



Standard Test Method for Determining the Effectiveness of Liquid, Gel, Cream, or Shampoo Insecticides Against Human Louse Ova¹

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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 the standard. The values given in parentheses are for information only.

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, dried, 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 shrunk 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 dipped. 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*—Sixty mL of solution known to give 65 % – 95 % mortality of louse eggs when used under these test conditions.

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

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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.