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**Textiles — Test methods for  
determining the efficiency of products  
against house dust mite**

*Textiles — Méthodes d'essai pour déterminer l'efficacité des produits  
contre les acariens*

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 38, *Textiles*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

## Introduction

The World Health Organization's (WHO) statement on the correlation of house dust mite to asthma and other allergic disorders resulted in the increased number of textile products treated against house dust mite available to the consumer.

However, the testing method to evaluate the efficacy against house dust mite of textiles has not been standardized to date. This has caused confusion among consumers because of various testing methods and results.

The purpose of this test method is to standardize the testing method of efficacy of products against house dust mite in textiles.

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# Textiles — Test methods for determining the efficiency of products against house dust mite

## 1 Scope

This document specifies test methods for efficiency of chemically or physically treated textile products against house dust mites.

For the products treated by chemicals against house dust mites, the test methods specified in a) to c) are applied. For the physically treated products, the test method specified in d) is applied.

a) Repelling method by using Petri dish

This method is applied to carpet, bedding surface fabric, bed sheeting, bed covering and blanket.

b) Repelling method by using glass tube (Methods A and B)

This method is applied to wadding (bedding, etc.) with a fibre content of cotton, wool or synthetic fibre, feathers/down.

c) Proliferation method by using Petri dish (Method A) and using vial (Method B)

Method A is applied to carpet, bedding surface fabric, bed sheeting, bed covering and blanket. Method B is applied to wadding.

d) Penetration method

This method is applicable to the outer fabric of a futon, bed sheeting and bed covering. However, this method is not applicable to the multiple component non-woven fabrics and fibre products with the high stretch properties such as jersey fabrics.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 105-F02, *Textiles — Tests for colour fastness — Part F02: Specification for cotton and viscose adjacent fabrics*

ISO 3310-1, *Test sieves — Technical requirements and testing — Part 1: Test sieves of metal wire cloth*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 9237, *Textiles — Determination of the permeability of fabrics to air*

## 3 Terms and definitions

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

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

**3.1 house dust mite**  
universal dominant species belonging to Pyroglyphidae, observed in or on floor surfaces, carpets and bedding with house dust as example

**3.2 efficiency of repellency**  
efficiency of the treatment in repelling house dust mites

**3.3 rate of repellency**  
ratio of the number of live mites in treated material against the number of live mites in untreated material

Note 1 to entry: The rate of repellency is expressed in percentage (%) and represents the efficacy of repellency of treated materials.

**3.4 rate of suppression of house dust mite reproduction**  
ratio of the number of live mites in treated material against the number of live mites in untreated material tested by the proliferation method

Note 1 to entry: The rate of suppression for reproduction of house dust mite is expressed in percentage (%) and represents the degree of population control of treated materials.

**3.5 culture medium**  
diet for breeding house dust mite

**3.6 mite medium**  
mix of diet and live house dust mite

**3.7 live mite**  
mites which react when stimulated from the outside

Note 1 to entry: It includes all of larva, nymph (protonymph and tritonymph) and adult mites but exclude eggs.

**3.8 population density**  
degree of live mites in a mite medium

Note 1 to entry: The number of live mites in 1 g of the mite medium.

**3.9 quiescent period**  
period in the stage in which activity of mites almost stops and is observed in the latter half of each development period of a larva, protonymph and tritonymph

## 4 Principle

### 4.1 Repelling method by using Petri-dish

The small Petri dish is placed in the centre position of the large Petri dish. The specimen or the reference fabric is placed in the small dish with the culture medium. The mite medium with 10 000 mites is spread in the large Petri dish for the mites to migrate to the small dish. After the designated time has elapsed, the number of live mites that have intruded into the small Petri dish where the test specimen and culture medium have been placed is counted. The efficiency of repellency is calculated by comparing the numbers of live mites for the reference test.

## 4.2 Repelling method by using glass tube, Method A and Method B

The specimen or the reference fabric is placed in one end of the glass tube in the following order: stuffing, culture medium and the adhesive tape at the end. The mite medium with 10 000 mites is placed at the opposite end of the glass tube and the mites migrate in the glass tube all the way. After the designated time has elapsed, the number of live mites in the stuffing, the culture medium and adhesive tape which are passing through the specimen or reference are counted. The efficiency of repellency is calculated by comparing the numbers of live mites for the reference test.

Glass tube Method A is a test method for wadding sample. Glass tube Method B is a test method for down and feather sample. For Method B only, use a stainless steel mesh disc to fix the position of the specimen in the glass tube.

## 4.3 Proliferation method by using Petri-dish Method A and using vial Method B

Mite medium with 50 to 80 live mites per 0,1 g is placed on the specimen in a Petri dish or vial. After the designated time has elapsed, the numbers of live mites on the Petri dish or vial, the specimen and the mite medium are counted and summed. The suppression effect of house dust mite reproduction is calculated by comparing the numbers of live mites for the reference test.

The Petri dish Method A is for the carpet samples, etc. and the vial Method B is for wadding samples.

## 4.4 Penetration method

The specimen or the reference is placed at the upper end of the glass tube and wrapped by plastic wrap tightly. The mite medium with 10 000 mites are placed at the bottom of the glass tube on the paper filter which is tightly sealed. After the designated time has elapsed, the number of the mite on the specimen or the reference which is passing through is counted through the plastic wrap. Check the efficiency for prevention by comparing the number of house dust mite passing through the fabric in the specimens and the reference.

## 5 Preparation of test

### 5.1 Reagents

The reagents shall be as follows.

**5.1.1 Water**, grade 3 according to ISO 3696.

**5.1.2 Dried yeast**, refined beer yeast, dried, mashed and filtered by a sieve.

**5.1.3 Saturated sodium chloride solution**, 392 g sodium chloride (NaCl) dissolved in 1 000 ml of water.

**5.1.4 Nonionic surfactant solution**, 0,1 g of nonionic surfactant [Polyoxyethylene sorbitan mono-oleate (Polysorbate 80) (CAS Number 9005-65-6)] dissolved in 100 ml of water.

#### 5.1.5 Colouring liquid.

Dissolve,

— 6,0 g of crystal violet ( $C_{25}H_{30}ClN_3 \cdot 9H_2O$ )

or

— 0,6 g of methylene blue ( $C_{16}H_{18}N_3S \cdot Cl \cdot 3H_2O$ )

in ethanol ( $C_2H_5OH$ ) of 100 ml,

then,

— dilute with water to make 1 000 ml.

## 5.2 Apparatus

**5.2.1 Oven**, capable of maintaining a temperature of  $70\text{ °C} \pm 2\text{ °C}$ .

**5.2.2 Incubator (or incubation room)**, capable of maintaining a temperature of  $25\text{ °C} \pm 2\text{ °C}$  in dark conditions.

**5.2.3 Erlenmeyer flask**, with a nominal volume of 50 ml.

**5.2.4 Beaker**, with a nominal volume of 50 ml and 100 ml.

**5.2.5 Large Petri dish**, made of glass, with an internal diameter of approximately 90 mm and an internal height of approximately 20 mm.

**5.2.6 Small Petri dish**, made of glass, with an external diameter of approximately 45 mm and an internal height of approximately 15 mm.

**5.2.7 Glass tube A**, hard-coated glass type with an external diameter of  $22,0\text{ mm} \pm 0,6\text{ mm}$  (wall thickness  $1,2\text{ mm} \pm 0,2\text{ mm}$ ) and a length of approximately 100 mm.

**5.2.8 Glass tube B**, hard-coated glass type with an external diameter of  $40,0\text{ mm} \pm 0,6\text{ mm}$  (wall thickness  $2,0\text{ mm} \pm 0,2\text{ mm}$ ) and a length of approximately 55 mm.

**5.2.9 Rubber band**, approximately 70,0 mm lay-flat length and approximately 15,0 mm width.

**5.2.10 Vial**, made of glass with an external diameter of approximately 30 mm, an internal height of approximately 63 mm and a volume of approximately 30 ml.

**5.2.11 Hot-melt adhesive**, with appropriate adhesive strength and no effect to mites.

**5.2.12 Filter paper**, used for counting mites with a diameter of 70 mm or 90 mm and a grid pattern of 5 mm to 10 mm in square.

**5.2.13 Adhesive tape**, with appropriate adhesive strength and no effect to mites.

**5.2.14 Sticky sheet**, with an adhesive strength with the ability to anchor mites that may escape.

**5.2.15 High-density fabric**, with air permeability of  $1\text{ cm}^3/\text{cm}^2\cdot\text{s}$  to  $10\text{ cm}^3/\text{cm}^2\cdot\text{s}$  as specified in ISO 9237 with a fibre content of 100 % cotton.

**5.2.16 Standard woven fabric**, 100 % cotton fabric used for the reference of the colour fastness test specified in ISO 105-F02.

**5.2.17 Airtight container**, made of polypropylene used for food preservation.

**5.2.18 Powder diet**, for small laboratory animals (mouse, rat, hamster, etc.) and used for breeding mites.

**5.2.19 Balance**, with a minimum indication of 1 mg with a scale in graduations of 0,1 mg.

**5.2.20 Stereoscopic microscope**, with an epi-illumination device with 20 × magnification.

**5.2.21 Test sieve**, as specified in ISO 3310-1.

The sieve opening shall be as follows.

- a) The mesh opening size is the range of 500 µm to 700 µm, used in [Annex B](#).
- b) The mesh opening size is 300 µm, used in [Annex A](#).

**5.2.22 Stainless mesh disk**, a circular-shaped wire mesh of approximately 20 mm diameter to fit the glass tube A ([5.2.7](#)), used for both ends of the specimen to keep thickness 20 mm ± 2mm for feather and down sample in [Annex D](#).

**5.2.23 Suction unit**, capable of performing suction filtration with an aspirator (or a suction pump) with a Buechner funnel attached to a vacuum flask. The bypass can be included for adjusting suction force if necessary.

**5.2.24 Counter**, capable of counting from 0 to 9 999.

**5.2.25 Plastic wrap**, as used for food preservation made of polyethylene or polypropylene.

**5.2.26 Stuffing**, staple fibre such as 100 % polyester with a fineness of 5 dtex to 8 dtex and fibre length 51 mm to 75 mm for counting the number of mites in [Annex D](#).

## 6 Reference sample

For all test methods, prepare the reference specimens. Reference specimens are untreated products similar to the test sample. If it is not available, use the same category of the products with the same structure to the testing sample.

## 7 Preparation of mite medium

The mite medium used for the test shall be prepared in accordance with [Annex A](#).

After the colonization procedure specified in [Annex A](#), just before the test, prepare the mite medium for inoculation using the following procedure.

- a) Take 0,025 g or 0,050 g of the mite medium which has been well stirred.
- b) Count the number of live mites in the mite medium according to [B.2.1](#) or [B.2.2](#). If the amount of mite medium used is 0,025 g, count the number of each live mites 8 times. If the amount of mite medium used is 0,05 g, count the number of each live mites 4 times.
- c) Calculate the number of live mites in 1 g of the mite medium and the mass of mite medium with 10 000 mites for inoculation according to [Formula \(1\)](#):

$$q = \frac{10\ 000}{Nm} \quad (1)$$

where

$q$  is the mass of the mite medium (g) with 10 000 live mites;

$Nm$  is the number of live mites in 1 g of the mite medium.

- d) Calculate the coefficient of variation values by using [Formula \(2\)](#) for the judgment of test effectiveness.

$$Cv = \frac{\sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{(n-1)}}}{\bar{x}} \times 100 \quad (2)$$

where

$Cv$  is the coefficient of variation ( $Cv$  %);

$x_1, x_2, \dots, x_n$  is the counting value of live mites in 0,025 g (or 0,050 g) of mite medium;

$\bar{x}$  is the average of live mites in 0,025 g (or 0,050 g) of mite medium;

$n$  is the number of times of counting of mite medium,  $n = 8$  (or  $n = 4$ ).

- e) Prepare the quantity of the mite medium with 10 000 live mites for inoculation in [Annexes C, D](#) and [F](#).
- f) From the measured value taken using step b), mix the mite medium including live mites with the culture medium without mites so that the number of live mites is between 50 and 80 in 0,1 g of the medium. This is used for inoculation in [Annex E](#).

## 8 Test conditions

### 8.1 Condition for work area

Temperature  $23 \text{ }^\circ\text{C} \pm 5 \text{ }^\circ\text{C}$  and relative humidity  $(55 \pm 15) \%$  is used for the work area.

### 8.2 Testing conditions

The culture medium for breeding and all testing apparatus assembly are placed in the airtight container ([5.2.17](#)) with the saturated sodium chloride solution ([5.1.3](#)) with a concentration of 10 % to control relative humidity at  $(75 \pm 5) \%$ . The containers are then placed in the incubator at the temperature of  $25 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ .

## 9 Test methods

### 9.1 Repelling method by using Petri dish

The test is carried out according to [Annex C](#).

### 9.2 Repelling method by using glass tube

The tests are carried out according to [Annex D](#).

### 9.3 Proliferation method by using Petri dish (Method A) and using vial (Method B)

The tests are carried out according to [Annex E](#).

### 9.4 Penetration method

The test is carried out according to [Annex F](#).

## 10 Test report

### 10.1 Overview

The test report shall contain the following information.

### 10.2 General

- a) a reference to this document, i.e. ISO 21326:2019;
- b) test method used;
- c) name of species of mites used for test (system, source, origin, etc.);
- d) type of sample;
- e) identification of sample (product name, etc.);
- f) measurement result of population density of mite medium;
- g) number of live mites in 1 g of mite medium;
- h) any deviation from this document.

### 10.3 Repelling methods

- a) Number of invasion mites on the reference assembly (in the case of glass tube method, number of attractant mites).
- b) Number of invasion mites of the testing assembly (in the case of glass tube method, number of attractant mites).
- c) Rate of repellency.

### 10.4 Proliferation methods

- a) Number of live mites in 0,1 g of the mite medium for inoculation (initial population).
- b) Number of live mites of the reference assembly at the time of each observation.
- c) Number of live mites of the testing assembly at the time of each observation.
- d) Rate of suppression of house dust mite reproduction at the time of each observation.

### 10.5 Penetration method

- a) The number of mites which passed through the standard woven fabric (each of the adult, the larva and the nymph).
- b) The number of mites which passed through testing specimens (each of the adult, the larva and the nymph).

## Annex A (normative)

### Preparation of mite medium

#### A.1 Species of mites

The following species of mites after colonization shall be used for these test methods.

- *Dermatophagoides pteronyssinus*, or
- *Dermatophagoides farinae*

#### A.2 Colonization procedure

##### A.2.1 Apparatus

**A.2.1.1 Breeding container**, a Petri dish is used as a glass container with suitable volume.

**A.2.1.2 Airtight container for breeding**, with the saturated sodium chloride solution (5.1.3) with the concentration of 10 % to control relative humidity at  $(75 \pm 5)$  % and the Petri dish used for breeding container (A.2.1.1) with mite medium is placed in this container (5.2.17) as shown in Figure A.1.

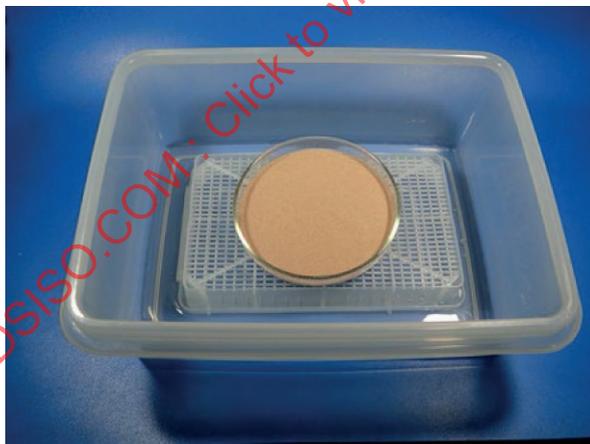


Figure A.1 — Airtight container with the breeding Petri dish

##### A.2.2 Preparation of culture medium

- a) Sieve (5.2.21) the powder diet (5.2.18).
- b) Mix the sieved powder diet for colonization and the dried yeast (5.1.2) in a mass ratio of 1:1.
- c) Spread the mixed diet in the breeding container (A.2.1.1) to a thickness of approximately 10 mm and heat the container with the mixed diet at  $70 \text{ °C} \pm 2 \text{ °C}$  for 2 h by using the oven (5.2.1).
- d) Store the mixed diet after heating in an airtight container (5.2.17) for 24 h to 48 h as shown in Figure A.1. This becomes the culture medium.

### A.2.3 Bleeding method

- a) Inoculate the mite medium into the Petri dish with the culture medium [A.2.2, d)] directly.
- b) Place the Petri dish into an airtight container (5.2.17) as shown in [Figure A.1](#).
- c) Stir the mite medium as needed and check that no other mites or insects exist during the breeding period.

When other mites or insects are observed in the mite medium, stop the bleeding immediately and discard the mite medium.

When the population density of the mite medium begins to decrease, stop the bleeding and restart this bleeding procedure from step a).

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## Annex B (normative)

### Counting methods for live mites

#### B.1 General

The number of live mites is counted by one of the following four methods according to the kind of sample shape being tested:

- a) floatation method;
- b) whole counting method;
- c) wet sieving methods;
- d) heat escape method.

#### B.2 Methods for counting

##### B.2.1 Floatation method

The counting procedure is as follows.

- a) Place the material for counting into an Erlenmeyer flask (5.2.3).
- b) Add several drops of a non-ionic surfactant solution (5.1.4) and about 1,0 ml of a colouring liquid (5.1.5) into the Erlenmeyer flask.
- c) Add a suitable amount of saturated sodium chloride solution (5.1.3) and stir well.
- d) Pour the saturated sodium chloride solution (5.1.3) into the Erlenmeyer flask c) up to the mouth of the flask and wait at the state for 10 min.
- e) After 10 min, perform suction filtration for the supernatant of the liquid d).
- f) Count the number of live mites on the filter paper (5.2.12) under a stereoscopic microscope (5.2.20) by using the counter (5.2.24).

Dead mites or the mites in a quiescent period shall be excluded and not be counted. The mites tend to die in the saturated sodium chloride solution, so the counting of the number of live mites shall be completed within 20 min.

NOTE There is data which shows that the number of dyed mites can increase over 20 min.

##### B.2.2 Whole counting method

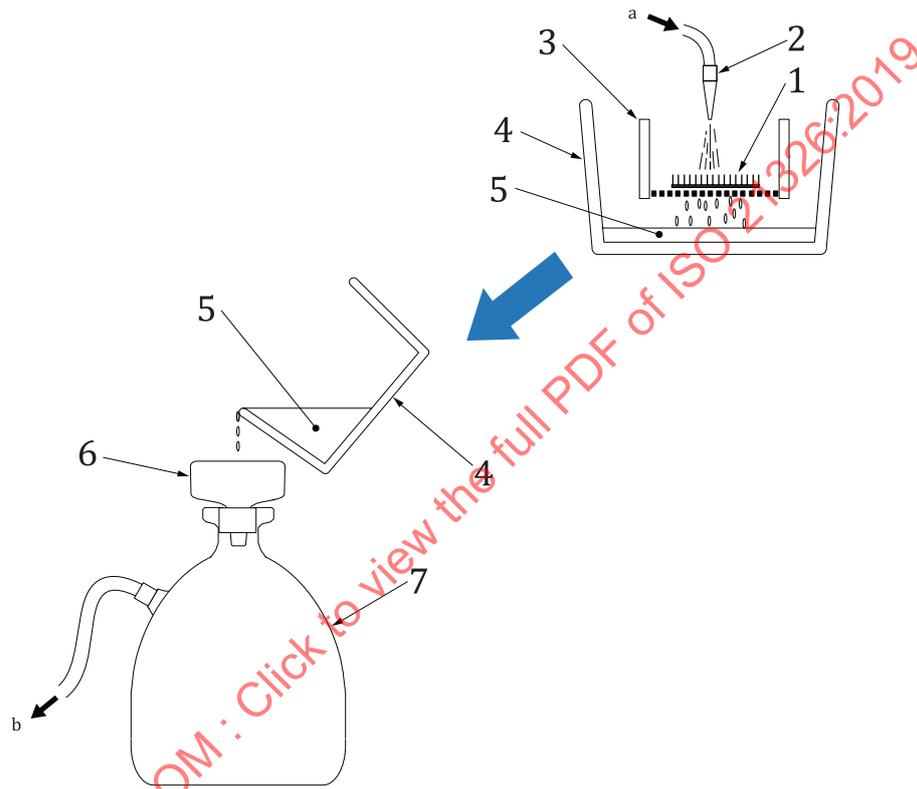
The counting procedure is as follows.

- a) Place the material for counting into a beaker (5.2.4).
- b) Add 20 ml to 30 ml of water (5.1.1) and 0,5 ml to 1,0 ml of colouring liquid (5.1.5) into the beaker and stir it well.
- c) Perform suction filtration for all liquid of the beaker.

- d) Pour water in the beaker for rewashing and filtrate the washing liquid again.
- e) Count the number of live mites on the filter paper under a stereoscopic microscope ([5.2.20](#)).
- Dead mites or the mites in a quiescent period shall be excluded and not be counted.

### B.2.3 Wet sieving method

**B.2.3.1 Apparatus assembly**, for wet sieving method as shown in [Figure B.1](#). The sieve used for this method is described in [5.2.21](#), a).



#### Key

- 1 specimen
- 2 nozzle
- 3 sieve
- 4 container
- 5 washing liquid
- 6 Buechner funnel with paper filter
- 7 vacuum flask
- a Water.
- b To aspirator or suction pump.

**Figure B.1 — Apparatus assembly for wet sieving method**

#### B.2.3.2 Counting procedure

The counting procedure is as follows (see [Figure B.1](#)).

- a) Place the specimen (1) on the sieve (3) then place the container (4) under the sieve (3) to collect the washing liquid.

- b) Spray water (2) on the specimen (1) and collect the washing liquid in the container (4).
- c) Perform suction filtration of the washing liquid b).
- d) Wash the inside of the container sufficiently and perform suction filtration of all liquid. Count the number of live mites on the filter paper (5.2.12) (see [Figure B.1](#), key 6) under a stereoscopic microscope (5.2.20).

The colouring liquid may be added to the washing liquid to make easy counting during filtration or after finished.

- e) Repeat this procedure b) until no live mites cannot be found.
- f) Take the sum of each value counted at repeating as the number of mites.

#### **B.2.4 Heat escape method**

**B.2.4.1 Apparatus assembly**, in which the specimen is placed between the heating plate and adhesive sheet. The live mites hate the heat and migrate to adhesive sheet.

#### **B.2.4.2 Counting procedure**

- a) Place the specimen on the heating plate at the initial temperature of  $40\text{ °C} \pm 1\text{ °C}$ .
- b) Place the adhesive sheet on the specimen.
- c) Keep the temperature of the heating plate between  $35\text{ °C}$  and  $40\text{ °C}$  for  $60\text{ min} \pm 5\text{ min}$ .
- d) Count the number of live mites on the adhesive sheet through a magnifying apparatus viewer.
- e) Record the temperature during test of the heating plate and describe it in the testing report.

## Annex C (normative)

### Repelling method by using Petri dish

#### C.1 General

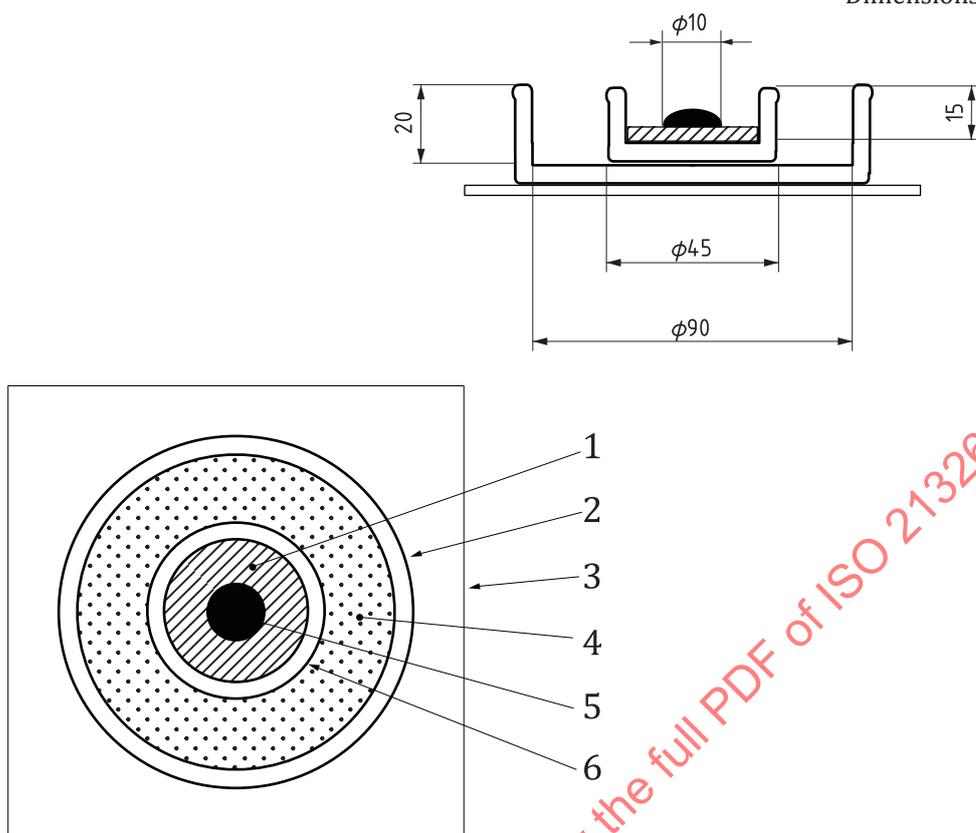
This method is applied to evaluate the efficiency of repellency for carpet, bedding surface fabric, bed sheeting, bed covering or blanket which have been treated to resist house dust mite.

#### C.2 Apparatus

**C.2.1 Testing assembly of apparatus**, constituted by two Petri dishes (large and small), in which the small Petri dish (5.2.6) is placed in the large Petri dish (5.2.5). [Figure C.1](#) shows a diagram and [Figure C.2](#) shows a photo as an example. The placement of the testing materials is as follows.

- In the small Petri dish, the test specimen is placed over the bottom and the culture medium as bite is placed on the centre of the specimen.
- In the centre of the large Petri dish, the small Petri dish with the testing materials is placed after the mite medium with 10 000 live mites is put uniformly in the large Petri dish as shown in the photo of [Figure C.2](#).

Dimensions in millimetres



**Key**

- 1 specimen
- 2 large Petri dish
- 3 sticky sheet
- 4 mite medium
- 5 culture medium
- 6 small Petri dish

**Figure C.1 — Testing apparatus assembly of Petri dish method**



**Figure C.2 — Photo of the testing apparatus assembly**

**C.2.2 Airtight container**, with the testing Petri dishes as shown in [Figure C.3](#).



**Figure C.3 — Airtight container with testing Petri dishes**

### C.3 Specimen

The specimen shall have a thickness of less than 15 mm. If the specimen have a thickness over 15 mm, the part of the specimen shall be peeled off or cut to reduce the thickness to less than 15 mm.

- a) Cut approximately 40 mm in diameter from the sample with 5 pieces.
- b) Cut the reference sample (see [Clause 6](#)) as in step a).
- c) Spread each of the specimens and each of the reference samples in a small Petri dish ([5.2.6](#)), respectively, as shown in [Figure C.1](#).

### C.4 Test procedure

- a) Place 0,05 g of the culture medium prepared according to [A.2.2](#) on centre of the specimen or the reference sample in the small Petri dish as shown in [Figure C.1](#), within approximately 10 mm in diameter as shown in [Figure C.1](#) and [Figure C.2](#).
- b) After stirring the mite medium well, prepare the mite medium with the mass containing 10 000 live mites as shown in [Clause 7](#).
- c) Put uniformly the mite medium in the large Petri dish ([5.2.5](#)) as shown in [Figure C.2](#).
- d) Place the small Petri dish in the centre position of the large Petri dish to complete the test apparatus assembly.
- e) Place the test apparatus assembly on a sticky sheet ([5.2.14](#)) as shown in [Figure C.1](#) and place it into an airtight container ([5.2.17](#)) as shown in [Figure C.3](#).
- f) Place the container in the incubator ([5.2.2](#)) according to [8.2](#) for 24 h  $\pm$  1 h.

### C.5 Counting method

- a) Take out the small Petri dish and wipe off the outside surface of the small Petri dish.
- b) Count the number of live mites of the testing assembly as follows:
  - the culture medium placed in the small Petri dish by using [B.2.1](#);
  - the specimen and the inside of the Petri dish by using [B.2.3](#) or [B.2.4](#).

- c) Take the sum of all counted number of invading mites.

## C.6 Test result

### C.6.1 Judgement of test effectiveness

When the conditions of steps a) and b) are satisfied, the test is judged to be effective. When the test is judged to be ineffective, a retest shall be carried out.

- a) The coefficient of variation of the number of live mites in the mite medium for the inoculation calculated in [Clause 7](#) d) shall be less than 10 %.
- b) The average of the number of invading mites in the standard woven fabric shall be 1 000 or more.

### C.6.2 Calculation of rate of repellency

The rate of repellency is calculated by [Formula \(C.1\)](#). The numerical value is rounded to one decimal place.

$$E_V = \frac{\sum_{i=1}^n C_{S_i} - \sum_{i=1}^n T_{S_i}}{\sum_{i=1}^n C_{S_i}} \times 100 \quad (C.1)$$

where

$E_V$  is the rate of repellency (%);

$C_{S_1}, C_{S_2}, \dots, C_{S_5}$  are the number of invasion mites in the standard woven fabric;

$T_{S_1}, T_{S_2}, \dots, T_{S_5}$  are the number of invasion mites in the testing materials;

$n$  is the number of the test specimens and the reference sample used in the test respectively,  $n = 5$ .

## Annex D (normative)

### Repelling method by using glass tube

#### D.1 General

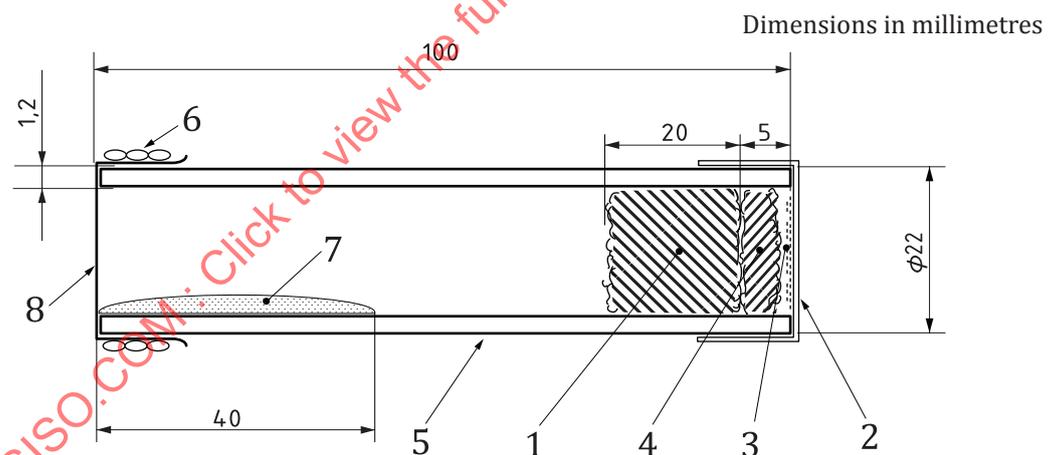
The glass tube method is used to evaluate the efficiency for repellency of wadding as Method A (see [D.4.1](#)), and down and feather as Method B (see [D.4.2](#)), which has been treated to resist house dust mite.

#### D.2 Apparatus

##### D.2.1 Testing apparatus assembly for the glass tube Method A

Method A is applied to wadding with a fibre content of cotton, wool or synthetic fibre.

The testing apparatus assembly for the glass tube Method A is shown in [Figure D.1](#) and [Figure D.2](#) as an example. The dimensions are approximate values.



#### Key

- 1 specimen
- 2 pressure sensitive adhesive tape
- 3 culture medium
- 4 stuffing
- 5 glass tube A
- 6 rubber band
- 7 mite medium
- 8 high-density fabric

**Figure D.1 — Testing apparatus assembly for the glass tube Method A**

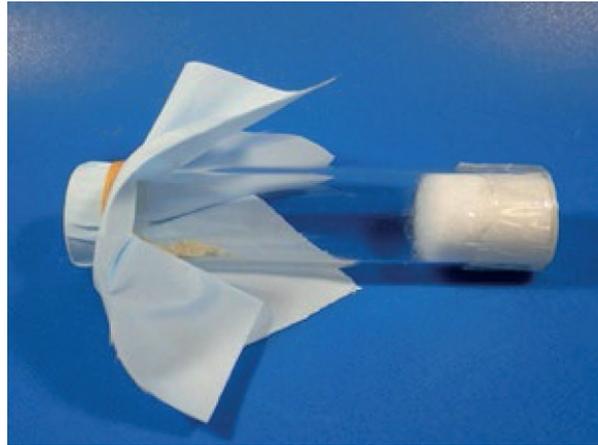


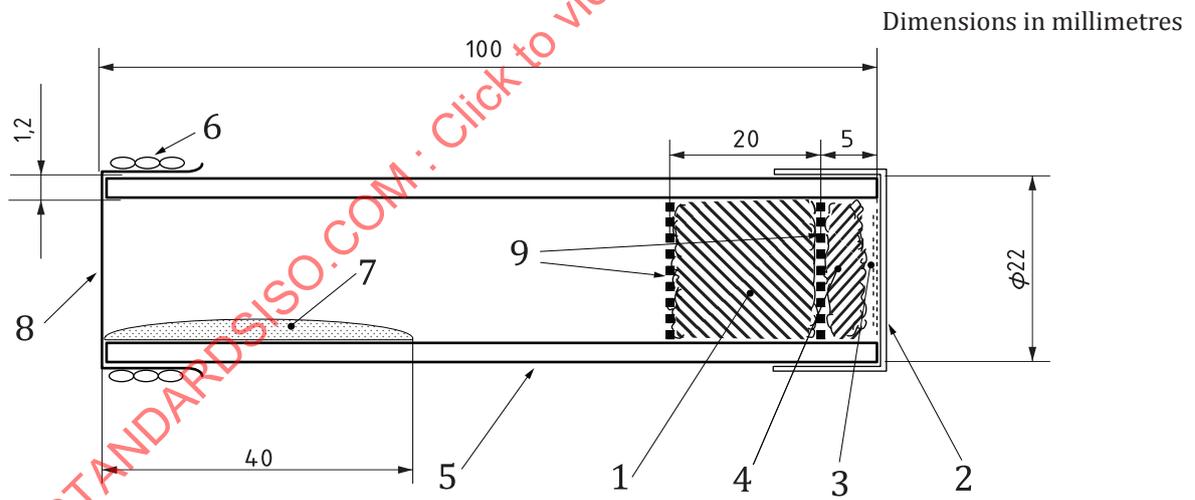
Figure D.2 — Glass tube Method A

**D.2.2 Testing apparatus assembly for glass tube Method B**

Method B is applied to wadding composed of down and feathers.

The testing apparatus assembly for the glass tube Method B is shown in [Figure D.3](#) and [Figure D.4](#) as an example.

The difference between Method A and Method B is that Method B uses the stainless wire mesh disks at both side of the test specimen to keep the thickness of the specimen as determined as shown in [Figure D.3](#).



**Key**

- 1 specimen
- 2 pressure sensitive adhesive tape
- 3 culture medium
- 4 stuffing
- 5 glass tube A
- 6 rubber band
- 7 mite medium
- 8 high-density fabric
- 9 stainless wire mesh disks

Figure D.3 — Testing apparatus assembly of the glass tube method B



Figure D.4 — Glass tube method B

### D.3 Specimen

The specimens are wadding or feathers and/or down.

### D.4 Preparation

#### D.4.1 Glass tube Method A

- a) Seal one end of glass tube A (5.2.7 and Figure D.3) by a pressure sensitive adhesive tape (5.2.13) as shown in Figure D.3.
- b) Put 0,01 g of the culture medium prepared in A.2.2 into a glass tube as shown in Figure D.1, and adhere uniformly onto the pressure sensitive adhesive tape.
- c) Put 0,025 g of stuffing (5.2.26 and Figure D.1) into a glass tube and stuff it to become the thickness of about  $5 \text{ mm} \pm 1 \text{ mm}$  as shown in Figure D.1.
- d) Insert 0,4 g of the specimen into the glass tube so that the thickness of the specimen becomes  $20 \text{ mm} \pm 2 \text{ mm}$  as shown in Figure D.1.
- e) Repeat steps a) to d) and prepare five glass tubes assemblies for each specimen. For the standard woven fabric, prepare five assemblies as same as for the specimen.
- f) Place the glass tube assemblies into separate airtight containers (5.2.17) so that the test assemblies from the same sample are put into one container and keep it horizontally for a minimum of 8 h.
- g) Prepare the mite medium with 10 000 live mites according to Clause 7.

#### D.4.2 Glass tube Method B

- a) Take the same procedure of glass tube Method A from steps a) to c).
- b) Place the stainless wire mesh disk (5.2.26) in the glass tube in a way that it is attached to the stuffing as shown in Figure D.3.
- c) Put 0,08 g of the specimen, and then place another stainless wire mesh disk (see Figure D.3) until the thickness of specimen becomes  $20 \text{ mm} \pm 2 \text{ mm}$  as shown in Figure D.3.
- d) Then, take the same procedure of glass tube Method A from steps e) to g).

## D.5 Test procedure

Perform the following procedure for both glass tube Method A and Method B.

- a) Inoculate the mite media prepared in [Clause 7](#) with 10 000 live mites after stirring well in the opposite end of the glass tube A as shown in [Figure D.1](#) and [D.3](#). Spread it approximately 40 mm as shown in [Figure D.1](#) and [Figure D.3](#).
- b) Place the high-density fabric ([5.2.15](#)) on the open end of the glass tube A as shown in [Figure D.1](#) and [Figure D.3](#) and fix the fabric by rubber band as shown in the [Figure D.1](#) and [Figure D.3](#) (see [Figure D.2](#) and [Figure D.4](#)).
- c) Place the test apparatus assembly into an airtight container ([5.2.17](#)) for 48 h ± 1 h under the designated conditions in [8.2](#).

## D.6 Counting method

- a) Take off the pressure sensitive adhesive tape, the culture medium and the stuffing from the testing apparatus assembly, to count the number of live mites.
- b) Count the number of live mites as follows:
  - the adhesive tape by [B.2.3](#);
  - the culture medium by [B.2.3](#) or [B.2.4](#);
  - the stuffing by [B.2.3](#) or [B.2.4](#).
- c) Sum all live mites counted at b).

NOTE Direct count or other suitable counting methods can also be used.

## D.7 Test result

Follow to [Annex C](#) and [Clause 6](#).

## Annex E (normative)

### Proliferation method

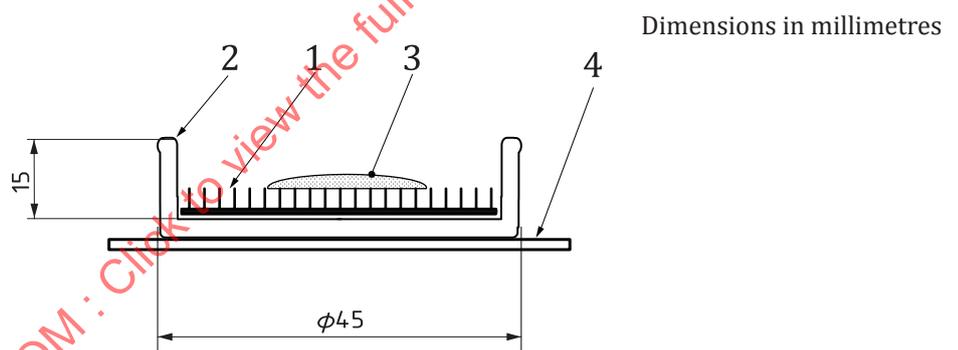
#### E.1 General

This test methods can evaluate the suppression effect against the reproduction of house dust mites by using a Petri dish as Method A for carpet, etc. and by using a vial for wadding as Method B as described in the scope.

#### E.2 Testing apparatus assembly

##### E.2.1 Petri dish Method A

**E.2.1.1 Testing apparatus assembly for Petri dish Method A**, a Petri dish is used for flat testing materials as shown in [Figure E.1](#) and [Figure E.2](#).



#### Key

- 1 specimen
- 2 small Petri dish
- 3 mite medium for inoculation (0,1 g, live mites of 50 to 80)
- 4 sticky sheet

**Figure E.1 — Testing apparatus assembly for Method A**



**Figure E.2 — Photo for Petri dish Method A**

E.2.1.2 **Airtight container**, with the testing Petri dishes as shown in [Figure E.3](#).

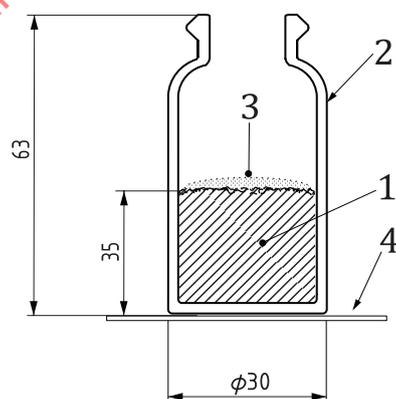


Figure E.3 — Airtight container with the testing Petri dishes

## E.2.2 Method B

E.2.2.1 **Testing apparatus assembly for vial Method B**, for wadding testing materials as shown in [Figure E.4](#) and [Figure E.5](#).

Dimensions in millimetres



### Key

- 1 specimen
- 2 vial
- 3 mite medium for inoculation (0,1 g, live mites of 50 to 80)
- 4 sticky sheet

Figure E.4 — Testing vial for Method B

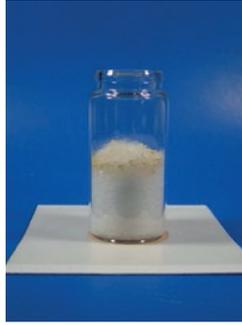


Figure E.5 — Photo for vial Method B

### E.3 Specimen

#### E.3.1 Method A

The specimen such as carpet shall be the thickness less than 15 mm, and if the test specimen with the thickness is over 15 mm, the part of the specimen shall be peeled off or cut to reduce the thickness to less than 15 mm.

#### E.3.2 Method B

Take approximately 1 g of the specimen, such as wadding, and fill a vial until the thickness of specimen becomes  $35 \text{ mm} \pm 5 \text{ mm}$ . If the thickness is out of this range, adjust the weight of the specimen.

### E.4 Preparation

#### E.4.1 Method A

- a) Take a testing specimen and a standard woven fabric of approximately 40 mm in diameter from the testing sample and the standard woven fabric, respectively.
- b) Spread the specimen and the standard woven fabric on a small Petri dish (5.2.6) as shown in Figure E.1 and Figure E.2.
- c) Prepare 9 Petri dishes for both testing samples and the standard woven fabric as shown in Figure E.3.
- d) Put the Petri dishes and the vials with the testing specimens and the standard woven fabric into the airtight containers (5.2.17) so that each container holds 9 Petri dishes and 9 vials and condition for minimum of 8 h.
- e) Prepare the mite medium of 0,1 g with 50 to 80 live mites for inoculation according to Clause 7 f).

#### E.4.2 Method B

- a) Weigh 1 g of the testing sample and the standard woven fabric respectively and put it into a vial (5.2.10) as shown in Figure E.4 and Figure E.5.
- b) Prepare 9 vials for 9 testing samples and 9 vials for 9 standard woven fabric respectively as in Method A.
- c) Put the Petri dishes and the vials with the testing specimens and the standard woven fabric into the airtight containers (5.2.17) so that each container holds 9 Petri dishes and 9 vials and condition for minimum of 8 h.

- d) Prepare the mite medium of 0,1 g with 50 to 80 live mites for inoculation according to [Clause 7 f\)](#).

## E.5 Testing procedure

Perform the following testing procedure for both Petri dish Method A and vial Method B.

- a) Spread 0,1 g of the well-stirred mite medium with 50 to 80 live mites for inoculation over the surface of the testing specimens and the standard woven fabric as shown in [Figure E.1](#) and [Figure E.4](#).
- If the specimen is a carpet, place the mite medium for inoculation into the deepest parts of the pile tufts and pay attention that the mite medium does not overflow from the Petri dish.
- b) Place the test apparatus on a sticky sheet.
- c) Put these testing apparatuses into an airtight container ([5.2.17](#)) as shown in [Figure E.3](#).
- d) Keep them under the testing condition ([8.2](#)) for a maximum of 8 weeks.
- e) Count the number of the live mites after 4 weeks and 6 weeks from the start of the test as periodical checking to know the progress of testing. If necessary, count the number of the live mites after between 7 weeks and 8 weeks.
- f) Repeat the test 3 times and at each observation period, count the number of live mites in or on each testing specimen and the standard woven fabric.

## E.6 Counting method

- a) Take out the test apparatus and wipe the outside of test container.
- b) Count the number of the live mites on the testing assembly as follows:
- the mite medium spread on the testing specimen or the standard woven fabric by [B.2.1](#);
  - the testing container and the specimen respectively by [B.2.3](#) or [B.2.4](#).
- c) Take the sum for the counted in step b) as the test value.

## E.7 Test result

### E.7.1 Judgement of test effectiveness

The test is deemed to be effective when the conditions of the steps below are satisfied. If the test is judged to be ineffective, a retest shall be carried out.

- a) The coefficient of variation of the number of live mites in the mite medium for the inoculation calculated in [Clause 7 d\)](#) shall be less than 10 %.
- b) The average of the initial population obtained in [E.4.2 d\)](#) and [Clause 7 f\)](#) shall be 50 to 80 live mites.
- c) The average of the number of live mites of the standard woven fabric after 4 weeks from the start of test shall be 3 times or more the average of the initial population.