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Standard Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test[1]

This standard is issued under the fixed designation F 720; 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.

ε[1] NOTE—Editorial changes were made throughout in June 2002.

1. Scope

1.1 This practice is intended to determine the potential for a substance, or material extract, to elicit contact dermal allergenicity.

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. Referenced Documents

2.1 *ASTM Standards:*

F 619 Practice for Extraction of Medical Plastics[2]

3. Summary of Practice

3.1 After a two-stage induction employing Freund's complete adjuvant and sodium lauryl sulfate, the substance or extract is placed on patches and then placed on the skin of guinea pigs. After 24 h, the patches are removed and the skin examined for allergic reaction, and the intensity of the reaction scored at the time of removal and 24 and 48 h subsequent to removal.

4. Significance and Use

4.1 In selecting a new material for human contact in medical applications, it is important to ensure that the material will not stimulate the immune system to produce an allergic reaction. The reaction would be due to substances which could leach out of a material. Therefore, this practice provides for using material extracts. The rationale for this practice is based on the fact that the guinea pig has been shown to be the best animal model for human allergic contact dermatitis. The use of Freund's complete adjuvant and sodium lauryl sulfate tends to enhance the potential of a material to cause an allergy. Therefore, this test, while not guaranteeing that a material is nonallergenic, is the most severe animal test in common use today.

5. Materials and Manufacturer

5.1 *Hartley Strain Guinea Pigs*, male, 300 to 500 g.

5.1.1 Ten animals are used for each test material.

5.2 *Freund's Complete Adjuvant.*

5.3 *Occlusive Surgical Tape*, 3.75 cm in width.

5.4 *Elastic Bandage*.

5.5 *Sodium Lauryl Sulfate* (10 weight %) in USP petroleum jelly.

5.6 *Positive Control Substance*.

5.6.1 5 % formaldehyde for water-soluble test substances.

6. Preparation of Test Samples

6.1 *Samples for Intradermal Injection*:

6.1.1 *Water-Soluble Constituents or Water Extract Liquids*:

6.1.1.1 Dissolve the water soluble constituent up to its maximum solubility, not to exceed a concentration of 10 weight %, or obtain a water extraction liquid as described in Practice F 619.

6.1.1.2 Combine equal volumes of the liquid described in 6.1.1.1 and Freund's complete adjuvant. Homogenize by continuous and vigorous vortex mixing for a minimum of 5 min. Emulsification is complete when a drop placed on the surface of a water-ice bath remains intact.

6.1.1.3 Also prepare the constituent or extract to the same concentration in water without Freund's complete adjuvant.

6.1.2 *Oil Soluble Constituents*:

6.1.2.1 Dissolve oil-soluble constituents in Freund's complete adjuvant to concentration of 10 weight %.

6.1.2.2 Combine equal volumes of the 10 % Freund's adjuvant solution with an equal volume of water by slowly adding the water to the adjuvant while homogenizing with a rotating stirrer. Homogenize by continuous and vigorous mixing for a minimum of 5 min. Emulsification is complete when a drop placed on the surface of a water-ice bath remains intact.

6.1.2.3 Also prepare the constituent to an equal concentration without Freund's complete adjuvant.

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[1] This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

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[2] *Annual Book of ASTM Standards,* Vol 13.01.

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6.1.3 *Vegetable Oil Extract Liquids*:

6.1.3.1 Mix equal volumes of oil extract liquid obtained in accordance with Practice F 619 with an equal volume of Freund's complete adjuvant.

6.1.3.2 Also prepare extract to an equal concentration in water without Freund's complete adjuvant.

6.1.4 Prepare control substances consistent with 6.1.1-6.1.3.2.

NOTE 1—If the final concentration of the suspected allergen results in deleterious effects such as ulceration, necrosis, or systemic toxicity, use the maximum tolerable concentration.

6.2 *Samples for Topical Application*:

6.2.1 *Liquids*—For all liquids, use highest concentration not causing excessive irritation or deleterious to general health of test animals. (See Note 1.)

6.2.1.1 For oil-miscible liquids, dilute with petroleum jelly if necessary.

6.2.1.2 For water-miscible liquids, dilute with water if necessary.

7. Procedure for Test and Control Substances

7.1 *Induction*:

7.1.1 *Intradermal Injection*:

7.1.1.1 Clip the shoulder region of each guinea pig free of hair exposing a 4 by 6-cm area.

7.1.1.2 Three injection sites on each side of the spine should be identified with at least 1.5 cm between sites. Intradermal injections are then made as indicated below, with Site 1 being closest to the animal's head.

(1) Site 1—0.1 mL of adjuvant without test sample

(2) Site 2—0.1 mL of test sample without adjuvant

(3) Site 3–0.1 mL of test sample emulsified in complete adjuvant

7.1.2 *Topical Application*:

7.1.2.1 One week after the intradermal injections, clip test area of guinea pigs.

7.1.2.2 If test sample is nonirritating, treat each test area with 10 % sodium lauryl sulfate (SLS) in petroleum jelly, 24 h before applying test patches. Massage SLS into skin with a glass rod.

7.1.2.3 Apply test sample to a 2 by 4-cm patch of qualitative filter paper. Apply test agent in petroleum jelly to filter in a thick, even layer. Apply test liquid to test paper until saturated.

7.1.2.4 Apply filter paper to injection site of guinea pigs. Occlude with 3.75-cm occlusive surgical tape, and wrap an elastic bandage around the torso to secure tape.

7.1.2.5 Leave in place for 48 h.

7.2 *Challenge*:

7.2.1 Perform two weeks following induction described in 7.1.

7.2.2 Shave a 5 by 5-cm area on animal's flank.

7.2.3 *Delivery of Test Sample*:

7.2.3.1 Test solids as a 25 % concentration (providing this concentration is not irritating) in petroleum jelly by weight. Apply on a 2 by 2-cm piece of filter paper as outlined in 7.1.2.3.

7.2.3.2 Test liquids in undiluted form consistent with 6.1.5. Apply on a 2 by 2-cm piece of filter paper as outlined in 7.1.2.3.

7.2.4 Occlude filter paper, and sample with occlusive surgical tape and elastic bandage as described in 7.1.2.4.

7.2.5 Leave samples in place for 24 h.

8. Interpretation of Results

8.1 Read the challenge sites within 1 h after removing the patches and at 24 and 48 h after removing the patches. Grade each site for erythema and edema in accordance with Table 1.

8.2 Prepare a tabular listing of the reactions at 1, 24, and 48 h. Any animal showing a reaction at 24 or 48 h of two or greater for erythema and edema shall be considered sensitized.

8.3 Rate the allergenicity of the test material in accordance with Table 2.

8.3.1 If a significant number (more than 50 %) of animals show a reaction score of one, repeat the test with an additional ten animals.

8.3.2 If 60 % of the animals in the positive control group do not show a reaction of two or greater, repeat the test.

9. Report

9.1 The report shall include the following:

9.1.1 Test material description, generic name, product name, and lot number.

9.1.2 Method of preparation, that is, extraction liquid used, and form of exposure.

9.1.3 Any necessary dilutions due to severe reactions.

9.1.4 General conditions of animal health.

9.1.5 Scoring of erythema and edema for each animal.

9.1.6 Rating of sensitization response.

10. Keywords

10.1 acute toxicity tests; allergenicity; biocompatibility; guinea pigs; sensitization; test animals

TABLE 1 Scoring Criteria for Test Reactions

Reaction	Description	Score
Erythema	erythema and eschar	
	no erythema	0
	very slight erythema (barely perceptible)	1
	well-defined erythema (pale red in color)	2
	moderate to severe erythema (red and area well defined)	3
	severe erythema (beet redness to slight eschar formation)	4
Edema	edema formation	
	no edema	0
	very slight edema (barely perceptible)	1
	slight edema (edges of area well-defined by definite raising)	2
	moderate edema (edges raised approximately 1 mm)	3
	severe edema (raised more than 1 mm and extending beyond area of exposure)	4

TABLE 2 Rating of Sensitization Response

% Sensitized	Grades	Classification
0 to 8	I	no different than control
9 to 28	II	mild
29 to 64	III	moderate
65 to 80	IV	strong
81 to 100	V	extreme

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