Guideline to Efficacy Test Method of Mite Repellent

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MINISTRY OF FOOD AND DRUG SAFETY

National Institute
of Food and Drug Safety Evaluation

Cosmetics Evaluation Division,

Biopharmaceuticals and Herbal Medicine Evaluation Department

This guideline is described to suggest general principles and analytical methods for efficacy test of mite repellent among quasi-drugs in the level of the current scientific technology by reflecting the opinions of persons concerned in the industry and experts. The guideline may be amended additionally according to the development of the scientific technologies in the future. In addition, this guideline describes the view of the MFDS and it does not have legal effect publicly. It may be applied differently depending on individual cases.

※ If you have comments on this guideline, please contact us at the following.

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I. Introduction

Mites among harmful insects are parasitic on animals but frequently attack humans to cause irritation and the secondary infection by prick and bite. Therefore, the development of more effective mite repellent is being required. Especially, Haemaphysalis longicornis Neumann has 1% or less virus retention ratio, but it mediates severe fever with thrombocytopenia syndrome (SFTS) to cause death. In addition, trombicula mediates Tsutsugamushi fever or scrub typhus to cause fever, headache and muscle pain through latent period after infection (varies from 6 to 21 days, but commonly, 10 ~ 112 days). It requires attention because it may cause death if early treatment cannot be provided.

The mite repellent refers to the products that do not have the effect of killing mites, but contain substances mites dislike, and prevent mites to suck the blood as they are applied or sprayed on the skin or clothes. These products are currently classified as quasi-drugs, which are approved and managed by the MFDS.

To evaluate the accurate repelling effect of mite repellent on the human body, a variety of factors need to be considered to obtain more accurate and reproducible results. This guideline attempts to provide standardized test method which can be helpful for efficacy test and evaluation of mite repellent.

This guideline provides the guideline to test the repelling effect through *In vivo* clinical test and *In vitro* test among indoor tests of mite repellent.

For some parts that cannot be handled in this guideline, it may be helpful to refer to the contents of Guideline to Efficacy Evaluation of Mosquito Repellent.

II. Efficacy Test Method of Mite Repellent

1. General Statement

① Mite Species

The efficacy test of mite repellent should be conducted by using the mite species known to mediate diseases. The general species of hard tick inhabiting in Korea include *Haemaphysalis longicornis*, *Haemaphysalis flava, Ixodes nipponensis, and Amblyomma testudinarium* that belong to Family Ixodidae. Trombicula include *Leptotrombidium pallidum* or *Leptotrombidium scutellare* which belong to Family Trombiculidae. The mites used in the test should be identified by species and genus.

② Developmental Stage and Gender of Mites

The mites used in the test should be in the life cycle which seeks host. If testing with hard tick, both nymph and imago are appropriate. However, with regard to the efficacy test for the same study drug, the uniform stage of development (nymph or imago) should be unified and used. In addition, species not inhabited in Korea, for instance, if the species that does not suck the blood from humans in nymph stage such as American dog tick (*Dermacentor variabilis*) is used, only imago, the adequate stage of development, is recommended. If testing with trombicula species, larva should be sued. The gender and developmental stage of all testing mites must be recorded.

③ Breeding and Storage Condition of Mites

Before the test, mites should be stored under condition of temperature (22±3℃), relative humidity (50 ~ 80%), and light cycle (light:dark=16:8 hours). If other storage conditions are to be applied, the sufficient validity of them should be described.

④ Preparation of Test Mites

In principle, the test mites should be the species that does not mediate diseases. However, if the species known to mediate disease such as Haemaphysalis longicornis Neumann is used, the method for which 3rd person can logically understand enough that the mite species has no possibility of mediating diseases to the subjects should be used, and this method should be clearly described.

⑤ Use of Test Mites

The mites used in behavior screening in which mites move to find bloodsucking area in host and repelling efficacy test should be used only once, and they must not be reused. The mites used in the test should be disposed immediately after the test.

⑥ Test Condition

During the test period, temperature (20~25℃), relative humidity (not less than 35%), and indirect light (50~80%) should be maintained. The light must be turned on.

⑦ Positive Control

Generally, as the positive control for efficacy test of mite repellent, 20% N,N-diethyl-3-methylbenzamide(Deet)[1] ethanol solution should be treated at ratio of 1 mL per 600 cm^2.

2. Type of Test Method

1) *In vivo* Clinical Efficacy Test of Mite Repellent

① Subject Selection and Management

1) 20% N,N-diethyl-3-methylbenzamide(Deet) ethanol solution: Weigh about 2 g of N,N-diethyl-3-methylbenzamide, melt it in ethanol, and make 10mL.

The repellent test conducted in persons is the method to derive similar results as the actual use condition by using the end consumers of the repellent. As subjects, healthy adult volunteers who have no hypersensitivity or weak reaction to mite bite should be selected. The subjects should not be exposed to specific infectious risk in the same environment as the test being conducted. Before the test, the approval of Institutional Review Board (IRB) is required, and overall progress should be conducted in accordance with Good Clinical Practice (GCP).

To minimize various factors that may change the aggression of mites against people and factors that may affect the analysis results, the subjects should not use alcohol or perfume and other repellents 12 hours before the test and during the test. If possible, the volunteers should be non-smokers, and they should not smoke cigarettes at least 12 hours before the test and during the test.

② Number of Subjects

The number of subjects in the test differ by the time of repelling effect. If the repelling effect of 1 ~ 4 hours is to be investigated, at least 5 adult subjects should participate, and if the repelling effect of 5 hours and longer is to be investigated, at least 10 subjects should participate. It is desirable to recruit the same number of male and female subjects, if possible. The age of subjects is ranged 18 ~ 55 years old. To decrease the possible changes between subjects, health adults should be selected.

③ Size of Study Drug Treatment Area and Preparation

Each subject should put one hand on the flat floor with the forearm and wrist at angle of 30°. The top and bottom of the boundary of the study drug treatment area should be covered by materials to which mites cannot permeate. Both arms of each subject should be washed with odorless soap, rinsed with water, rinsed with at least 50% ethanol (or isopropanol), and then dried with a towel. The surface area (cm^2) of each subject should be calculated by multiplying the mean of circumferences of wrist and elbow by the length from the wrist to elbow (see Attachment 1). Treat the study drug from the wrist to elbow of one arm of each subject, and then draw 2 lines on the wrist[The first line is ‘boundary line’ at the

boundary of the study drug treatment area, and the second line is ‘release line’ at distance of 3 cm from the boundary line to fingers]. Do not treat the study drug on the other arm, and draw the same 2 lines to test.

④ Amount of study drug

Store the study drug at the room temperature and maintain constant humidity before the test. Record the manufactured date of study drug, and the drug should be the one manufactured within 1 year. Apply or spray 1g of the drugs such as aerosol or liquid, or 1 ~ 1.5g of cream, lotion, stick, etc. per 600cm^2 in arms of each subject evenly. If 1 g or 1.5 g are inadequate according to the characteristics of study drug, content, dose and administration, establish the amount of use applied by 95% confidence interval, and report the result. If the study drug is measured not weight but volume, it should converted to weight (mg/cm^2 or g/cm^2) based on the density of the product, and the results should be reported as well.

⑤ Test Procedures

Once it is proved whether mites move actively on the arms of the subjects not treated with the study drug (see ⑥ negative control below), use an appropriate apparatus (e.g. paint brush, pipette and cotton swab) to put 2 entities of mites on the release point (3 cm below the area treated with the study drug) in the arms of the subjects treated with the study drug at once. Observe whether mites move toward the upper direction of the arm treated with the study drug for 3 minutes. If mites cross[2] toward boundary line for study drug treatment within 3 minutes or mites stay in the treatment area for 3 minutes, record ‘crossing’ or ‘not repelled’. On the other hand, for mites that do not go to the study drug treated area, or they return immediately or fall to the ground even if they go, record ‘repelled’. What is cautious is to make sure that mites should not bite, and if it is suspected that mites are biting because they stay still for 20 seconds or more, push them gently with a brush so that they should not bite.

2) Crossing: Refers to the activity that mites move from the skin not treated with study drug to the skin treated with study drug. With crossing, it is possible to record the distance of mite movement or how long they remain in the skin treated with the study drug to quantify them.

시험약물처리지역: study drug-treated area

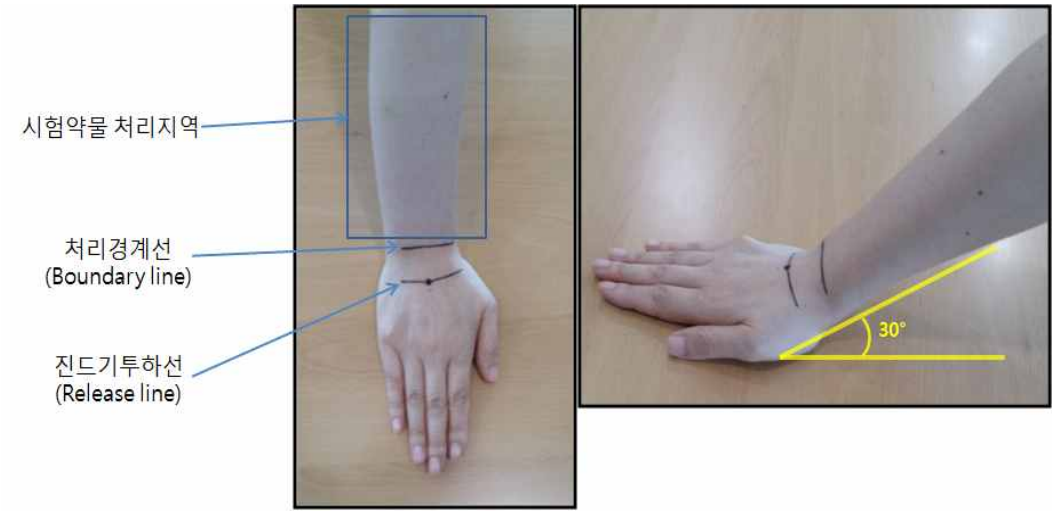

Figure 1. *In vivo* Clinical Efficacy Test Method of Mite Repellent

Begin the first exposure test after 10 minutes from the treatment of study drug, observe them for 30 minutes, and take a rest for 20 minutes. After 1 hour of the treatment, put new mites on the release line in the arms untreated with study drug, observe whether they move actively, and then put the adequate mites on the release line in the arms treated with the study drug and test them. If 2 entities show crossing (or not repelled) behavior (e.g. 1 entity cross at the same time line, and 1 entity cross at the next time line, or both 2 entities show crossing behavior at the same test) in the consecutive tests, record the time and report it as complete protection time (CPT).

⑥ Negative Control

Use the arms of each subject not treated with the study drug in screening behavior of mites to move to find host. Only the mites moving actively should be used in the repellent efficacy test. Under the same condition of the arms treated with the study drug, wash with odorless soap, rinse it with water, rinse it with at least 50% ethanol (or isopropanol), and then dry it with a towel. The investigator should use appropriate apparatus (e.g. paint brush, pipette and cotton swab) to put mites on the release line in the arms of the subjects not treated with the study drug carefully so that the trunk or fore-legs of mites should not

be damaged. The mites moving toward boundary line of the arms not treated with the study drug in the direction of elbow constantly are the mites moving actively which are adequate for the repellent efficacy test. The mites that failed in this screening test should be disposed immediately.

⑦ Recording of Test Results

The result of crossing records during the exposure time and repelled mites should be managed by the investigator. The investigator and assistant investigators should record the number of mites and repellent time in all repellent results occurring in the test. The repelling effects may be expressed as reduction ratio (%) of the number of mites crossing.

For the test results, the followings should be recorded.

ⅰ) 95% Repellency: In terms of mites, repellency means that there is no mite that climbs up to the body part applying the repellent. When compared to control group, it refers to that the repellency of mites for each test shows 95%, and time at this point should be recorded. Regarding the test species of each mite, the mean and SD of the time when 95% repellency occurs should be recorded. The deviation between repeated tests should be considered through statistical analysis, and the reason to use each statistical method should be explained.

ⅱ) Complete Protection Time (CPT): Record the repellent time when 2 entities of mites cross consecutively or both 2 entities cross after treating study drug in each test. Record the mean and SD of CPT for each mite test species. The deviation between repeated tests should be considered through statistical analysis, and the reason to use each statistical method should be explained.

⑧ Calculation of Repellency

Repellency percentage (%) can be determined in the following formula.

Repellency (%) = [(C − T) / C)] x 100

Here, T refers to the number of mites not repelled (crossing) in the group treated with the study drug, and C refers to the number of mites not repelled (crossing) in the group treated with negative control. To record the change of efficacy of the repellent by time, repeat the calculation by each time.

⑨ Judgment of Repellent Efficacy

CPT of the study drug should be 2 hours and longer, and 95% repellency should be maintained. In most cases, CPT is very different by species of mites.

2) *In vitro* Warm Plate Efficacy Test Method of Mite Repellent

As an efficacy test of mite repellent, *in vivo* efficacy test method is recommended. For inevitable cases, the following *In vitro* efficacy test method may be used.

① Test Equipment

The behavioral reaction of each mite for study drug is recorded on the test plate (area 20 X 20 cm, thickness 0.4 cm) which maintains warm temperature. The various materials can be used for the plate including plastic, glass, polymer, etc., and it is important to maintain constant test temperature. For example, the test plate can maintain the temperature with aluminum hotplate to maintain temperature of 25 ~ 35℃. Regarding the entire test environment setting, tilt 15° of angle from the vertical line, draw a circle in the plate (diameter of 13 cm for hard tick species, and diameter of 4 cm for trombicula), and draw a horizontal line.

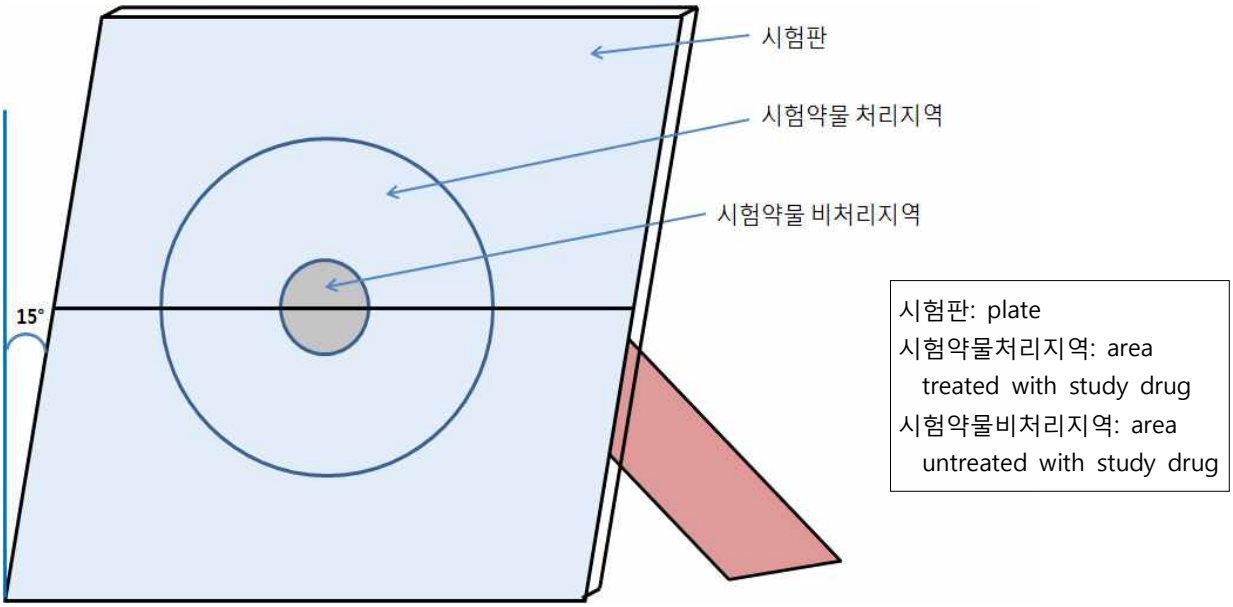

Figure 2. *In vitro* Warm Plate Efficacy Test Method of Mite Repellent

② Test Method

Except the circle center (diameter of 3 cm for hard tick species, and diameter of 1 cm for trombicula), treat the rest of the parts with study drug. Here, cover the circle center with adequate object (e.g. a coin) so that study drug should not be applied, treat the plate with the study drug, and them remove it. With regard to the amount of study drug, apply or spray 1g of the drugs such as aerosol or liquid, or 1 ~ 1.5g of cream, lotion, stick, etc. per 600cm^2 evenly. If 1 g or 1.5 g are inadequate according to the characteristics of study drug, content, dose and administration, establish the amount of use applied by 95% confidence interval, and report the result. If the study drug is measured not weight but volume, it should converted to weight (mg/cm^2 or g/cm^2) based on the density of the product, and the results should be reported as well.

The investigator should treat study drug, and after 10 minutes, use an appropriate apparatus (e.g. paint brush, pipette and cotton swab) to put mites on circle center not treated with study drug carefully so that trunk or fore-legs of mites should not be damaged. Then observe them for 5 minutes. Test 5 ~ 10 entities of test mites on the plate at once.

③ Negative Control

It is possible to screen the behavior of mites to find host through negative control test. Only the mites moving actively should be used in the repellent efficacy test. In the negative control group, test at least 30 entities and more mites on the test plate treated with 50% ethanol (or isopropanol) instead of study drug. The investigator should use appropriate apparatus (e.g. paint brush, pipette and cotton swab) to put mites on the on the circle center not treated with study drug in the plate carefully so that the trunk or fore-legs of mites should not be damaged. The mites moving toward study drug-treated area constantly are the mites moving actively which are adequate for the repellent efficacy test. The mites that failed in this screening test should be disposed immediately.

④ Observation of Test Results

To evaluate the repellent efficacy of study drug, observe whether mites move toward the study drug-treated area for 5 minutes, and then record the following behavioral reactions of mites.

- The number of mites move downward to cross toward the area treated with the study drug
- The number of mites falling outside from the areas untreated or treated with the study drug
- The number of mites that do not leave the area untreated with the study drug within 5 minutes
- The number of mites moving upward to cross to the area treated with the study drug
- The number of mites moving horizontally to cross to the area treated with the study drug

If mites cross toward the area treated with study drug and stay there within 5 minutes, record ‘crossing’ or ‘not repelled’. On the other hand, for mites that do leave the area untreated with the study drug and do not go to the study drug-treated area, or they return immediately or fall to the ground even if they go, record ‘repelled’.

If 2 entities of mites show crossing (or not repelled) behaviors in the consecutive tests (e.g. 1 entity cross at the same time line, and 1 entity cross at the next time line, or both 2 entities show crossing behavior at the same test), record the time and report it as CPT.

⑤ Recording of Test Results

The result of crossing records during the exposure time and repelled mites should be managed by the investigator. The investigator and assistant investigators should record the number of mites and repellent time in all repellent results occurring in the test. The repelling effects may be expressed as reduction ratio (%) of the number of mites crossing the study drug.

For the study results, the followings should be recorded.

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⑥ Calculation of Repellency

Repellency percentage (%) can be determined in the following formula.

$$\text{Repellency (\%)} = [(C - T) / C)] \times 100$$

Here, T refers to the number of mites not repelled (crossing) in the group treated with the study drug, and C refers to the number of mites not repelled (crossing) in the group treated with negative control. To record the change of efficacy of the repellent by time, repeat the calculation by each time.

⑦ Judgment of Repellent Efficacy

CPT of the study drug should be 2 hours and longer, and 95% repellency should be maintained.

Attachment 1.

○ The surface area of the skin in arms for efficacy test of the study drug can be measured by approximate value based on the cylindrical surface area. To calculate the surface area, the length of treatment area, the length of wrist circumference in the treated area and the length of elbow circumference are required.

○ The study drug should be applied evenly to overall area of the arm from wrist to elbow (A). With regard to the treated surface area, calculate the approximate value by measuring the elbow circumference (cm) when the arm is stretched (cm)(C), 3 places at constant interval (cm)(B1-3), and the distance from the elbow to wrist (cm)(D).

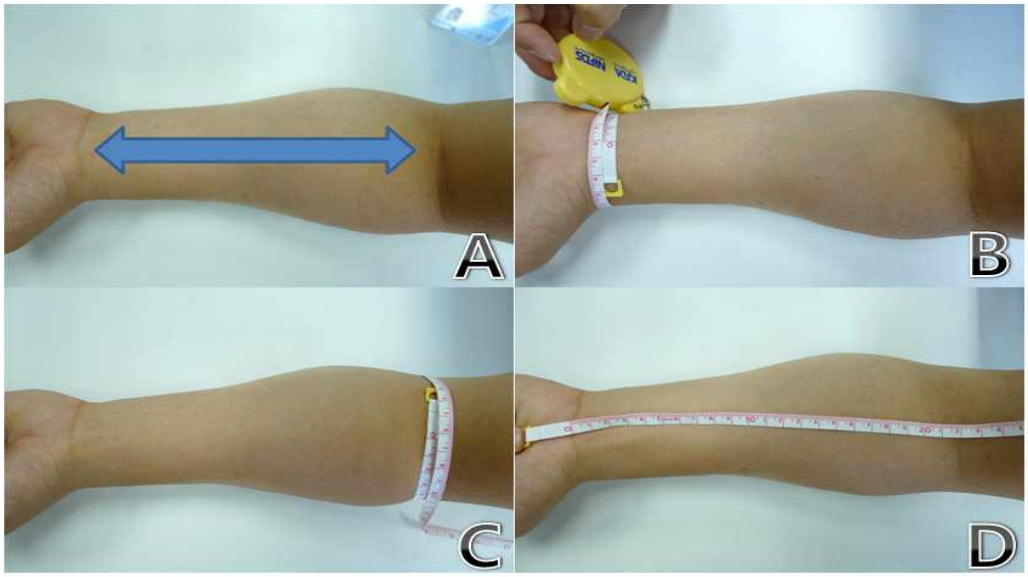

Figure 1. Area treated with repellent(A), measurement of wrist circumference (B), circumference of elbow (C) and length of arm (D)

○ The surface area of the skin (cm^2) should be calculated as below.

$$\text{Surface area} = \frac{(C_{w1}+C_{w2}+\text{---}+C_{w5})}{5} \times D_{we}$$

C_{wi} (i=1,2,3,4,5): Circumference measured in 5 places at constant interval from the wrist to elbow (cm), D_{we}: distance between C_{w1} and C_{w5} (cm)

<Example> Repellent Test using Arm

When the mean of the circumferences measured in 5 places at constant interval between the wrist and elbow of the subject is 20 cm, and the distance from the wrist to elbow is 26 cm, the following calculation can be made using the above formula.

Surface area = 20×26 cm^2 = 520 cm^2

III. Reference

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