



## Standard Test Method for Evaluation of Delayed Contact Hypersensitivity<sup>1</sup>

This standard is issued under the fixed designation E 993; 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.

<sup>ε1</sup> NOTE—Section 16 was added editorially in October 1996.

### 1. Scope

1.1 This test method determines whether a test substance will elicit delayed-contact hypersensitivity in guinea pigs. The test method is applicable to individual chemical compounds, simple and complex mixtures, and also finished products.

1.2 *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. Summary of Test Method

2.1 The test material or a solution of the material is placed on the shaved backs of 20 guinea pigs under occlusive patches. Six hours later, the patches and test material are removed from the animals. This procedure is repeated at the same site once a week for two more weeks for a total of three 6-h exposures. These are called the induction exposures.

2.2 Two weeks after the last induction exposure, the test material is applied to a freshly clipped skin site on each test animal that has not been exposed previously. The same patching procedure for the induction exposures is used. Ten additional untreated animals are patched in the same manner to serve as controls. This treatment is called the primary challenge.

2.3 Twenty-four hours after the primary challenge application, the animals are depilated, and a minimum of 2 h later, the test sites are graded on a scale of zero to three. This grading is repeated 24 h later and is called a 48-h grade.

2.4 Grades of one or greater in the test group indicate sensitization, provided grades of less than one are noted on control animals.

2.5 Test animals may be rechallenged 7 to 15 days after primary challenge. Test material is applied to freshly clipped, previously unexposed skin sites (same procedure as challenge). Ten previously untreated animals serve as controls.

### 3. Significance and Use

3.1 This test method can be used to determine whether a test

material will elicit skin sensitization or delayed contact hypersensitivity in guinea pigs.<sup>2,3,4,5</sup>

3.2 The results of this test method are a good indication of the potential for a given test material to produce delayed contact hypersensitivity in human beings under the appropriate conditions.

3.3 This test method can be used as a screening technique before conducting a similar test with human subjects.

### 4. Reagents and Materials

4.1 *Bandage*, a 20 by 20 mm pad on a 37 by 40 mm adhesive square<sup>6</sup>, or a chamber.<sup>7</sup>

4.2 *Cream or Lotion Hair Remover*.<sup>8</sup>

4.3 *Rubber Dam*, elastoplast, or other suitable materials.

4.4 *Small Animal Clipper*.

4.5 *Metal Restrainers*.<sup>9</sup>

### 5. Test Animals

5.1 Young, adult Dunkin Hartley outbred guinea pigs are preferred. Usually 20 test animals, 10 control animals, and 4 to 8 animals for primary irritation are employed for each test substance.

5.2 Equal numbers of males and females in both test and control groups should be used.

### 6. Pretest Conditioning

6.1 Examine the animals on arrival for overt signs of disease, and condition them to the environment for a minimum

<sup>2</sup> Buehler, E. V., *Arch. Dermat.*, Vol 91, 1965, pp. 171–177.

<sup>3</sup> Griffith, J. F. and Buehler, E. V., “Prediction of Skin Irritancy and Sensitizing Potential with Animals and Man”, *Cutaneous Toxicity*, Academic Press, NY, 1977, pp. 162–173.

<sup>4</sup> Ritz, H. L., and Buehler, E. V., “Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests”, *Cutaneous Toxicity*, Academic Press, NY, 1980, pp. 25–40.

<sup>5</sup> “Delayed-type Contact Sensitization”, *Principles for Evaluation of the Toxicity of Household Substances*, National Academy of Sciences, Washington, DC, 1977, pp. 36–44.

<sup>6</sup> Parke-Davis Read Bandage available from Professional Medical Products, Inc., P.O. Box 3288, Greenwood, SC 29648.

<sup>7</sup> Hill Top chamber is available from Hill Top Research Inc., Miamiville, OH 45147, and has been found suitable for this purpose.

<sup>8</sup> Neet Cream or Lotion Hair Remover, available from Whitehall Laboratories, Inc., Medical Dept., 685 Third Ave., New York, NY 10017 has been found suitable for this purpose.

<sup>9</sup> Metal restrainers available from Suburban Surgical, 275 12th St., Wheeling, IL 60890, have been found suitable for this purpose.

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