Designation: E1517 – 13

Standard Test Method for Determining the Effectiveness of Liquid, Gel, Cream, or Shampoo Insecticides Against Human Louse Ova[1]

This standard is issued under the fixed designation E1517; 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 ovicidal materials in liquid, gel, cream, or shampoo form against the ova (that is, eggs or nits) of the human louse, *Pediculus humanus*.

1.2 This test method is intended for use by those wishing to develop efficacy data or compare ovicidal formulations for human louse ova control.

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

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard, except for temperature measurements in Section 5.

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 *hatched*—those eggs (nits) from which the nymph has emerged completely; an empty, clear egg case with the operculum clearly open.

2.1.2 *unhatched*—those eggs that are opaque; the operculum is closed or the nymph is partly emerged.

3. Summary of Test Method

3.1 Five replicates of 30 eggs are immersed in a test compound for a set period of time, washed, rinsed, blotted dry, and incubated.

3.2 Five control replicates are attached to human hair and processed as the treatment replicates, but with immersion in water.

3.3 Percent egg mortality, corrected by Abbott's Formula, is determined.

4. Significance and Use

4.1 This test method is a standardized test for the gathering of efficacy data for human louse ovicides.

4.2 Data collection in this manner is suitable for product development and comparison, and for review by regulatory agencies, to support the registration of human louse ovicidal products.

5. Apparatus and Materials

5.1 *Applicators*—Egg-infested hairs are attached to the end of a wooden applicator stick with duct tape such that 30 nits are on 1 to 3 hairs. Each replicate of 30 eggs is examined under a dissecting microscope to confirm viability. Any eggs that are shrunken or with other indications of being nonviable are excluded.

5.2 *Beakers*—A 100-mL beaker is used to contain 60 mL of test ovicide and another to contain 60 mL of water (control), into which the applicators are submerged. A 1000-mL beaker is used for washing the eggs.

5.3 *Heating Surface*—A slide dryer that provides heat of approximately 32°C (90°F).

5.4 *Incubator,* capable of maintaining a temperature of 31.7 ± 0.5°C (89°F) and a relative humidity of 60 ± 10 %.

5.5 *Water Bath,* capable of maintaining a temperature of 32°C (90°F).

5.6 *Wash Bottle, Stop Watch, and Dissecting Scope.*

5.7 *Test Insect*—The human louse, *Pediculus humanus*.[2]

5.8 *Positive Control Treatment (Optional)*—60 mL of solution known to give 65 to 95 % mortality of louse eggs when used under these test conditions.

[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] A strain of the human body louse, *Pediculus humanus*, is maintained by Insect Control and Research, Inc., Baltimore, MD 21228-1199. The strain was established from a U.S. Department of Agriculture Gainesville colony. It is a susceptible strain and, through selection, has been adapted to the New Zealand White rabbit.

6. Rearing of Test Insects

6.1 The adult human lice are blood fed on the shaven belly of a restrained rabbit.

6.2 The lice are transferred to human hair cuttings, held in a petri dish, and incubated for 24 h for oviposition to occur.

6.3 The lice are then allowed to crawl off the egg-infested hairs by placing them on a rabbit's belly, leaving the hairs with attached eggs for exposure.

7. Procedure

7.1 Use five replicates of each test formulation and five control replicates.

7.2 Prepare 5 cohorts of eggs for each treatment to be tested including the control treatments. Each cohort consists of 30 eggs (one to three hair shaft(s)) attached with duct tape to a wood applicator stick.

7.3 Heat the test samples to 32 ± 1°C in the waterbath.

7.4 Insert the taped ends (hairs) of the applicator sticks into the test samples for 10 min of immersion.

7.5 Wash the eggs in 900 mL of 32°C tap water for 1 min by vigorous up and down movement of the applicator sticks with the hairs attached.

7.6 Rinse the eggs with water from the wash bottle for 1 min.

7.7 Blot excess water with paper toweling.

7.8 Transfer the hair with attached eggs to labelled petri dishes and incubate.

7.9 Follow the same procedure for the control replications, except substitute tap water for the test solution.

7.10 When all control eggs have hatched (after approximately 12 days), examine all replicates under a dissecting microscope to determine the numbers hatching and failing to hatch. Failure to hatch is recorded as mortality. Categorize eggs failing to hatch as follows:

7.10.1 Early stage (no visible differentiation of the embryo when viewed under 30×);

7.10.2 Late stage (visible differentiation of embryo when viewed under 30×, typically eye spot is visible); and

7.10.3 Emergent (nymphal louse has opened operculum and begun to emerge, but died before emerging completely—part of nymph's body still within egg shell).

8. Analysis of Data

8.1 Calculate the percentage of control eggs failing to hatch; if this exceeds 15 %, the results should be discarded and the test repeated.

8.2 Correct all counts of treated eggs failing to hatch by Abbott's Formula (corrected % killed = (% alive control – % alive treated) × 100 % ÷ % alive control).[3] Confirm that the corrected mortality experienced by the positive controls is 65 to 95 %; if it is not, the results should be discarded and the test repeated.

8.3 Mortality data will be analyzed by appropriate statistical procedures, such as analysis of variance (ANOVA), followed by a standard statistical test to separate the means.

9. Precision and Bias

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

10. Keywords

10.1 effectiveness; human louse ovicides; insecticides

[3] Finney, D., *Probit Analysis*, Cambridge University Press, Cambridge, England, 1962, pp. 88–92.