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Textiles — Anti-mosquito performance test method using the attractive blood feeding apparatus

Textiles — Méthode d'essai de performance anti-moustiques au moyen du dispositif d'attraction par apport de sang

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Foreword

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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.

Introduction

In recent years, threats of infectious diseases mediated by insects such as mosquitoes are rising. In response to consumers' concerns about this, demand for high-performance anti-mosquito fabrics has been expanding. However, there has been no standard for evaluating the performance of such anti-mosquito fabrics, and it has been difficult to provide fair technical information on their performance. Therefore, a new test method was established in order to evaluate anti-mosquito performance of fabrics.

For this purpose, an apparatus was developed for attraction and blood feeding of blood-unfed mosquitoes through fabrics without using human or animal as a feeding source.

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Textiles — Anti-mosquito performance test method using the attractive blood feeding apparatus

1 Scope

This document specifies a method for evaluating the function of reducing mosquito contact and blood feeding through the anti-mosquito fabric regardless of whether chemicals are treated or not. It provides the test method for evaluating this function without using human or animal as blood feeding sources.

In addition, this document is only concerned with evaluation of anti-mosquito performance, and not concerned with evaluation of preventive method of diseases caused by anti-mosquito performance.

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 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 https://www.electropedia.org/

3.1
anti-mosquito fabric
fabric for preventing blood sucking by treating chemicals or processing fabric structure

3.2
landing
state of mosquito alighting and staying on a surface within an arbitrary section (on the test specimen)

3.3
blood feeding rate
percentage of the number of blood-fed mosquitoes through specimen to the total number of mosquitoes

3.4
probing behaviour
behaviour of mosquito while looking for a place to insert its proboscis for blood feeding

Note 1 to entry: This gesture is recognized as an up and down movement of the head.

3.5
blood feeding prevention
effectiveness of test sample in preventing blood feeding of mosquitoes

Note 1 to entry: In this document, it is indicated by the blood feeding prevention index.

4 Principle

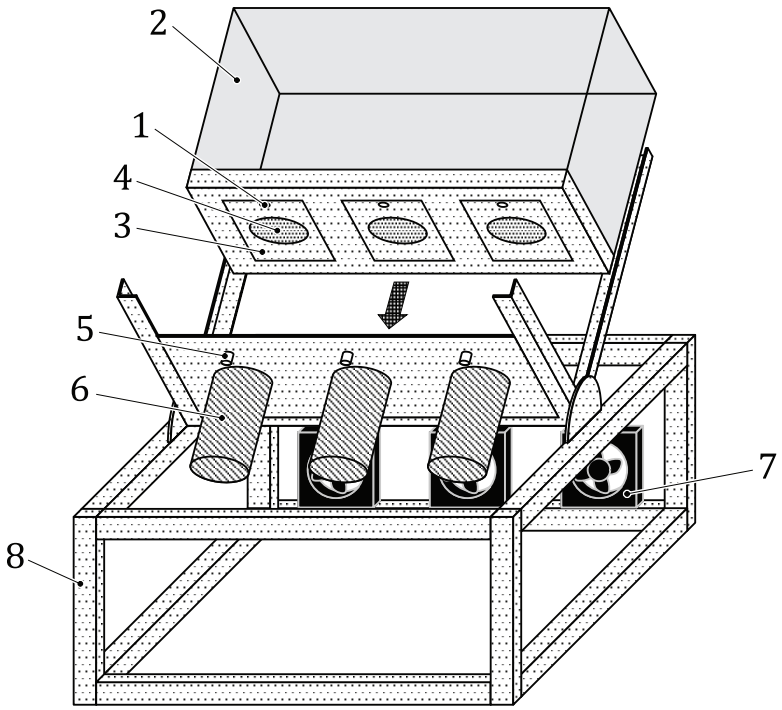
Using the attractive blood feeding device, obtain the blood feeding prevention index and determine the landing and probing behaviour of blood eager female mosquitoes when exposed to a test sample.

5 Apparatus and materials

5.1 Attractive blood feeding device

5.1.1 General

Attractive blood feeding device consists of parts described in 5.1.2 to 5.1.7. An example of the configuration of attractive blood feeding device is shown in Figure 1.



Key

1	hole for supplying carbon dioxide gas	5	nozzle for supplying carbon dioxide gas
2	test cage	6	feeding device
3	specimen holder	7	ventilation fan
4	test specimen	8	frame

Figure 1 — Configuration example of attractive blood feeding device

5.1.2 Feeding device

The feeding device is composed of a feed reservoir for pouring test blood and a thermostat temperature regulator. An example of the configuration is shown in Figure 2 and Figure 3.

5.1.1.1 Feed reservoir, composed of a metal or glass container holding test blood and membrane covering the container. The surface covered with membranes serves as the feeding surface.

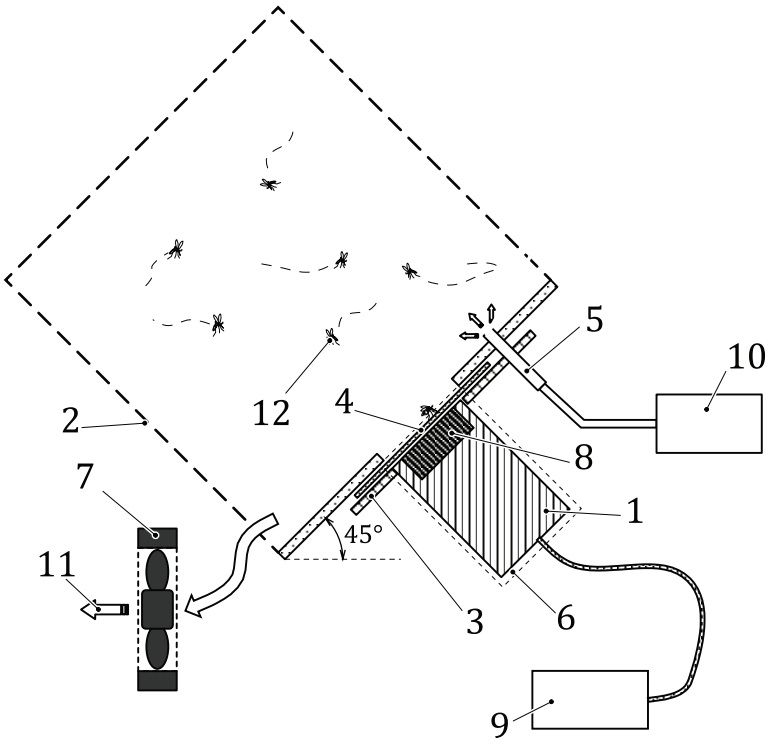
The diameter of feeding surface of feed reservoir shall be 35 mm ± 2 mm. The capacity of feed reservoir should be at least 3 ml.

5.1.1.2 Membrane

An animal intestine, animal skin, collagen film, sausage casing, or artificial skin to be used as a membrane through which a test mosquito can blood feed. Selected membrane should be agreed upon between the interested parties.

5.1.1.3 Thermostat temperature regulator, device connected to feed reservoir that can control temperature to a constant temperature ±0,5 °C.

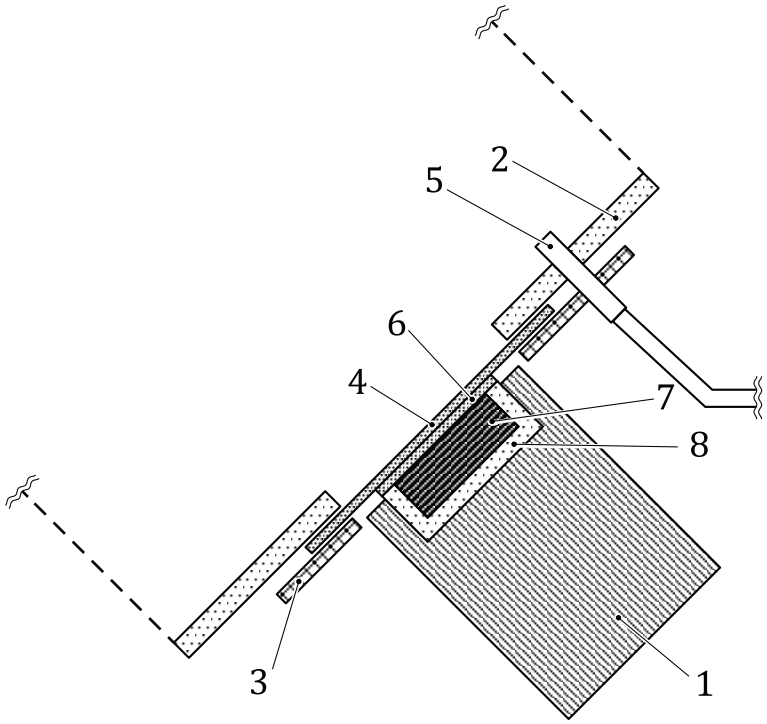
An electrically controllable heater, a constant temperature water circulation device, and the like are examples.



Key

1	thermostat temperature regulator	7	ventilation fan
2	test cage	8	feed reservoir
3	specimen holder	9	temperature control unit
4	test specimen	10	carbon dioxide gas supply unit
5	nozzle for supplying carbon dioxide gas	11	exhaust direction
6	feeding device	12	test mosquito

Figure 2 — Configuration example of periphery of attractive feeding device (cross sectional view)



Key

1	thermostat temperature regulator	5	nozzle for supplying carbon dioxide gas
2	test cage	6	membrane
3	specimen holder	7	test blood
4	test specimen	8	feed reservoir

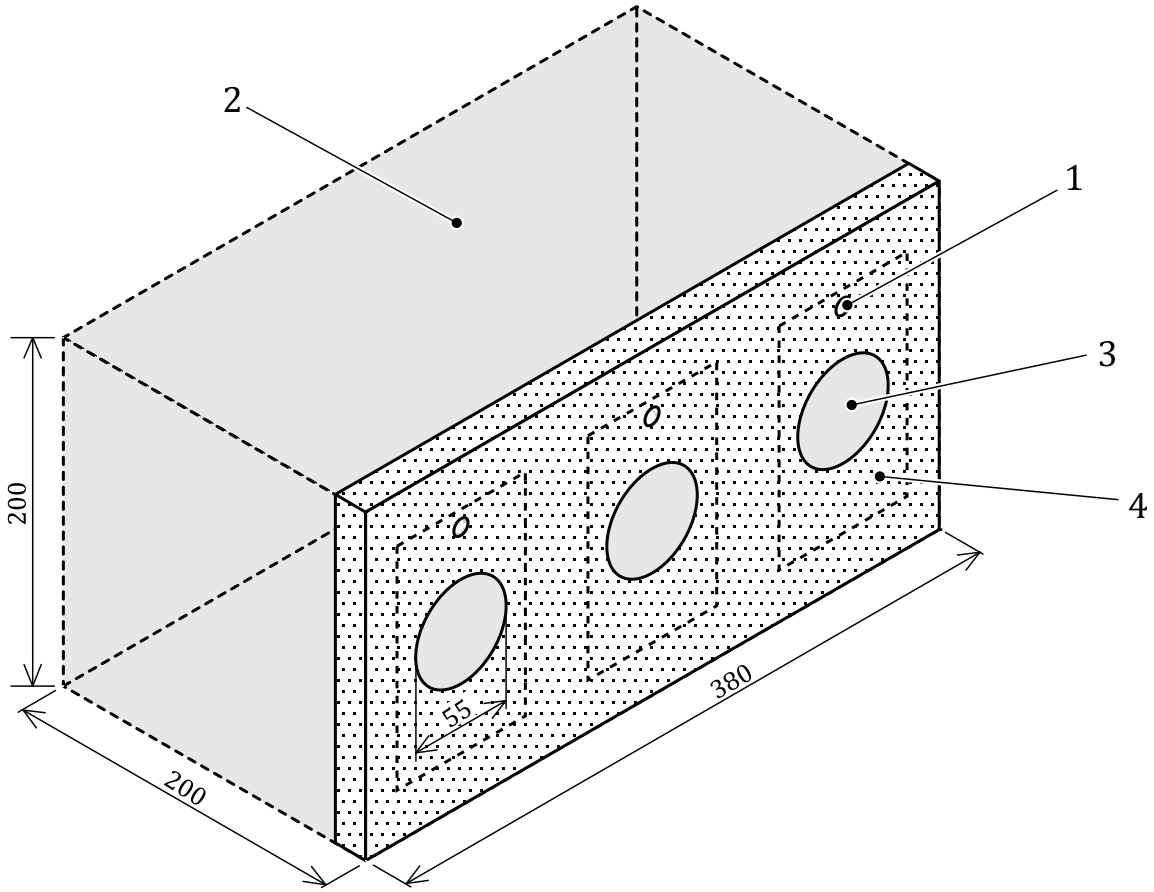
Figure 3 — Configuration example of periphery of feed reservoir (cross sectional view)

5.1.3 Test cage

The test cage is a cuboid (width: 380 mm ± 10 mm, height: 200 mm ± 5 mm, depth: 200 mm ± 5 mm) with three openings on one side that can be connected to a feeding device having a structure designed to allow test mosquito to be released inside. Three openings with diameter 55 mm ± 2 mm are jointed to feed reservoirs via specimen holder which size is such that feeding surface is sufficiently exposed through the openings. Three openings should be covered with lids before and after the test, and should be tightly connected with specimen holder during the test to prevent the test mosquitoes from escaping from the cage. Mesh is used for five faces of test cage. Mesh is fine enough so as not to allow test mosquitoes to escape, but not too tight so that the behaviours of test mosquitoes inside can be observed.

An example of test cage is shown in Figure 4.

Dimensions in millimetres



Key

1 hole for supplying carbon dioxide gas
2 mesh (5 faces)
3 opening
4 fitting part of specimen holder

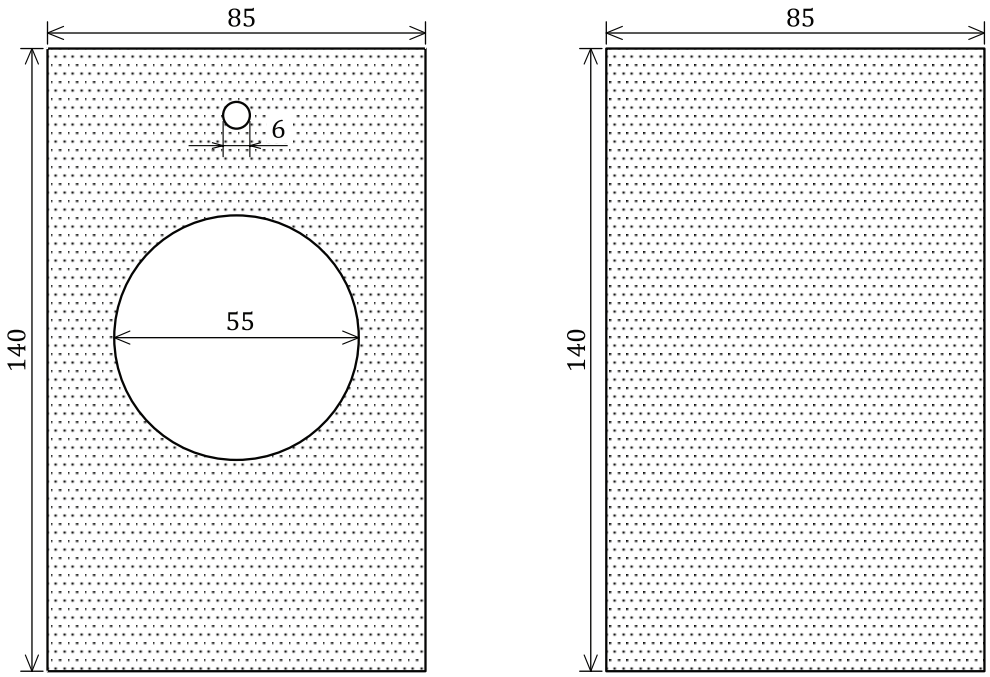
Figure 4 — Example of test cage

NOTE In order to reduce the effect of the examiner's exhalation on the test mosquito, for example, a face shield may be worn or a transparent acrylic sheet may be placed on the observation surface.

5.1.4 Specimen holder and lid

The specimen holder is used for immediate removing of test specimen (or validation specimen) after test. The lid is used for avoidance of escape of test mosquitoes from test cage before and after test. The specimen holder is quickly removed from test cage immediately after completion of test and opening of test cage is immediately closed by lid. An example of specimen holder and lid is shown in Figure 5.

Dimensions in millimetres



a) Specimen holder **b) Lid**

Figure 5 — Example of specimen holder and lid

5.1.5 Frame

A frame that can connect and fix the test cage and feeding device, and has a structure that can fix feeding surface at a position 45° from the horizontal plane.

5.1.6 Carbon dioxide gas supply unit and ventilation fan

The carbon dioxide gas supply unit is composed of a carbon dioxide gas cylinder, a gas regulator, a flow meter, carbon dioxide gas supply nozzle with an inner diameter of approximately 3 mm and a solenoid valve with timer for releasing a certain amount of carbon dioxide (not less than 99,5 % purity) gas at constant time intervals on the surface of test specimen. Carbon dioxide gas is blown from the upper part in the vicinity of each test specimen (or validation specimen) of test cage. Air flow rate is 1,0 l/min ± 0,1 l/min, and air supplying time is 10 s per 1 min.

In order to prevent carbon dioxide gas residence in test cage, ventilation of air is done with ventilation fan or the like. The wind speed shall be about 1 m/s immediately under test cage.

5.1.7 Infrared surface thermometer

With a measurement range of 0 °C to 50 °C and accuracy of ±2 °C.

5.2 Test blood

Blood in Alsever's solution, a solution prepared by mixing animal blood with an anticoagulant. Alternatively, a solution containing the required amount of blood components or a solution containing similar components except of animal or human blood to promote the mosquito blood feeding can be used by agreement between the interested parties.

5.3 Test mosquito

Unfed female adults of Asian tiger mosquito, *Aedes albopictus* (Skuse) (see Figure 6), with blood feeding eagerness are used for test.

If other mosquito species are used for testing, the test mosquito information shall be described in the test report as specified in Clause 7.

Figure 6 — Asian Tiger Mosquito, *Aedes albopictus* (Skuse), females

For *Aedes albopictus*, in order to obtain test mosquitoes with blood feeding eagerness, test mosquitoes 7 days to 20 days old after emergence should be used, which are kept in breeding cages with male adult mosquitoes. The mosquito breeding conditions used for test is shown in Annex A.

5.4 Validation sample

Validation sample is medical gauze, bleached and purified, 100 % cotton woven, plain weave, warp (11 ± 1) threads/cm weft (9 ± 1) threads/cm. It is used for testing to confirm the blood feeding activity of test mosquitoes.

Alternatively, a stainless steel mesh can be used by agreement between the interested parties.

NOTE Examples of medical gauze include types 18, 20, and 22 as specified in EN 14079:2003.

6 Test sample and test specimen

6.1 General

Two types of fabric samples, test sample and validation sample, are prepared as test specimen and validation specimen, respectively.

6.2 Handling of test sample and test specimen (Conditioning)

Preparation of test sample and test specimen is done by keeping them in an environment without direct sunlight, at a temperature of 27 °C ± 2 °C, relative humidity within range of from 50 % to 85 % for more than 16 h. Test sample and test specimen are individually placed in a sealed container or the like in order to avoid sample contamination and laboratory contamination caused by diffusion and adhesion of anti-mosquito repellent chemicals.

6.3 Preparation of specimen

Preparation of test specimens shall be done as follows.

a) Prepare nine test pieces. Test for each sample is repeated 3 times. One set of 3 specimen is used for each test.

b) Each test specimen shall have a dimension of 8 cm × 8 cm.

c) Attach the test piece to the test piece holder.

7 Test environment

Test shall be conducted in an environment not exposed to direct sunlight at a temperature of 27 °C ± 2 °C, relative humidity within range of from 50 % to 85 %, illuminance of 600 lx or more. Also, ensure that the exhalation gas and body temperature of the observer does not affect test mosquitoes in attractive blood feeding device.

When using test mosquitoes other than *Aedes albopictus*, test environment may be changed to appropriate environmental conditions depending on species.

8 Preparation

8.1 Separation of test mosquitoes

Openings of test cage shall be closed in advance with lids. To collect test mosquitoes with blood feeding eagerness, mosquitoes showing probing behaviour to human hands held over breeding cage shall be collected with aspirator tube. Thirty test mosquitoes are released in test cage. Test cage containing test mosquitoes is kept in breeding environment described in Annex A until the start of test.

8.2 Preparation of thermostat temperature regulator

Feeding device of thermostat temperature regulator shall be pre-heated before starting test.

8.3 Preparation of feed reservoir

Test blood is poured into feed reservoir covered with membrane.

8.4 Preparation of feeding device

Feed reservoir is attached to thermostat temperature regulator. Temperature of feeding surface should be confirmed to reach above 34 °C with tolerance 4 °C using an infrared surface thermometer.

9 Procedure

9.1 Test procedure

Test operating procedure shall be as follows.

a) Replace the lids of all openings with the test specimen holders to which the test specimen is attached. Expose the front side of test specimen inside the test cage.

b) Install the test cage on the frame.

c) The three test specimens are kept close to each feeding surface and are preheated. The distance between the test specimen and the feeding surface should be approximately 7 mm to prevent mosquitoes from blood feeding before testing.

d) Adhere the feeding surface and test specimen, and start the test. During the test, make sure that the observer does not breathe on the feeding surface.

e) Release carbon dioxide gas with a supply cycle described in 5.1.6.

f) Record the number of landing test mosquitoes on the feeding surface for a 10 min test period. Recordings shall be taken every 30 s for the first 6 min and every 60 s after 6 min.

g) At the completion of the final recording at 10 min, separate the test specimen from the feeding surface to prevent additional feeding.

h) Stop supplying of carbon dioxide gas.

i) Replace the test specimen holders with the lids.

j) Kill the test mosquitoes by an appropriate method such as freezing treatment.

k) Count the number of blood-fed and blood-unfed test mosquitoes. Crush the test mosquito abdomens to examine whether they are blood-fed or not.

l) Wash the specimen holders, test cages, feed reservoirs, feeding devices, etc. that might be contaminated with repellent treatment from the test specimen.

9.2 Validation check procedure

Validation check shall be carried out using validation specimen and the operating procedure shall be as described in 9.1.

9.3 Calculation

9.3.1 Calculation of landing rate

The landing rate for each measurement time and average landing rate for each measurement section are calculated using Formulae (1) to (4).

a) Landing rate for each measurement time

$$L_t = \frac{N_{Lt}}{N} \times 100 \quad (1)$$

where

L_t is the landing rate (%) at arbitrary time t (min);
For example, $L_{0,5}$ represents landing rate after 0,5 min (30 s) of measurement start;
N_{Lt} is the number of landing (number of test mosquitoes) at arbitrary time t (min);
N is the total number of test mosquitoes.

b) Average landing rate per measurement section

$$\bar{L}_e = \left(L_{0,5} + L_1 + L_{1,5} + L_2 + L_{2,5}\right)/5 \quad (2)$$

$$\bar{L}_m = \left(L_3 + L_{3,5} + L_4 + L_{4,5} + L_5 + L_{5,5}\right)/6 \quad (3)$$

$$\bar{L}_l = \left(L_6 + L_7 + L_8 + L_9 + L_{10}\right)/5 \quad (4)$$

where

$\bar{L}_e$ is the initial average landing rate (%) (0 min to 3 min after start of test);

$\bar{L}_m$ is the mid-term average landing rate (%) (3 min to 6 min after start of test);

$\bar{L}_l$ is the late average landing rate (%) (6 min to 10 min after test).

The average landing rate for each measurement section repeated three times is averaged and rounded to the integer position.

9.3.2 Calculation of blood feeding rate

Blood feeding rate is calculated by Formula (5):

$$F = \frac{N_F}{N} \times 100 \quad (5)$$

where

F is the blood feeding rate (%);

N_F is the number of blood-fed test mosquitoes;

N is the total number of test mosquitoes.

Blood feeding rates with three times repetition are averaged (hereinafter referred to as "average blood feeding rate F_{ave}") and rounded to the integer position.

9.3.3 Calculation of blood feeding prevention index

Blood feeding prevention index is calculated by Formula (6)

$$E_F = 100 - F_{ave} \quad (6)$$

where

E_F is the blood feeding prevention index;

F_{ave} is the average blood feeding rate (%).

Anti-mosquito performance is classified according to blood feeding prevention index in Annex B.

9.4 Blood feeding validation check

In order to confirm blood feeding activity of test mosquitoes, every test day or with each new breeding lot of test mosquitoes, at least one validation check with validation specimen is conducted in accordance with the test procedure of 9.1. If the blood feeding rate, when calculated using Formula (5), does not reach 50 % the blood feeding activity of test mosquitoes is judged as insufficient.

If blood feeding activity of test mosquitoes is insufficient, another validation check shall be conducted. Testing shall not begin until blood feeding activity of the test mosquitoes is validated to exceeds 50 %.

10 Test report

The test report shall state the following items.

a) date of test

b) a reference to this document, ISO 24461:2022;

c) environmental conditions of the test site;

d) test mosquito information (name of mosquito species, colony name, place of breeding or purchase, age after emergence);

e) test blood, membrane;

f) sample information (product name, product number, chemical name of ingredients, etc.);

g) test results (Initial average landing rate, average landing rate in the middle term, average landing rate in late stage, total number of test mosquitoes, number of blood-fed mosquitoes, blood feeding rate, blood feeding prevention index);

h) notices that seem to be required reporting (such as probing behaviour of test mosquitoes);

i) deviations from this test method.

For reference, examples of test reports are shown in Table 1.

Table 1 — Example of test report

Date of test	Year Month Day								
Standard number	ISO 24461								
Test condition	°C, %RH								
Test mosquito information	Type of mosquito, colony name, subculture place or purchase site, post-emergence age								
Type of membrane covering test blood, bait container (product name, etc.)	Animal preserved blood, sausage casing								
Sample information	Product name, product number etc.								
Sample		$\bar{L}_e$	$\bar{L}_m$	$\bar{L}_l$	**Total number of test mosquitoes, *N***	**Number of blood-fed test mosquitoes, N_F**	**Blood feeding rate, *F***	**Blood feeding prevention index, E_F**	**Classification of anti-mosquito performance**
Validation specimen	1								
Test specimen	1								
	2								
	3								
	average						(F_{ave})		

Annex A
(informative)

Breeding environment of mosquitoes used for testing

Mosquitoes used for test are kept under the following environmental conditions. Conditions not shown in this annex are based on general rearing techniques.

a) Environmental conditions of breeding room

   Same as the test conditions described in 7, temperature 27 °C ± 2 °C, and relative humidity within range of from 50 % to 85 %.

b) Lighting: Illumination of the breeding room shall be a cycle of 16 h light and 8 h dark.

c) Rearing: Adult mosquitoes are reared as follows until they are used for test.

   1) Adult mosquitoes are released in a breeding cage while mixing the sexes.

   2) Adult mosquitoes are fed with 3 % to 5 % aqueous sucrose solution and are not fed with blood.

   3) Female adults that reached the age after emergence prescribed in 5.3 are used for test.

Annex B
(informative)

Evaluation of blood feeding prevention

B.1 General

Blood feeding prevention index is indicated by stepwise classification.

B.2 Evaluation of anti-mosquito performance

Anti-mosquito performance is classified according to Table B.1 based on blood feeding prevention index obtained by 9.3.3.

Table B.1 — Evaluation of anti-mosquito performance

Blood feeding prevention index	95 or more	80 or more and less than 95	50 or more and less than 80	Less than 50
Symbolization	E95+	E80	E50	E50-

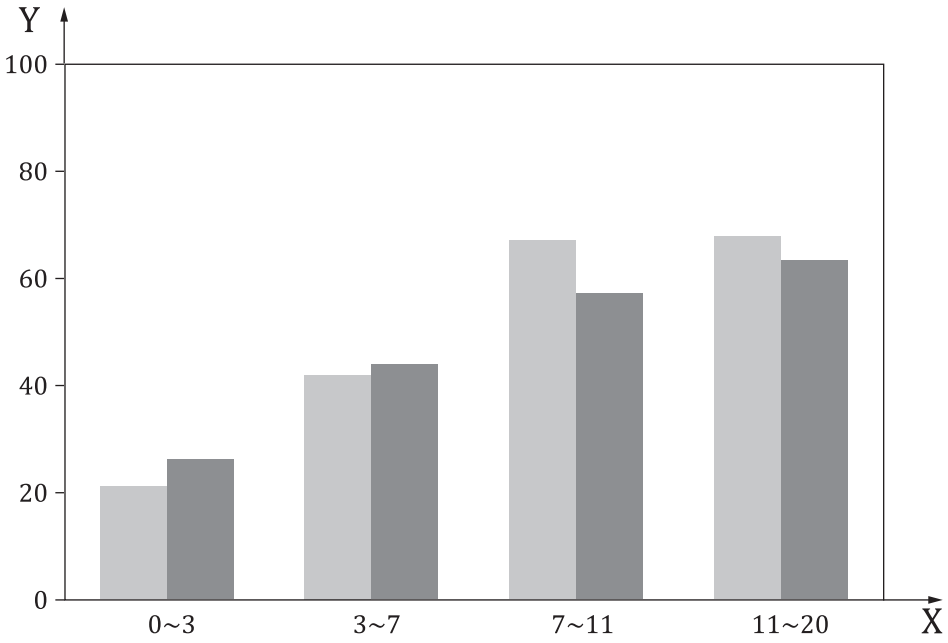
Annex C
(informative)

Blood feeding rate in relation to ages of test mosquitoes

The blood feeding rate of test mosquitoes at different days old after emergence was measured using the validation specimen.

Two colonies of blood-unfed female test mosquitoes (Colony ID: SCC and NGC) were used for the test when they reached the relevant age range in days old after emergence.

Blood feeding rate of both of the colonies exceeded 50 % after 7 days old after emergence (see Figure C.1).



Key

Aedes albopictus (SCC)

Aedes albopictus (NGC)

X age range (days old after emergence)

Y blood feeding rate (%)

Figure C.1 — Example of blood feeding rate in relation to ages of test mosquitoes

Bibliography

[1] EN 14079:2003, *Non-active medical devices. Performance requirements and test methods for absorbent cotton gauze and absorbent cotton and viscose gauze*

[2] TSURUKAWA C., KAWADA H., Experiment on mosquito blood feeding using the artificial feeding device. Med. Entomol. Zool. 2014, **65** pp. 151–155

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