Points to Consider in Suitability Testing, etc. of Microbial Tests〈*G4-12-190*〉

(微生物学的試験法の適合性試験等における留意事項〈*G4-12-190*〉)

This general information provides points to consider in the suitability tests of Microbiological Examination of Non-sterile Products *<4.05>*, Sterility Test *<4.06>*, Microbial Limit Test for Crude Drugs and Preparations containing Crude Drugs as Main Ingredient *<5.02>* and Preservatives-Effectiveness Tests *<G4-3-170>*.

1. Performing tests

Suitability testing of microbial test methods is preferable to be conducted before or simultaneously with the first microbiological test on the product to be examined to confirm the ability of a chosen test method to detect micro-organisms. If there are any changes in the test method or the product formulation that may affect the test results, the suitability should be confirmed again.

2. Selection and preparation of test strains

Micro-organisms have a variety of natures, which affect their responses to antimicrobial activity and neutralizing methods required for their recovery. Depending on the purpose of a test, representative test strains are selected from bacteria, yeasts, and moulds.

The growth state and the preparation method of the test strains affect the physiological activity of the cells of the test strains. The use of standardized and stable suspensions or the use of a freshly prepared inoculum prepared on a specified liquid medium or agar medium allow for homogeneous and less variable preparation or reproducible preparation. In Preservatives-Effectiveness Tests *<G4-3-170>*, it is preferable to use a freshly prepared inoculum because the bioactivity of the test strains is likely to affect the results.

3. Preparation of test samples

If the preparation processes of test samples may affect micro-organisms present in the product to be examined, confirm the effects of the preparation processes. If the sample preparation is allowed to stand before testing, confirm that there is no change in the number of micro-organisms, etc.

4. General additional procedures for test samples that have antimicrobial activity

When antimicrobial activity is observed in the product to be examined and the test method is changed to remove the antimicrobial activity, repeat the suitability test using the changed test method to verify the reproducibility of the results.

4.1. Dilution

Neutralize the antimicrobial activity of the product to be examined by dilution. The effective dilution factor varies depending on the product to be examined. If the dilution factor required for neutralization is too large, neutralize the antimicrobial activity by other methods.

If dilution makes it difficult to interprete results based on the microbial acceptance criterion in Microbial Attributes of Non-sterile Pharmaceutical Products *<G4-1-170>* specified in the product testing, the number of plates or media may be increased as appropriate.

4.2. Use of neutralizing agents

For the use of neutralizing agents, refer to 3.4.3. of "I. Microbiological Examination of Non-sterile products: Microbial Enumeration Tests" in Microbiological Examination of Non-sterile Products *<4.05>*. For common neutralizing agents for interfering substances, refer to Table 4.05-I-2 in Microbiological Examination of Non-sterile Products *<4.05>*.

If the product to be examined is an antibiotic, it may not be susceptible to neutralization by general neutralizing agents, but rather by enzymatic treatments (penicillinase, β-lactamase, etc.). In the case of enzymatic treatment, an appropriate concentration referring to the information such as Minimum Inhibitory Concentration (MIC) is preferred.

4.3. Membrane filtration

Generally, membrane filters are used. Membrane filters are made of various materials, so select appropriate material by considering the properties and composition of the product to be examined. For example, cellulose mixed ester filters are used for test samples without antimicrobial activity, cellulose nitrate filters are used for aqueous, oily, or weakly alcoholic solutions, and cellulose acetate filters are used for strongly alcoholic solutions. Low binding filters such as polyvinylidene fluoride (PVDF) are also used to reduce the binding of antimicrobial substances on the filter.

Filters can be washed with an appropriate solution to remove antimicrobial substances from the product to be examined. If the suitability is verified, a surfactant such as polysorbate 80 (concentration: 1 g/L) may be added to the washings to neutralize residual or bound antimicrobial substances on the filter or to suspend the test sample.

5. Inoculation of test strains

In the microbial enumeration tests of Microbiological Examination of Non-sterile Products *<4.05>* and Microbial Limit Test for Crude Drugs and Preparations containing Crude Drugs as Main Ingredient *<5.02>*, when a test sample is prepared, the initial prepared test sample (lowest dilution) should be inoculated with test strains to confirm the effect of antimicrobial activity. Even if the test sample is further diluted and tested, inoculate the initial test sample with test strains. Even when a diluent containing a neutralizing agent is used, inoculate the initial test sample with test strains. If antimicrobial activity cannot be removed or neutralized by

various means, such as dilution, use of a neutralizing agent, or membrane filtration, test strains may be added to a test sample diluted to as maximum as possible extent or to the final washings of the filter after membrane filtration. If the product to be examined is used for membrane filtration as it is, the final washings of the filter after membrane filtration is inoculated with test strains.

6. Addition of culture media

In principle, the type of culture medium specified in a microbial test cannot be changed. However, depending on the properties and composition of the product to be examined or the type of contaminating micro-organisms, it may be more appropriate to use a culture medium other than that specified.

6.1 Non-selective media such as for microbial enumeration tests or sterility tests

Perform a growth promotion test to verify the ability to detect micro-organisms, referring to Microbiological Examination of Non-sterile Products <*4.05*> and Sterility Test <*4.06*>, etc.

Some microorganisms require particular components for growth, while others cannot grow in the presence of particular components. It should be noted that the number and type of micro-organisms detected and the appearance of grown colonies may differ depending on the medium composition, even using non-selective media.

6.2 Selective media for tests for specified microorganisms

Perform a growth promotion test to verify the ability to detect micro-organisms, referring to Microbiological Examination of Non-sterile Products <*4.05*>, etc. Note that indication reactions may differ depending on the composition of a culture medium.

7. Incubation time

In the growth promotion tests and the suitability tests, adopt incubation time specified respectively.

However, adopt an appropriate time when it is verified that the growth of the test organisms can be observed and the number of organisms can be measured.

If the shortest incubation time during the specified incubation times is used in product testing using the test method whose suitability is established, the validity of the use of the shortest incubation time should be verified.

8. Counting of the number of colonies in product testing

The counting of the number of colonies by the plate-count method is performed using an appropriate dilution factor. In the product testing, the number of colonies of less than 250 CFU per plate for bacteria and yeast, and less than 50 CFU per plate for moulds as a guide is generally counted. After the counting, take an arithmetic mean for each medium and multiply by the dilution factor to calculate the enumeration of viable micro-organisms.