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JAPANESE INDUSTRIAL STANDARD

**Testing methods for
determining bacterial retention
of membrane filters**

JIS K 3835—1990

Translated and Published

by

Japanese Standards Association

In the event of any doubt arising,
the original Standard in Japanese is to be final authority.

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Testing methods for determining bacterial
retention of membrane filters

K 3835-1990

1. Scope

This Japanese Industrial Standard specifies the testing methods for determining bacterial retention of filter disc, filter cartridge, and disposable filter of microfiltration membrane used for filtering air or liquid using *Pseudomonas diminuta* as the challenge organism.

Remarks 1. The applicable standards to this Standard are shown in Attached Table 1.

2. The units and numerical values given in { } in this Standard are based on the traditional units and are currently the criteria in force.

2. Definitions of Terms

For the main terms used in this Standard the definitions in JIS K 3802 apply, and the rest of the terms shall be as follows:

- (1) bacterial retention The value defined in the following formula that is used for evaluating element and module of microfiltration membrane.

$$M = \log_{10} \frac{A}{B}$$

where, M : bacterial retention ⁽¹⁾ (no dimension)

A : total number of organisms in the challenge on micro-filtration membrane.

B : number of organisms retained on the analysis filter for test

Note ⁽¹⁾ This can be represented as the logarithm reduction value or LRV (Log Reduction Value).

- (2) filter disc Sheet-type filter element.
- (3) filter cartridge Pleat-type or cylinder-type filter element.
- (4) disposable filter Throwaway-type filter assembly.
- (5) filter assembly Housing or holder installed with a filter element.
- (6) holder Container for keeping a filter disc.

3. Organisms, Reagents and Culture Media

3.1 Organisms Organisms shall be *Pseudomonas diminuta* ATCC 19146 or IFO 14213.

3.2 Reagents Reagents shall be as follows:

- (1) Water Distilled water or demineralized water specified in 2.(9) (water) of JIS K 0101, or water at least equal to those.
- (2) Sodium Chloride Specified in JIS K 8150.

3.3 Culture Media Culture media shall be as follows:

- (1) Salt Lactose Broth Culture Medium (Corresponding to Saline Lactose Broth described in ASTM) Put 970 ml of water into a 2000 ml Erlenmeyer flask, add and dissolve 7.6 g of sodium chloride in it. Separately, dissolve 1.3 g of powdery or granular lactose broth in 100 ml of water, and pipette 30 ml of this solution into the Erlenmeyer flask. Adjust its pH to 6.9 ± 0.1 , and autoclave at 121°C for about 20 min.
- (2) Standard Agar Culture Medium Dissolve 5.0 g of meat extract, 10.0 g of peptone, 1.0 g of D-glucose, and 15 to 18 g of agar in 1000 ml of water; prepare it so that its pH becomes 7.0 to 7.2 after sterilization ⁽²⁾; then autoclave at 121°C for about 20 min.

Note ⁽²⁾ Powdered dried culture medium of ready made may be used for the preparation.

- (3) Lactose Broth Add 5.0 g of peptone, 3.0 g of meat extract, and 5.0 g of lactose to 1000 ml of water; and adjust its pH to 6.9 ± 0.2 ⁽²⁾.

4. Instruments Instruments shall be as follows:

- (1) Analytical Microfiltration Membrane 142 mm diameter, not more than $0.45 \mu\text{m}$ pore size.
- (2) Petri Dishes ⁽³⁾ About 50 mm and about 150 mm diameter, made of glass.

Note ⁽³⁾ Petri dishes shall have been hot-air sterilized using a hot-air sterilizer at 170°C for 3 h. A sterilized plastic Petri dish on the market may be used.

- (3) Erlenmeyer Flask Specified in JIS R 3503.
- (4) Hot-air Sterilizer Adjustable its temperature from 150 to 200°C .
- (5) Autoclave Usable at 121°C or higher with the inside absolute pressure not lower than 210 kPa $\{2.1 \text{ kgf/cm}^2\}$.
- (6) Incubator Adjustable its temperature at $30 \pm 2^\circ\text{C}$.
- (7) Clean Bench Specified in JIS B 9922.

- (8) Pressure Vessel Stainless steel vessel of the capacity not less than 12 l and equipped with a pressure gauge from 0 to 350 kPa {0 to 3.5 kgf/cm²}.
- (9) 142 mm Filter Holder Made of stainless steel.
- (10) Housing Made of stainless steel.
- (11) Filter Assembly Holder or housing equipped with an element or module of microfiltration membrane.
- (12) Manifold Branch pipe autoclavable at 121°C.
- (13) Pressure Gauge Diaphragm-protected 0 to 400 kPa{0 to 4 kgf/cm²}, pressure gauge for pressure reading, or at least equivalent in performance to it.
- (14) Thermometer Thermometer specified in JIS B 7411, Digital thermometer of semiconductor sensor method, or other methods of thermometer calibrated previously with the standard thermometer.
- (15) Flowmeter The flowmeter, specified in JIS B 7551, scaled least in 5 to 10% of measured flow amount and measuring approximately 1.5 to 2 times flow amount at most of the measured flow amount, or at least equal to it in performance.

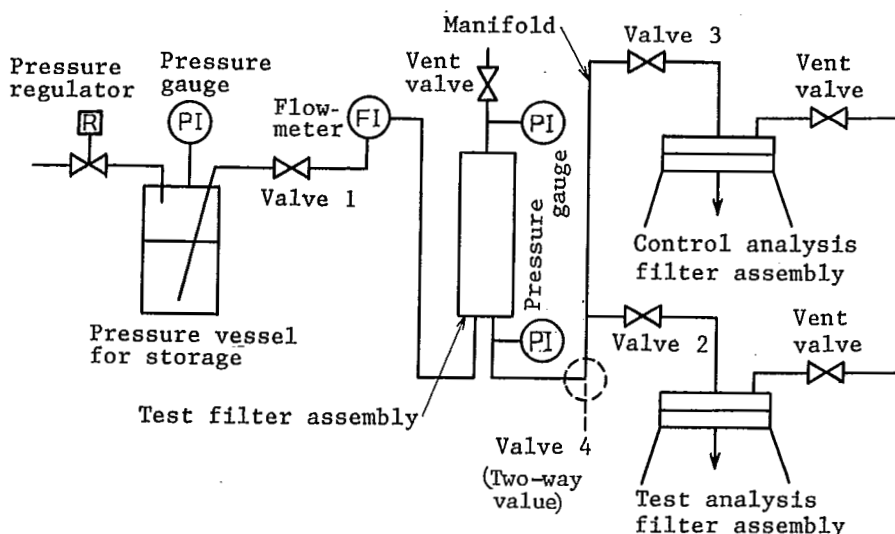
5. Construction and Parts of Testing Apparatus

Testing apparatus shall be composed of pressure regulator, pressure vessel for storage, flowmeter, test filter assembly, control analysis filter assembly, test analysis filter assembly, and so on, and the parts shall be as follows. The example of construction is shown in Fig. 1.

Remarks: Valve 4 shall be two-way valve, be fitted according to the necessity, and be used on draining dipping liquid, etc. of test filter.

- (1) Test Filter Assembly Filter disc, filter cartridge, and disposable filter subjected to test (hereinafter referred to as test filter), and holder or housing fitted with it.
- (2) Control Analysis Filter Assembly 142 mm filter holder fitted with 142 mm diameter of filter disc for control test.
- (3) Test Analysis Filter Assembly 142 mm holder fitted with 142 mm diameter of filter disc for testing test filter.

Fig. 1. Apparatus for Testing Bacterial Retention of Test Filter



6. Preparation

6.1 Identification of Challenge Organism The identification of challenge organism shall comply with Annex 1.

6.2 Preparation of Bacterial Challenge Suspension Bacterial challenge suspension shall be prepared by adding bacterial challenge stock suspension prepared beforehand to sterilized salt lactose broth culture medium or to 0.9% sodium chloride solution ⁽⁴⁾. The methods for preparation of bacterial challenge stock suspension shall comply with Annex 2.

Note ⁽⁴⁾ The concentration of bacterial challenge suspension shall not be specified, but, when tested, filtration membrane shall contain 10^7 or more organisms per 1 cm^2 of effective filtration area.

6.3 Preparation of Testing Apparatus Testing apparatus shall be prepared as follows.

(1) Preparation of Test Filter Assembly

- (a) Install the test filter in holder or in housing ⁽⁵⁾, wrap the inlet and outlet connections of holder or housing with autoclave paper, and autoclave ⁽⁶⁾ according to manufacturer's instructions.

Notes ⁽⁵⁾ In case of using disposable filter for test filter, holder or housing shall be replaced.

⁽⁶⁾ The test filter may be in-situ steam sterilized.

Remarks: The sterilization procedure shall be validated using biological indicators ⁽⁷⁾ or thermocouples ⁽⁸⁾.