Control of Culture Media and Strains of Microorganisms Used for Microbial Test Methods

(微生物試験法に用いる培地及び微生物株の管理)

This General Information describes points to consider in the control of culture media and strains of microorganism used for microbial test methods in a laboratory.

Use apparatuses which are appropriately maintained, controlled, and calibrated.

1. Media preparation and quality control

1.1. Media preparation

Select culture media and medium components suitable for microbial tests to be conducted when preparing culture media. Dehydrated media are accompanied by component compositions and instructions for preparation. Because different media may have different preparation requirements (e.g., heating, additives, and pH adjustment), it is important to follow their instructions to prepare media with appropriate quality. Records of the date of preparation, name, lot number, mass of dehydrated media or medium components, the volume of water used, sterilization conditions, pH after sterilization, and equipment used, etc. are useful to investigate cause when a problem occurs.

Dehydrated media or medium components should be weighed appropriately. In addition, clean containers and tools should be used to prevent contamination with foreign matters during preparation. Water suitable for performing the relevant test should be used to prepare culture media, and purified water is most often used.

Dehydrated media should be dissolved in water before sterilization, or shaken thoroughly to disperse sufficiently. When dispensing before sterilization, media should be thoroughly dissolved in water. If heating is necessary to dissolve media, care should be taken not to overheat media. Browning of media by the Maillard reaction etc. is one of the indication of overheating. Appropriate equipment and tools should be used for heating, stirring and mixing in the preparation of media. When adding components that cannot be heated, they should be aseptically added to media cooled to an appropriate temperature after sterilization, and mixed thoroughly.

If poorly cleaned tools are used to prepare media, substances that inhibit the growth of microorganisms may contaminate the media. Inhibitory substances are derived from detergent residues after cleaning tools, etc., substances used before cleaning tools, or residues during manufacturing even when unused tools are used. In the cleaning process, residues and foreign matters should be removed certainly, and finally detergents, etc. should be washed out completely using purified water, etc.

Sterilization of media should be performed within parameters (temperature, pressure, exposure time, etc.) provided by a manufacturer or parameters validated by users. Sterilization in an autoclave is preferred, unless media contain medium components that are unstable to heat. Sterilization by filtration may be appropriate for some medium compositions.

When an autoclave is used, the sterilization should be performed in the sterilization cycles (which includes the process where the temperature of products to be sterilized rises to specified temperature and the process from the completion of sterilization until the falling to the temperature to be able to take out the objects) in which the temperature of all products to be sterilized (temperature of media) meets specified temperature and exposure time, depending on the loading format of loads (loading pattern: the shape, size, number and distribution of containers, and the type and liquid volume of a product to be sterilized, etc.). The sterilization cycles in which temperature rises slowly may result in the overheating of media. In general, the more the liquid volume of an object to be sterilized, the longer the sterilization cycle. However, since the cycle is affected by the size and number of dispensing containers even if the total volume of liquid is the same, so appropriate conditions should be selected. After the completion of sterilization cycle, the media should be taken out immediately, and cooled, if necessary. The effects of the sterilization cycles should be verified by the growth promotion test (refer to Microbiological Examination of Non-sterile Products *<4.05>*, Sterility Test *<4.06>*, etc.) together with confirmation of the sterility of media (no microbial contamination).

Take into consideration that improper preparation may result in the deterioration of growth promoting properties and inhibitory properties, and in the deviation of the properties, such as color, clarity, gel strength and pH, from the acceptable range.

The pH of a medium should be confirmed after it has cooled to room temperature or another specified temperature by aseptically withdrawing a test sample every each sterile batch (unit to be sterilized at one time, hereinafter called as "batch"). If it cannot be measured at the specified temperature, the pH should be corrected for the specified temperature. A flat pH probe is recommended for agar surfaces, and an immersion probe is recommended for liquids. The pH of media should be within a specified range. However, unless when it is confirmed that a wider range is acceptable by the growth promotion test or suitability test (refer to Microbiological Examination of Non-sterile Products *<4.05>*, Sterility Test *<4.06>*, etc.).

Prepared media (agar plate or media dispensed to test tubes etc.) are identified by name, batch number, preparation date, etc. Also, pay attention to the following terms.

(i) Container fracture
(ii) Unequal dispensing volume between containers
(iii) Dirt of containers due to adhesion of medium components, etc.
(iv) Browning or discoloration
(v) Air bubbles
(vi) Status of redox indicator (if applicable)
(vii) Hemolysis (if applicable)
(viii) Formation of crystals, etc.
(ix) Drying that causes cracks and dimples
(x) Microbial contamination

1.2. Media storage

When storing media and medium components, the following points should be noted, including transport conditions until acquisition, in order to prevent the deterioration of quality.

(i) Drying, evaporation, moisture absorption
(ii) Temperature
(iii) Microbial contamination
(iv) Contamination of foreign matters
(v) Fracture

In addition, media or medium components should be labelled with names, batch or lot numbers, storage conditions, expiration dates, etc. and identified.

The storage conditions and the expiration dates of the media after preparation is set after the stability is confirmed by verifying that the performance of the media meet the acceptance criteria up to the end of the expiration date by the growth promotion test and other necessary quality tests when media are stored under the set conditions.

For long-term storage, packaging materials, packaging types, containers and stoppers that can prevent evaporation of water should be selected. Also, protect from light, if necessary. The agar media should be stored avoiding freezing because freezing damage the gel structure of agar.

In addition, agar media that have been remelted after storage can be used within the confirmed expiration date, if performance tests are performed to confirm the suitability. Also, it is desirable to remelt agar media only once to avoid the possibility of deterioration and contamination due to overheating. It is recommended that remelting is performed in a heated water bath or in free-flowing steam. When using a microwave oven or a heating plate for melting media, care should be taken because whole media may not be uniformly heated, and deterioration of the media and breakage of the container due to overheating may occur.

Agar media immediately after sterilization, or remelted media should be held at 45 to 50℃ or another specified temperature, but holding for a long-time should be avoided in consideration of the risk of deterioration and contamination. In addition, if media are held in a water bath, be careful of contamination derived from water in a bath when pouring them into petri dishes.

When discarding used or expired media, sterilize as needed and take care to prevent contamination.

1.3. Quality control testing

Perform the following quality control tests for each batch or lot for all prepared media. The prepared media include ready-prepared media and ready-to use swabs, strips, etc.

(i) Growth promotion (growth promoting properties, and, as needed, inhibitory properties or indicative properties)
(ii) pH (as needed, for ready-prepared media)

Media purchased or stored under a refrigeration condition should be returned to room temperature or a temperature being specified separately, and confirmed.

(iii) Sterility (no microbial contamination)

Media used for environmental monitoring of Grade A and B in processing areas for sterile pharmaceutical products should be multiple-wrapped or carried into the areas according to a specified procedure after disinfecting or decontaminating the exterior. If sterilization after packaging is not performed, all media (100%) should be subjected to incubation prior to use and confirmed to be free of microbial contamination in order to prevent extraneous contamination from being carried into controlled environments and prevent false-positive results.

A certificate of analysis describing storage conditions and an expiration date accompanies ready-prepared media, as well as the standard microbial strain used in growth promotion testing. When the data of testing are obtained from a media manufacturer at the time of acceptance, if the data is reliable as the result of writing or site investigation etc. and the validity of the growth promotion testing result and the expiration date can be confirmed, the quality control test may be performed regularly instead of every batch or lot.

Similar to media, those requiring quality control tests include reagents for microbial identification tests such as Gram staining reagents and oxidase reagents. These should be subjected to quality control tests at the time of acceptance or use using appropriate standard microbial strains selected.

2. Maintenance and control of microbial strains

The appropriate treatment of stock microbial strains is very important to maintain the accuracy and repeatability of results of microbial tests. The storage and handling of microbial strains in a laboratory should be done in such a way that will minimize changes in the growth characteristics of the microorganism, paying attention to contamination. Microbial strains used in the compendial methods are

available in frozen, lyophilized, slant cultured or ready-to-use forms from an organization for culture collections or an appropriate supplier. Obtained microbial strains should be confirmed to be contaminated with no other strains before or at use for quality control testing by the observation of emerged colonies being single, etc. when spread on plate media having no selectivity. In addition, if necessary, confirm the distributed microbial species.

Stock microbial strains are resuscitated according to the method specified by an organization for culture collections, etc. The application of seed lot culture maintenance techniques (seed-lot systems) is recommended to control the preservation of microbial strains. The seed-lot system is a system to control the number of passage of stock microbial strains in order to avoid property changes due to passages. One passage is defined as a transfer to a fresh medium. Any form of subculturing is considered to be a passage. At least the following terms should be noted in the control.

(i) Count a culture obtained by resuscitating (the number of passage is first) a microbial strain obtained from an organization for culture collections, etc. as the first generation.

(ii) Control the number of passages.

(iii) Microbial strains must not be used more than 5 passages for growth promotion tests and suitability tests.

There are methods by freezing, by drying, by serial subculture, etc. for the preservation of microbial strains, and an example of the method by freezing is shown. A standard microbial strain obtained from an organization for culture collections etc. is resuscitated and grown in appropriate medium. Aliquot of this culture (the first generation) is suspended in a solution containing a protective agent that prevents freezing damage, transferred to a vial or the like, and cryopreserved at an appropriate temperature according to the microbial species. Many microbial strains can be stored for a long time by maintaining at the temperature not exceeding −70℃. If the second and subsequent generations are prepared in large quantities, the frequency of obtaining and preparing standard microbial strains can be reduced.

Microbial strains in once opened containers should be discarded without refreezing to avoid the risk of reduced viability and contamination of stored microbial strains.

References

1) WHO, WHO good practices for pharmaceutical microbiology laboratories(WHO Technical Report Series, No.961, Annex 2, 2011).
2) US Pharmacopeia 41 (2018), <1117> Microbiological Best Laboratory Practices.