Designation: E938/E938M – 12

Standard Test Method for Effectiveness of Liquid, Gel, Cream, or Shampoo Insecticides Against Adult Human Lice[1]

This standard is issued under the fixed designation E938/E938M; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method determines the effectiveness of pediculicidal materials in liquid, gel, or cream form, against the adult human louse, *Pediculus humanus humanus*, the surrogate subspecies for the human head louse (*P.h. capitis*). (Only gels or creams that liquefy at 32°C [90°F] can be tested).

1.2 This test method is for the use of those wishing to develop efficacy data on adult lice.

1.3 This test method consists of five replicates for a statistical comparison of formulations.

1.4 The values stated in either SI units or inch-pound units are to be regarded separately as standard. The values stated in each system may not be exact equivalents; therefore, each system shall be used independently of the other. Combining values from the two systems may result in non-conformance with the standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Terminology

2.1 *Definitions of Terms Specific to This Standard:*

2.1.1 *morbid*—unable to move towards heat 1 h after treatment: sickly, but not necessarily dying; may recover by 24 h.

2.1.2 *moribund*—unable to move towards heat (and therefore food) 24 h after treatment; dying.

3. Summary of Test Method

3.1 Five replicates of 25 lice shall be used for each test concentration or any other variable tested. Five water control replicates will be used on each day of testing.

3.2 Percent louse mortality, corrected by Abbott's Formula, is determined.[2]

4. Significance and Use

4.1 This test method should provide a consistent approach both in terms of test insects and test procedures for the gathering of efficacy data for pediculicides.

4.2 Data collection in this manner should be suitable for product development and comparison. In addition, it should be suitable for review by regulatory agencies.

5. Apparatus and Materials

5.1 *Test Container*—A 9-dram plastic vial, screened at the bottom with 20-mesh screen, shall be used as the dipping vessel. A plunger, made from a plastic rod, and a circular screen fits inside the vial. Plastics used should be as chemically unreactive as possible. Plastic vials are to be discarded after each test.

5.2 *Beakers*—A 100- to 500-mL beaker is used to contain the pediculicide into which the test container is dipped. A1000-mL beaker is used as the container in which the lice are washed after treatment.

5.3 *Heating Surface*—A slide warmer that provides heat of approximately 37°C [98°F].

5.4 *Incubator,* capable of maintaining a temperature of 31.7°C [89°F] and 60 % RH.

5.5 *Petri dishes,* 8.9 cm in diameter and 1.3 cm deep.

5.6 *Waterbath,* capable of maintaining 32°C [90°F].

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.12 on Insect Control Agents.

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[2] Abbott, W. S., "A Method of Computing the Effectiveness of An Insecticide," *Journal of Economic Entomology*, Vol 18, 1925, pp. 265–267.

5.7 *Dark Cotton Corduroy,* 4 by 4 cm.

5.8 *Paper Toweling, Stop Watch, Forceps or Spoon,* and *Wash Bottle*.

5.9 *Test Insect*—The test insect is the human body louse, *Pediculus humanus humanus*. The present strain was established from the USDA Gainesville strain.[3] It is a susceptible strain and, through selection, has adapted to a rabbit host.

5.10 *Host Animal*—New Zealand white rabbits.

6. Rearing of Test Insects

6.1 Collect eggs twice a week. This can be done when the corduroy patches are placed on the rabbit. The adult lice leave the patches to feed. The patches are then removed from the rabbit. Any lice that do remain on the patches should be removed.

6.2 Place the patch containing eggs in a plastic container (10 by 7 cm) with a screened lid, and note the date on the container. Place the container in an incubator that is maintained at 31.7°C and 60 % RH.

6.3 The eggs will hatch in approximately 7 days and a blood meal should be provided to the newly-hatched nymphs on day 7.

6.4 Provide blood meals six days a week. Allow the lice to feed on the shaved abdomen of a restrained rabbit. The rabbit is placed on its back on the restraining rack for approximately 30 min. Collect the lice after feeding by moving the corduroy patches back and forth gently over the shaved area of the rabbit. Most of the lice will attach to the patch. Pick up any remaining lice with a forceps or a spoon.

6.5 Lice used for testing are usually 17 ± 1 day old (as determined from the date of the first blood meal).

6.6 Keep adult lice, for egg laying purposes, approximately three weeks (from time of hatching) and then discard.

7. Procedure

7.1 Place 25 adult lice, mixed sexes, in the bottom of the 9-dram test container. Insert the screened plunger to keep the lice from floating to the surface.

7.2 Place the pediculicide to be tested in a 100- to 500-mL beaker and introduce the beaker into a waterbath maintained at 32°C. Allow the test formulation temperature to stabilize prior to testing.

7.3 Place the 9-dram vial in the 100-mL pediculicide beaker, and keep the lice under the pediculicide for 10 min.

7.4 Remove the test container and blot the bottom of the container to remove any remaining liquid.

7.5 Place the 9-dram vial into the 1000-mL beaker containing tap water at 32°C and agitate the container. At the end of 1 min, remove container, and gently wash the lice in a stream of tap water (32°C) from the wash bottle for 1 min.

7.6 Blot excess water with paper toweling.

7.7 Transfer the lice to a clean 4 by 4-cm patch of dark corduroy cloth. Use forceps to remove any lice that remain in the container. Place corduroy patch in a petri dish, then cover with an additional corduroy patch.

7.8 Place the petri dish with lice in an incubator maintained at 31.7°C and 60 % RH.

7.9 Make the first observation 1 h post treatment, and replace the petri dish in the incubator.

7.10 To make an observation, place the lice on top of one patch, which is on top of the second patch, in a petri dish. The petri dish is then placed on the slide warmer (37°C) or heating pad. Lice not dead or morbid will move to the lower patch within 5 min.

7.11 For the controls, repeat all of the above procedures, substituting tap water for the pediculicide.

8. Analysis of Data

8.1 Numbers of dead and moribund lice are summed to give mortality at 24 h.

8.2 All mortality counts are corrected to mean corrected percent mortality using Abbott's formula:

$$Corrected\,\%\ killed = [(\%\ alive\ control - \%\ alive\ treated)/\%\ alive\ control] \times 100^{3}$$

8.3 Prior to statistical analysis, percent mortalities will be transformed using Arcsine Tranformation Tables.[4] Then an Analysis of Variance (ANOVA) will be performed and the means will be separated by a suitable statistical procedure.

9. Precision and Bias

9.1 No precision data is available for this test method, however, Committee E35 is interested in conducting an interlaboratory test program and encourages interested parties to contact the staff manager, Committee E35, ASTM Headquarters.

[3] The present strain of *Pediculus humanus humanus* is maintained by ICR, Inc., Baltimore, MD 21228. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[4] Box, G., Hunter, W., Hunter, S., Statistics for Experimenters, Wiley, 1978.