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Aerosol bacterial retention test method for air-inlet filter on administration devices

Méthode d'essai de rétention bactérienne dans les aérosols pour les filtres d'admission d'air utilisés sur les dispositifs d'administration

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Foreword

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This document was prepared by Technical Committee ISO/TC 76, *Transfusion, infusion and injection, and blood processing equipment for medical and pharmaceutical use,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 205, *Non-active medical devices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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Introduction

Several methods are used to assess the retention ability of filter membranes, including the liquid bacterial retention test (e.g. ASTM F838-20[1]), the aerosol bacterial retention test (e.g. ASTM F2101-19[2]) and the liquid virus retention test (e.g. ASTM F1671-22[3]). The choice of test method depends on the characteristics of the filtered object. For liquid filters, liquid bacteria retention test is generally adapted. For air filters, the aerosol form of microorganisms is generally used, which is more representative of clinical use.

Since the aerosol bacterial retention test is a destructive test with more stringent requirements for test conditions and personnel operation, its application for routine quality controls is generally not viable.

Aerosol bacterial retention test method for air-inlet filter on administration devices

1 Scope

This document specifies a test method to assess bacterial retention ability of finished stand-alone and integrated air-inlet filters on administration devices for infusion and transfusion applications.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
aerosol
suspension of solid or liquid particles in a gas

3.2
bacterial retention ability
effectiveness of an air-inlet filter to prevent the ingress of microorganisms into the container into which the device is inserted

3.3
collecting fluid
fluid contained in the liquid impinger that is used to collect challenge bacteria for subsequent bacteria counting analysis

3.4
liquid impinger
glass vial used with an air sample pump to collect challenge bacteria into designated collection fluid for analysis

Note 1 to entry: Impingers have an inlet with an internal protrusion extending close to the bottom of the vial thus "bubbling" the sampling through the collection liquid. Air is drawn into the inlet via a port near the top of the glass vial.

Note 2 to entry: Impingers are also known as bubblers.

3.5
sterility assurance level
probability of a single viable microorganism occurring on an item after sterilization

[SOURCE: ISO 11139:2018, 3.275, modified — Note 1 to entry has been deleted.]

3.6
sterilization
validated process used to render product free from viable microorganisms

Note 1 to entry: In a sterilization process, the nature of microbial inactivation is exponential and thus the survival of a microorganism on an individual item can be expressed in terms of probability. While this probability can be reduced to a very low number, it can never be reduced to zero.

Note 2 to entry: See 3.5, sterility assurance level.

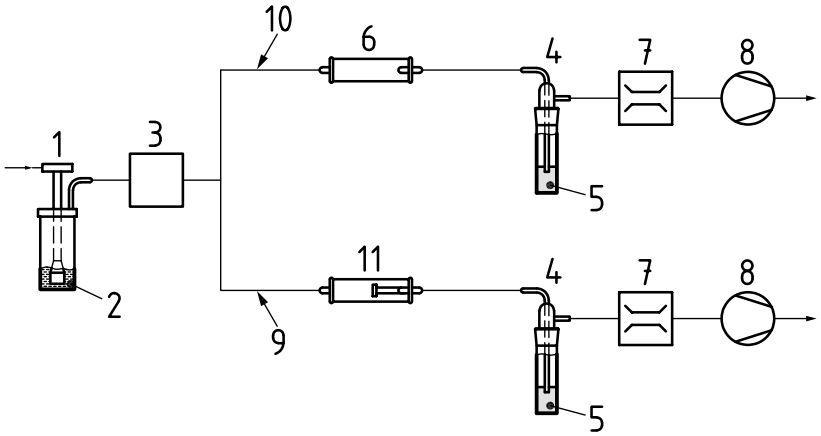
[SOURCE: ISO 11139:2018, 3.277]

4 Principle

The test is designed to simulate clinical applications. The method uses a *Staphylococcus aureus* aerosol representing natural microorganisms present in a clinical environment to challenge the air-inlet filters. The bacterial retention ability is assessed by calculating the amount of bacteria permitted to pass through the air-inlet filter. The bacterial concentration is higher and the aerosol size distribution is smaller than the conditions during the use of the final product. The sample challenge flow parameters are designed to be stricter than the infusion speed during clinical practise.

5 Apparatus

A schematic diagram of an aerosol challenge test apparatus is shown in Figure 1. An aerosol generator (1) is used to produce a bacterial aerosol from a challenge bacteria suspension (2) of specified concentration which is then sprayed into an aerosol chamber (3). A vacuum pump (8) pulls the bacterial aerosol through both the positive control pipeline (10) and the sample challenge pipeline (9) simultaneously. The sample challenge pipeline (9) has the air-inlet filter assembly [see examples in ISO 8536-4:2019, Figures 1 (key 3) and 3 (key 5)] attached to the sample test housing (11) outlet. Both pipelines terminate in liquid impingers (4) pre-filled with collecting fluid (5) from which bacterial counts can be assessed for the positive control and bacteria that are not retained by the air-inlet filter. Multiple sample challenge pipelines can be set in parallel.



Key

1	aerosol generator	7	flow meter
2	challenge bacteria suspension	8	vacuum pump
3	aerosol chamber	9	sample challenge pipeline
4	liquid impinger	10	positive control pipeline
5	collecting fluid	11	sample test housing (with sample inside)
6	positive control housing (without sample inside)		

Figure 1 — Example of the test apparatus (schematic)

5.1 Aerosol generator, capable of generating an aerosol of a particle size distribution with a mass median aerodynamic diameter (MMAD) of (2,5 ± 0,5) µm and a geometric standard deviation (GSD) of approximately 1,8. The MMAD and GSD are calibration parameters of the aerosol generator and the calibration shall be performed as recommended by the manufacturer.

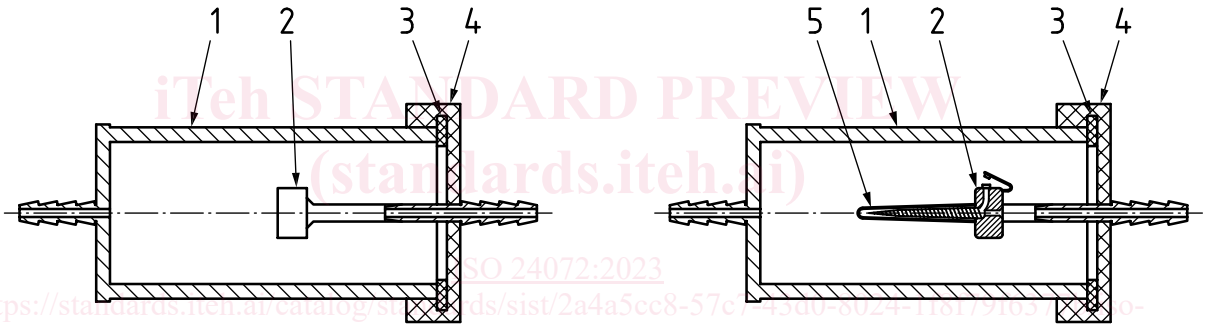
5.2 Aerosol chamber, designed with transparent materials to observe aerosol formation and shall withstand sterilization treatment. Appropriate measures shall be taken to disperse the aerosol uniformly and balance internal pressure.

5.3 Sample test housing, capable of loading stand-alone air-inlet filter assembly or integrated air-inlet filter assembly in such a manner to ensure the air-inlet filter membrane is the only channel through which aerosol passes, see Figure 2 a) (for stand-alone air-inlet filters) and Figure 2 b) (for integrated air-inlet filters).

5.4 Liquid impinger, prefilled with collecting fluid to collect bacteria.

5.5 Flowmeter, capable of measuring a flow rate of 50 ml/min with an accuracy of ±5 %.

5.6 Vacuum pump, capable of maintaining a flow rate of 50 ml/min.



a) For stand-alone air-inlet filters **b) For integrated air-inlet filters**

Key

1 sleeve with inlet port
2 air-inlet filter for test
3 sealing gasket
4 back cover with outlet port
5 closure

Figure 2 — Sample test housing for air-inlet filter (schematic)

6 Reagents and materials

6.1 Tryptone Soya Broth (TSB) or Nutrient Broth (NB) as enrichment medium.

6.2 Tryptone Soya Agar (TSA) or Nutrient Agar (NA) as counting medium.

6.3 Sterile saline as collecting fluid and diluent.

6.4 ***Staphylococcus aureus*** **ATCC®[1)] 6538™** or an equivalent strain. Inoculate *Staphylococcus aureus* ATCC® 6538™ into TSB or NB and incubate at (32,5 ± 2,5) °C for 18 h to 24 h. Then dilute it to appropriate concentration (as validated in Clause 7) with sterile saline as the challenge bacteria suspension.

6.5 **Analytical filter membrane**, with a nominal pore size of 0,45 µm.

7 Validation of testing system

7.1 Validation shall be performed on the first use of the test apparatus to ensure the whole test system, including the concentration of challenge bacteria suspension and spraying time meets the design requirements.

7.2 In order to maintain the validated status of the test apparatus, revalidation should be performed when changes are made which have potential effects on the operation of the test system challenge level, such as examples including changes in environmental conditions, concentration of challenge bacteria suspension, spraying time and apparatus modification.

7.3 For each validation procedure, a minimum of three runs should be conducted.

7.4 Prior to validation, the apparatus shall be properly sterilized (sterility assurance level 10^{-6}) and assembled as in Figure 1 under aseptic conditions inside a bio-safety cabinet. All sample test housings shall be connected without air-inlet filters for test.

7.5 The validation procedure is as follows:

a) Fill the aerosol generator (5.1) with challenge bacteria suspension in the appropriate concentration.

b) Start the aerosol generator (5.1), spray microbial aerosol into the aerosol chamber (5.2).

c) Start the vacuum pump (5.6), regulate flow to 50 ml/min, and observe bubble generation in the liquid impinger (5.4).

d) Turn off the aerosol generator (5.1) after spraying for appropriate time.

e) Turn off the vacuum pump (5.6) after sampling for 30 min.

f) Remove the liquid impinger (5.4) and perform a 10-fold serial dilution of the collecting fluid using sterile saline solution. Select appropriate dilution and analyse the collecting fluid with the membrane filtration method. Culture the analytical filter membrane at (32,5 ± 2,5) °C for 18 h to 24 h, and then count the colony forming units (CFU) and calculate the population of challenge bacteria.

7.6 A validated test system ensures a bacterial aerosol challenge level of $1,0 \times 10^4$ CFU to $5,0 \times 10^4$ CFU in 30 min at a flow rate of 50 ml/min. If it does not meet the aforementioned requirement, adjust the concentration of challenge bacteria suspension without reducing the level below $1,0 \times 10^4$, the spraying time of aerosol generator, or by modifying the apparatus to meet the challenge level requirement.

8 Challenge test

8.1 The challenge test shall be carried out after the validation of the test system.

1) *Staphylococcus aureus* ATCC® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.