the product to be examined to the appropriate amount of diluents in accordance with the confirmed method per *Suitability of the Counting Method*, filter immediately and rinse. For samples containing larger numbers of microorganisms, choose the suitable dilutions to filter. After rinsing, transfer the membrane to the surface of Soybean-casein digest agar or Sabouraud dextrose agar with the surface containing microorganisms upside.

Incubation and enumeration For incubation and enumeration, proceed as plate-count method. The number of colonies per membrane should not be more than 100 cfu.

Reporting criteria of microbial counts Report the number of microorganisms equivalent to 1 g, 1 ml or 10 cm² of the product. If no colony grows on the membrane, report the number of colony as <1 (1 g, 1 ml or 10 cm² of the product filtered by one piece of membrane), or report the count as <1 multiplied by the lowest dilution factor.

3. MPN method

Prepare and dilute the specified amount of sample using a

method that has been confirmed per *Suitability of the Counting Method*. Incubate all tubes for 3–5 days at 30–35°C. Subculture if necessary, using the procedure shown to be suitable. Record for each level of dilution the number of tubes showing microbial growth. Determine the most probable number of microorganisms per gram or millilitre or 10 cm² of the product to be examined from Table 3.

Interpretation of the Results

The total aerobic microbial count (TAMC) is considered to be equal to the total number of colonies found in Soybean-casein digest agar; if colonies of fungi are detected on this medium, they are counted as part of the TAMC. The total combined yeast/mold counts (TYMC) are considered to be equal to the number of colonies found in Sabouraud dextrose agar. If colonies of bacteria are detected on this medium, they are counted as part of the TYMC. When TYMC exceed the acceptance criterion due to the bacterial growth, Sabouraud dextrose agar containing antibiotics (such as chloramphenicol, gentamicin) or other selective media (such as rose bengal agar) may be used. When a selective medium is used, suitability of the medium should be tested. If the count is carried out by the MPN method the calculated value is TAMC.

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

10¹ cfu: maximum acceptable count = 20;

10² cfu: maximum acceptable count = 200;

10³ cfu: maximum acceptable count = 2000; and so forth.

If the results of the total aerobic microbial count and the total combined yeasts/mold count meet the specified requirements for the product being examined, the product is qualified. If any of them is out of the range, the product is unqualified.

Diluents, Rinsing fluids, Media

See *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms* (1106).

1106 Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms

The tests for specified microorganisms described hereafter will allow determination of the absence of, or limited occurrence of, specified microorganisms that may be detected in products. The tests are designed primarily to determine whether non-sterile pharmaceutical products, including raw materials and excipients, comply with Microbial contamination limit of pharmaceutical preparations. When used for such purposes, follow the instructions given below, including test amount and result interpretation.

If specified microorganisms or other pathogens are detected in products once, the product is unqualified. No retest should be taken.

The sample preparation and experimental environmental requirements are in accordance with that described in *Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests* (1105).

If the product to be examined has antimicrobial activity, this is insofar as possible removed or neutralized. If neutralizers or inactivators are used in tests, their efficacy and their absence of toxicity for microorganisms must be demonstrated.

If surfactants are used for sample preparation, their absence of toxicity for microorganisms and their compatibility with any neutralizers or inactivators used must be demonstrated.